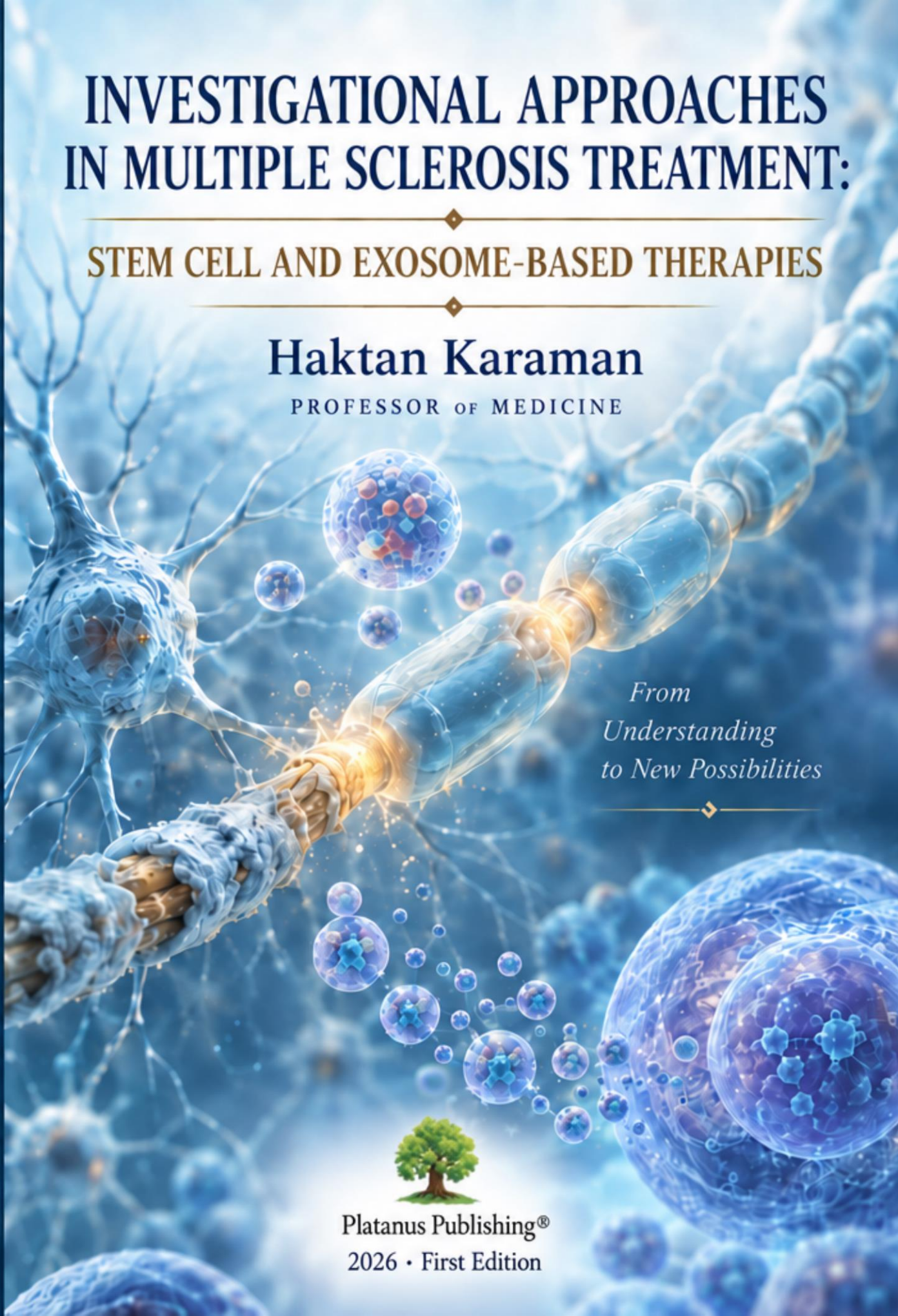
INVESTIGATIONAL APPROACHES IN MULTIPLE SCLEROSIS TREATMENT:

STEM CELL AND EXOSOME-BASED THERAPIES

Haktan Karaman

PROFESSOR OF MEDICINE



From Understanding to New Possibilities

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STEM CELL AND EXOSOME-BASED THERAPIES

Haktan KARAMAN

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*"I dedicate this book to my beloved daughter **Ranya***
*and my beloved son **Yekta**.*

Every page of this work carries a reflection of love,
hope, and inspiration you have brought into my life."

Preface

Multiple sclerosis (MS) imposes substantial neurological and social burdens despite major advances in disease-modifying therapy. This book examines biological rationale, preclinical work, and clinical evidence for stem cell and extracellular vesicle-based approaches. Established care, clinical investigation, and experimental mechanisms are distinguished throughout; biological plausibility alone does not demonstrate patient benefit.

Literature sources and scope: I consulted PubMed, Scopus, Web of Science, Embase, and Google Scholar. For recent research findings and contemporary developments, I focused on work published from 2010 through 2026. I did not impose a fixed publication-date limit on foundational information; earlier sources were included where they provided relevant historical, biological, or clinical context. This book is a narrative synthesis, not a systematic review. Evidence from controlled clinical studies is distinguished from uncontrolled observations, studies using human-derived samples, and preclinical models. Study design, prespecified outcomes, comparator groups, and follow-up inform the interpretation of therapeutic claims; a laboratory finding is not evidence of efficacy in patients.

Writing this book has been a deep personal and professional journey. It is my privilege to dedicate this work to those who have been my unwavering source of strength and inspiration: my beloved mother, Makbule, and my late father, Abdulcelil, whose wisdom and values continue to guide me; and my dear children, Ranya and Yekta, who are the light of my life and the driving force behind my pursuit of a better world for future generations.

May this book help clarify what these emerging approaches can and cannot yet offer, and stand as a tribute to the resilience and courage of those living with MS. Together, let us continue to test these ideas rigorously, so that the hope of those living with MS comes to rest on firmer evidence.

Prof. Dr. Haktan KARAMAN

2026

Foreword

Multiple sclerosis (MS) causes substantial neurological disability. Treatments that control inflammatory disease activity have improved, but established tissue damage and progression remain difficult to address. Stem-cell and extracellular-vesicle approaches are being investigated in response to these unmet needs.

I am pleased to introduce Investigational Approaches in Multiple Sclerosis Treatment: Stem Cell and Exosome-Based Therapies by Prof. Dr. Haktan Karaman. The book examines the biological rationale, clinical evidence, and practical limitations of these approaches within the wider context of MS treatment.

The chapters connect MS pathophysiology with cell and vesicle biology, then assess the findings that support further investigation and the uncertainties that constrain clinical use. This distinction gives clinicians and researchers a basis for judging emerging treatment claims.

Prof. Dr. Karaman's clinical experience in algology and regenerative medicine informs his interest in the questions addressed here. His concern for patients is reflected in the emphasis on clinically meaningful outcomes.

I hope this book will support careful reading of the evidence and well-designed research into treatments that can improve the lives of people with MS.

Prof. Dr. Kenan HASPOLAT

2026

TABLE OF CONTENTS

Chapter 1: Introduction to Multiple Sclerosis 1

1.1. DEFINITION AND GENERAL INFORMATION ABOUT MULTIPLE SCLEROSIS 2

1. Introduction 2
2. Pathophysiology and Disease Course 3
3. Etiology and Risk Factors 4
4. Diagnosis and Biomarkers 4
5. Clinical Manifestations and Impact 6
6. Treatment and Management 7

References 11

1.2. EPIDEMIOLOGY OF MS: PREVALENCE AND INCIDENCE GLOBALLY 19

1. Introduction 19
2. Global Prevalence and Incidence Trends 19
3. Regional Variations in MS Epidemiology 20
4. Factors Influencing MS Epidemiology 21
5. Climate Change and MS Epidemiology 22
6. Gender Disparities in MS Epidemiology 22
7. Temporal Trends in MS Epidemiology 22
8. MS Epidemiology in Special Populations 23
9. Challenges in MS Epidemiology Research 23
10. Implications for Healthcare and Research 24
11. Future Directions in MS Epidemiology Research 24
12. Conclusion 25

References 25

1.3. PATHOPHYSIOLOGY AND SYMPTOMS OF MULTIPLE SCLEROSIS 27

1. Introduction 27
2. Pathophysiology of Multiple Sclerosis 27
3. Disease Course and Clinical Subtypes 28
4. Symptoms of Multiple Sclerosis 29
5. Factors Influencing Symptom Presentation 31
6. Diagnostic Challenges 31
7. Impact on Quality of Life 32
8. Management Approaches 32
9. Conclusion 33

References 33

1.4. GENETIC AND ENVIRONMENTAL RISK FACTORS 36

1. Introduction 36
2. Genetic Risk Factors 36
3. Environmental Risk Factors 38
4. Gene-Environment Interactions 41
5. Epigenetic Mechanisms in MS 41
6. Protective Factors 42
7. Implications for Prevention and Treatment 42
8. Future Directions 43
9. Conclusion 43

References 44

1.5. CURRENT TREATMENT METHODS FOR MULTIPLE SCLEROSIS AND THEIR LIMITATIONS: A COMPREHENSIVE ANALYSIS 52

1. Introduction 52
2. Disease-Modifying Therapies (DMTs) 53
3. Symptomatic Treatments 53
4. Emerging and Experimental Therapies 55
5. Challenges and Future Directions 56
6. Conclusion 56

References 56

Chapter 2: Pathophysiology of Multiple Sclerosis 59

2.1. IMMUNE SYSTEM DYSFUNCTION IN MS 60

1. Immune System Dysregulation in MS 60
2. Demyelination and Axonal Damage 63
3. Blood-Brain Barrier Disruption 64
4. Oxidative Stress and Mitochondrial Dysfunction 64
5. Remyelination and Repair Mechanisms 65
6. Genetic and Environmental Factors 66
7. Emerging Concepts in MS Pathophysiology 66
8. Therapeutic Implications and Future Directions 67
9. Conclusion 68

References 68

2.2. MECHANISMS OF DEMYELINATION AND AXONAL DAMAGE IN MULTIPLE SCLEROSIS 74

1. Introduction 74
2. Immune-Mediated Demyelination 74
3. Mechanisms of Axonal Damage 77
4. The Interplay Between Demyelination and Axonal Damage 79
5. Remyelination and Repair Mechanisms 80
6. Emerging Concepts and Future Directions 81
7. Neuroplasticity and Adaptive Mechanisms in MS 82
8. The Role of Glial Cells in MS Pathogenesis and Repair 84
9. Emerging Therapeutic Approaches 84
10. The Impact of Environmental Factors on MS Pathogenesis 85

References 86

2.3. THE ROLE OF NEUROINFLAMMATION 93

1. Introduction 93
2. Pathophysiology of Neuroinflammation in MS 93
3. Molecular Mediators of Neuroinflammation in MS 94
4. Novel Molecular Players in MS Neuroinflammation 95
5. Neuroinflammation and Neurodegeneration in MS 95
6. Imaging Neuroinflammation in MS 96
7. Biomarkers of Neuroinflammation in MS 96
8. Therapeutic Targeting of Neuroinflammation in MS 97
9. Future Directions in MS Neuroinflammation Research 98
10. The Role of Glial Cells in MS Neuroinflammation 98

11. Oxidative Stress and Mitochondrial Dysfunction in MS Neuroinflammation 99
12. The Role of Adaptive Immunity in MS Neuroinflammation 100
13. Environmental Factors Influencing Neuroinflammation in MS 100
14. Neuroplasticity and Repair Mechanisms in MS 100
15. Chronic Neuroinflammation and Progressive MS 101
16. Emerging Therapeutic Approaches Targeting Neuroinflammation 101
References 102

2.4. BLOOD-BRAIN BARRIER DISRUPTION IN MULTIPLE SCLEROSIS 110
1. Introduction 110
2. Mechanisms of BBB Disruption in MS 111
3. Clinical Implications of BBB Disruption in MS 113
4. Therapeutic Approaches Targeting BBB Disruption 114
5. Future Directions and Challenges in BBB Disruption Research in MS 116
References 117

Chapter 3: Current Treatment Landscape 125

3.1. DISEASE-MODIFYING THERAPIES (DMTs) 126
1. Introduction 126
2. Historical Perspective and Evolution of DMTs 126
3. Current Landscape of DMTs 127
4. Mechanism of Action and Efficacy Profiles 127
5. Agent-by-Agent Overview of Approved DMTs 128
6. Challenges in DMT Selection and Management 131
7. Special Populations and Considerations 132
8. Emerging Trends and Future Directions 133
9. Conclusion 133
References 134

3.2. SYMPTOMATIC TREATMENTS 139
1. Introduction 139
2. Pharmacological Interventions: A Cornerstone of Symptom Management 139
3. Symptom-Specific Pharmacological Interventions 140
4. Non-Pharmacological Approaches: Complementing Medical Management 141
5. The Role of Technology in Symptom Management 142
6. Addressing Invisible Symptoms: The Hidden Burden of MS 143
7. The Importance of Holistic Care in MS Management 144
8. The Impact of Comorbidities on MS Symptom Management 145
9. Emerging Therapies and Future Directions 145
10. Conclusion 146
References 146

3.3. LIMITATIONS AND SIDE EFFECTS OF CURRENT TREATMENTS 152
1. Efficacy Limitations of Disease-Modifying Therapies 152
2. Side Effects and Safety Concerns 154
3. Treatment Adherence Challenges 155
4. Limitations in Addressing Disease Progression 156
5. Challenges in Treatment Selection and Sequencing 157
6. Pregnancy and Family Planning Considerations 157
7. Conclusion 158

References 158

Chapter 4: Stem Cell Therapy *161*

4.1. DEFINITION AND TYPES OF STEM CELLS (E.G., EMBRYONIC, MESENCHYMAL, HEMATOPOIETIC) **162**

1. Introduction 162
2. Defining Stem Cells 163
3. Types of Stem Cells 167
4. Clinical Applications of Stem Cells 174
5. Stem Cells and Technology: The Intersection with Artificial Intelligence 176
6. Challenges and Future Directions 177
7. Conclusion 179

References 180

4.2. SOURCES OF STEM CELLS FOR THERAPY **195**

A. TYPES OF STEM CELLS 195
1. Embryonic stem cells (ESCs) 195
2. Adult stem cells (ASCs) 196
3. Induced Pluripotent stem cells (iPSCs) 203
4. Perinatal Stem Cells 205
5. Parthenogenetic Stem Cells 206
6. Somatic Cell Nuclear Transfer (SCNT) 207
7. Cancer Stem Cells (CSCs) 208

B. STEM CELL THERAPY: CURRENT APPLICATIONS AND FUTURE PROSPECTS 209
C. ETHICAL AND REGULATORY CONSIDERATIONS IN STEM CELL RESEARCH 210

References 211

4.3. ISOLATION AND CULTURE OF STEM CELLS **223**

1. Introduction 223
2. Stem Cell Isolation: Techniques and Considerations 224
3. Challenges and Future Directions in Stem Cell Isolation 228
4. Stem Cell Culture: Maintaining Stemness and Directing Differentiation 229
5. Challenges in Stem Cell Culture 231
6. Future Directions in Stem Cell Culture 231
7. Applications of Stem Cells 231
8. Ethical Considerations in Stem Cell Research 233
9. Future Directions in Stem Cell Research 233
10. Conclusion 234

References 236

4.4. MECHANISM OF ACTION IN MS TREATMENT **248**

1. Immunomodulation 248
2. Myelin Regeneration 252
3. Neuroprotection 257

References 259

4.5. PRECLINICAL STUDIES: ANIMAL MODELS AND IN VITRO STUDIES **268**

1. Animal Models in MS Research 268
2. In Vitro Studies and Cellular Mechanisms 272
3. Molecular and Genetic Investigations 274

4. Emerging Therapeutic Approaches 275
5. Challenges in Translating Preclinical Findings 277
6. Future Directions and Emerging Technologies 278
7. Future Prospects and Potential Impacts 281
References 283

4.6. CLINICAL TRIALS AND OUTCOMES OF STEM CELL THERAPY IN MULTIPLE SCLEROSIS: 292
1. Introduction 292
2. Clinical Trials and Results 293
3. Discussion and Future Directions 299
4. Meta-Analyses and Reviews 301
Conclusion 301
References 303

4.7. ADMINISTRATION ROUTES AND METHODS 307
1. Introduction 307
2. Intravenous Administration 307
3. Intrathecal Administration 311
4. Localized Delivery 315
5. Conclusion 318
References 319

4.8. CHALLENGES AND CONSIDERATIONS 324
1. Introduction 324
2. Ethical Concerns 324
3. Graft-versus-Host Disease in Allogeneic Transplantation 326
4. Tumorigenicity 327
5. Conclusion 329
References 329

Chapter 5: Exosome Therapy 335

5.1. DEFINITION AND BIOLOGICAL ROLE OF EXOSOMES 336
1. Historical Perspective and Evolution of Exosome Research 336
2. Exosome Biogenesis and Secretion 337
3. Molecular Composition of Exosomes 338
4. Biological Roles of Exosomes 340
5. Exosomes in Diagnostics and Therapeutics 342
6. Challenges in Exosome Research and Clinical Translation 344
7. Future Directions and Emerging Trends 345
8. Conclusion 346
References 346

5.2. BIOGENESIS AND RELEASE OF EXOSOMES 356
1. The Endosomal Origin of Exosomes 356
2. Formation of Multivesicular Bodies (MVBs) 357
3. Cargo Sorting into Exosomes 358
4. Regulation of Exosome Release 358
5. Regulatory Mechanisms of Exosome Biogenesis and Release 359
6. Exosome Release in Response to Cellular Stress 360

7. Exosome Release in Different Cell Types 361
8. Technological Advances in Studying Exosome Biogenesis and Release 361
9. Exosome Biogenesis and Release in Pathological Conditions 362
10. Therapeutic Potential of Modulating Exosome Biogenesis and Release 362
11. Future Directions and Challenges 363
12. Evolutionary Aspects of Exosome Biogenesis and Release 363
13. Exosome Biogenesis and Release in the Context of Cellular Metabolism 364
14. Conclusion 365
References 365

5.3. CARGO OF EXOSOMES: PROTEINS, LIPIDS, RNA 372
1. Introduction to Exosomes 372
2. Composition of Exosomal Cargo 374
3. Mechanisms of Cargo Loading 379
4. Exosome Biogenesis and Secretion 382
5. Exosome Uptake and Internalization 385
6. Functional Implications of Exosomal Cargo Transfer 387
7. Exosomes as Biomarkers 392
8. Therapeutic Applications of Exosomes 393
9. Emerging Technologies in Exosome Research 394
10. Future Directions and Challenges 395
11. Conclusion 395
References 396

5.4. METHODS OF OBTAINING AND PURIFYING EXOSOMES 414
1. Introduction 414
2. Traditional Methods of Exosome Isolation 415
3. Size-Based Isolation Methods 416
4. Precipitation-Based Methods 416
5. Immunoaffinity-Based Methods 417
6. Microfluidics-Based Techniques 417
7. Comparative Analysis of Isolation Methods 417
8. Impact of Isolation Methods on Exosome Characteristics 418
9. Emerging Trends and Future Directions 418
10. Conclusion 418
References 419

5.5. MECHANISM OF ACTION IN MS TREATMENT 422
1. Exosomes: Nature's Cellular Messengers 422
2. Cell Communication in MS: The Exosome Paradigm 423
3. Reduction of Inflammation: Exosomes as Immunomodulators 424
4. Promotion of Remyelination: Exosomes as Regenerative Agents 425
5. Exosome Engineering for Enhanced Remyelination 427
6. Challenges and Future Directions in Exosome-mediated Remyelination 428
7. Ethical Considerations in Exosome-Based MS Therapies 431
8. Economic Impact of Exosome Therapies in MS 431
9. Global Perspective on Exosome Research in MS 431
10. Conclusion 432
References 432

5.6. PRECLINICAL STUDIES: ANIMAL MODELS AND IN VITRO STUDIES 438
1. Preclinical Studies: Animal Models and In Vitro Studies 440
2. Critical Evaluation of Current Limitations and Challenges 448
3. Future Directions and Recommendations 450
References 452

5.7. PRECLINICAL, HUMAN MECHANISTIC, AND BIOMARKER STUDIES OF EVs IN MS 454
Evidence synthesis 457
Conclusion 458
References 458

5.8. ADMINISTRATION ROUTES AND METHODS 461
1. Intravenous Delivery 461
References 469
2. Intrathecal Delivery 472
References 479
3. Localized Delivery 480
References 483

5.9. CHALLENGES AND CONSIDERATIONS 485
1. DOSAGE AND DELIVERY 485
References 488
2. IMMUNE RESPONSE 489
References 494
3. STANDARDIZATION AND QUALITY CONTROL 496
References 500

Chapter 6: Combined Treatment Approaches 505

6.1. RATIONALE FOR COMBINING STEM CELL AND EXOSOME THERAPIES 506
References 511

6.2. MECHANISM AND SYNERGISTIC EFFECTS OF COMBINED THERAPY 516
6.2.1. Enhanced immunomodulation: mechanistic rationale 516
6.2.2. Remyelination and neuroprotection: evidence synthesis 521
References 525

6.3. ADVANTAGES AND DISADVANTAGES OF COMBINED THERAPY 531
1. Advantages of Combined Therapy 531
2. Disadvantages and Challenges of Combined Therapy 533
3. Future Directions and Research Priorities 534
4. Conclusion 535
References 536

Chapter 7: Clinical Studies and Translational Experience 539

7.1. PUBLISHED CLINICAL STUDIES OF CELL-BASED THERAPIES AND LONG-TERM OUTCOMES 540
1. Historical Context and Early Studies 540
2. Mesenchymal stromal cell evidence: critical synthesis 540
3. Evidence hierarchy and clinical positioning 541
4. Hematopoietic Stem Cell Transplantation (HSCT) in Multiple Sclerosis 541

5. Neural stem/progenitor cell approaches in multiple sclerosis 543
6. Synthesis 544
7. Exosome Therapies in Multiple Sclerosis: Emerging Frontiers in Treatment 544
References 547

7.2. COMPARISON WITH TRADITIONAL TREATMENTS OF MS 552
1. Introduction 552
2. Traditional Treatments for Multiple Sclerosis 552
3. Stem Cell Therapies in Multiple Sclerosis 553
4. Exosome Therapies in Multiple Sclerosis 555
5. Comparative Analysis of MS Therapies 555
6. Emerging Combination Therapies 557
7. Future Directions and Challenges 558
8. Conclusion 559
References 559

Chapter 8: Conclusion and Future Perspectives 563

8.1. Introduction 564
8.1.1. Neuroprotection and Remyelination Strategies 564
8.1.2. Targeted Immunotherapies 566
8.1.3. Gene Therapy Approaches 566
8.1.4. Nanoparticle-based Therapies 567
8.1.5. Stem cell-based Approaches 568
8.1.6. Microbiome-based Interventions 568

8.2. Integration of Treatment Strategies into Clinical Practice 569
8.2.1. Multi-disciplinary Approaches 569
8.2.2. Clinical Decision Support Tools 569
8.2.3. Telemedicine and Remote Monitoring 569
8.2.4. Patient Education and Self-Management 570
8.2.5. Integration of Complementary Therapies 570
8.2.6. Pharmacoeconomic Considerations 571
8.2.7. Implementation Science in MS Care 571

8.3. Regulatory and Ethical Considerations 571
8.3.1. Regulatory Challenges in Stem Cell Therapies 571
8.3.2. Ethical Implications of Gene Editing 571
8.3.3. Equity and Access to Advanced Therapies 572
8.3.4. Ethical Considerations in AI and Big Data 573
8.3.5. Informed Consent and Patient Autonomy 573

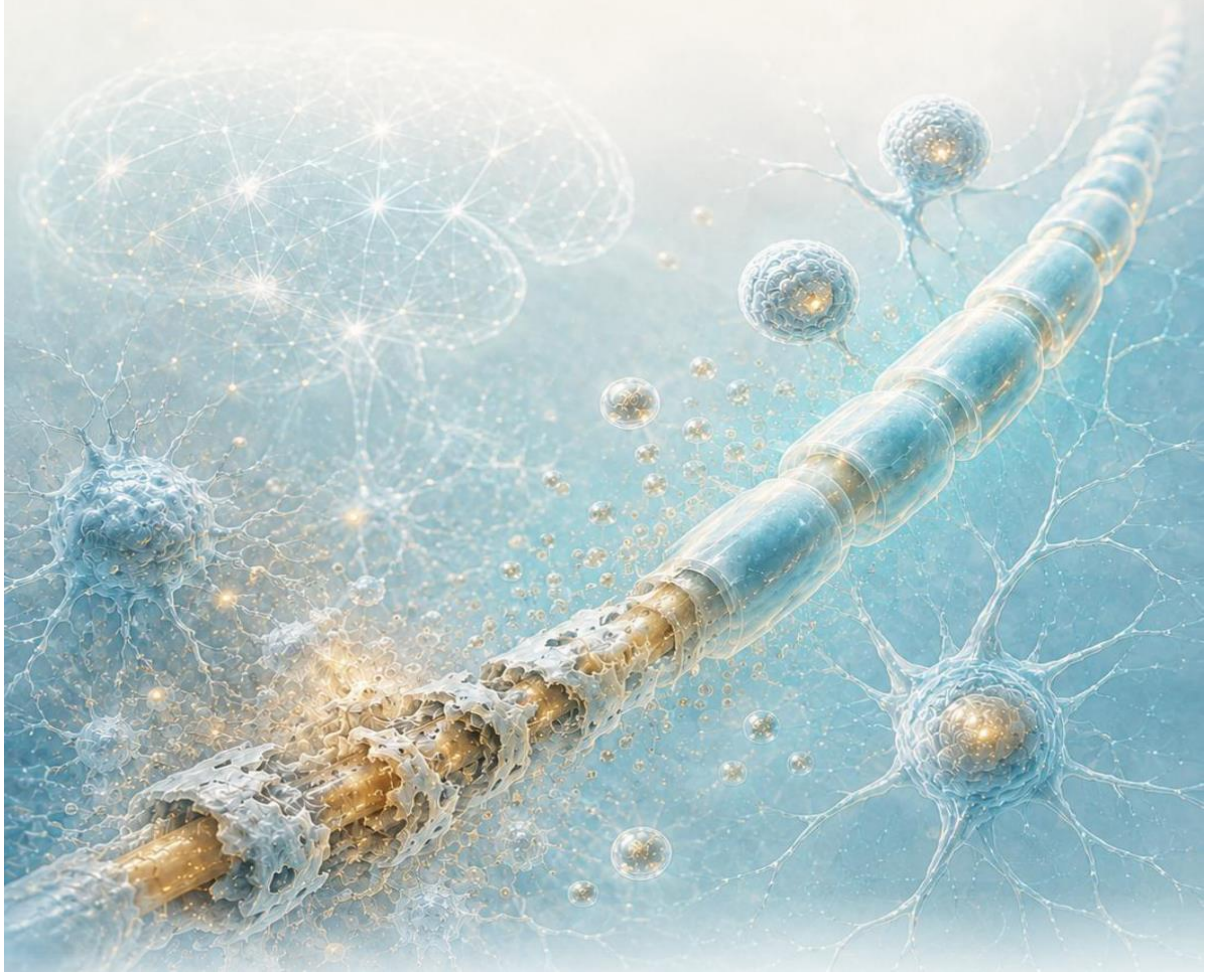
8.4. The Role of Technology and AI in Advancing MS Treatment 574
8.4.1. AI in Diagnosis and Prognosis 574
8.4.2. AI in Treatment Optimization 574
8.4.3. AI in Drug Discovery 575
8.4.4. Natural Language Processing in Clinical Documentation 575
8.4.5. AI in Personalized Rehabilitation 576
8.4.6. AI in Predicting and Managing Comorbidities 577
8.4.7. Challenges and Future Directions 577

8.5. Concluding Remarks 577

References 579

CHAPTER 1

INTRODUCTION TO MULTIPLE SCLEROSIS



1.1. DEFINITION AND GENERAL INFORMATION ABOUT MULTIPLE SCLEROSIS

1. Introduction

Multiple Sclerosis (MS) is a complex, chronic autoimmune disease characterised by inflammation, demyelination, and neurodegeneration within the central nervous system (CNS) (Haacke et al., 2009). This complex neuroinflammatory condition involves a genetically mediated immune response targeting myelin, the protective sheath surrounding nerve fibres in the CNS (Grigoriadis & van Pesch, 2015). The resulting formation of lesions disrupts nerve signal transmission, leading to a wide range of neurological symptoms and varying degrees of disability (Compston & Coles, 2008). As one of the most common neurological disorders affecting young adults, MS poses significant challenges to patients, healthcare systems, and society at large, necessitating a detailed understanding of its pathophysiology, diagnosis, and management strategies. The four disease courses and the descriptors used to qualify them are summarized in Figure 1.1.1.

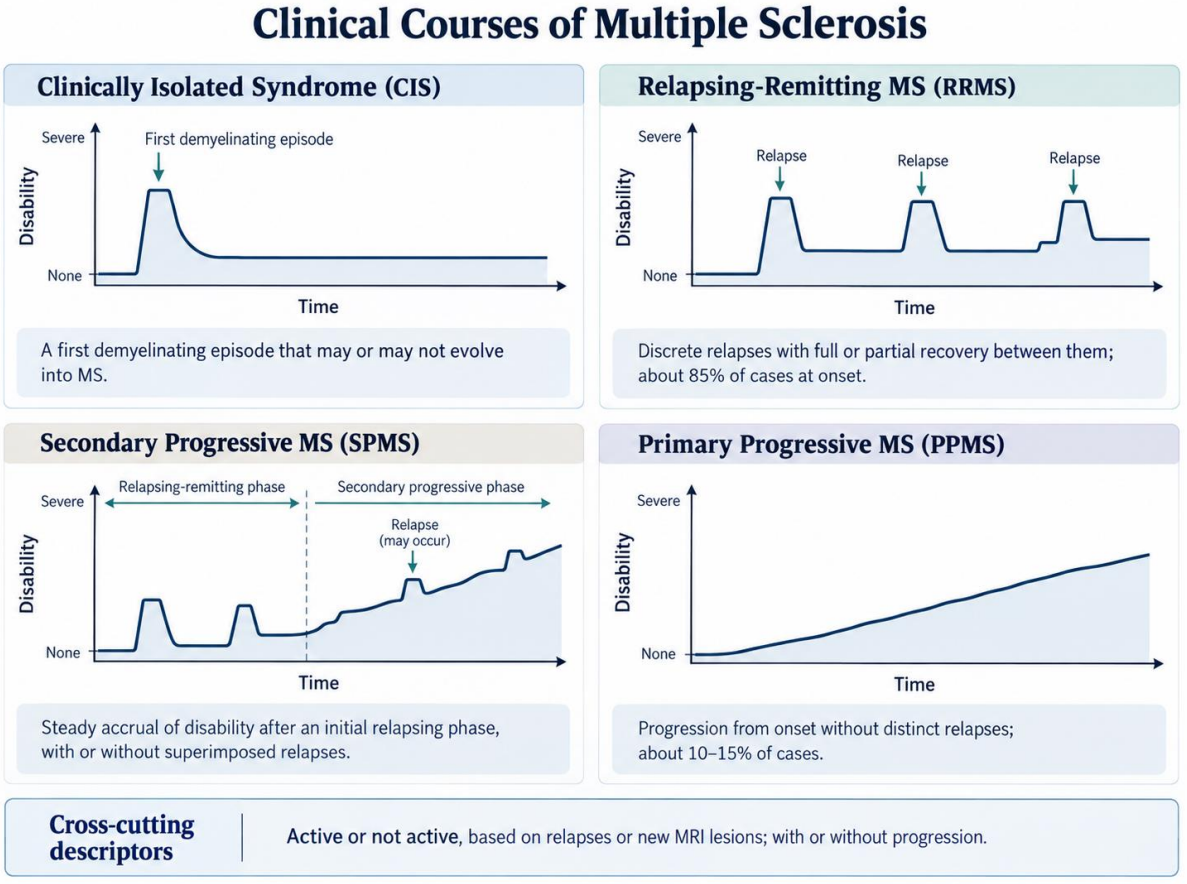


Figure 1.1.1. Clinical courses of multiple sclerosis, shown as disability over time. Proportions refer to disease course at onset and follow the currently used descriptors of activity and progression.

1.1. Historical Perspective

The history of MS as a recognised medical condition dates back to the early 19th century, with the first detailed clinical description attributed to Jean-Martin Charcot in 1868. Charcot's observations of the characteristic sclerotic plaques in the CNS laid the foundation for our modern understanding of the disease. Over the subsequent decades, advancements in medical science, particularly in the fields of neurology and immunology, have significantly enhanced our comprehension of MS pathogenesis and led to the development of increasingly effective therapeutic interventions. See Figure 1.1.2.

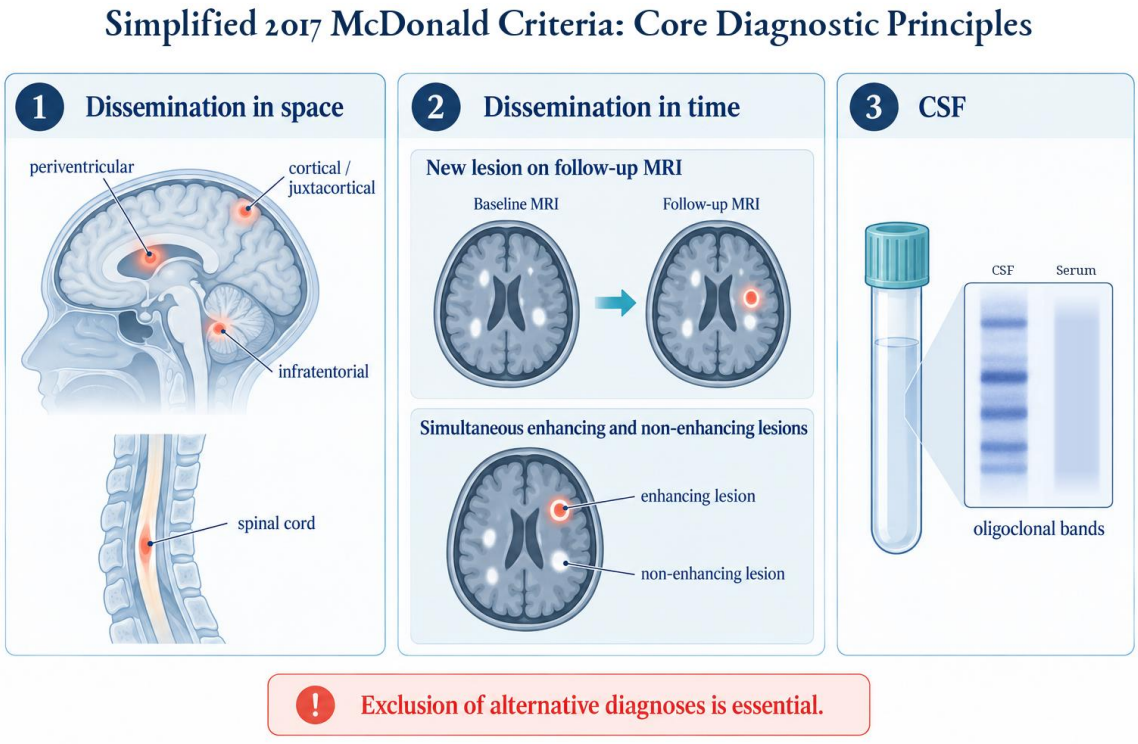


Figure 1.1.2. Simplified core diagnostic principles under the 2017 McDonald criteria: dissemination in space, dissemination in time, and cerebrospinal-fluid-specific oligoclonal bands absent from paired serum. Exclusion of alternative diagnoses remains essential. This schematic is not a complete representation of the 2024 revisions, which are discussed in the text.

1.2. Epidemiology

The Atlas of MS 2026 estimates that approximately 3.1 million people live with MS worldwide, using data gathered for its 2024 update; the earlier Atlas estimate was 2.8 million in 2020 (Multiple Sclerosis International Federation [MSIF], 2026; Walton et al., 2020). Prevalence remains particularly high in North America and Europe. MS most often begins in early adulthood, commonly between 20 and 40 years of age, with a peak incidence around age 30. Women are affected more often than men, with a female-to-male ratio of approximately 3:1 in many populations (Rejdak et al., 2010). The reasons for this sex difference remain incompletely defined and probably involve hormonal, genetic, and environmental influences.

The geographic distribution of MS exhibits a distinct pattern, with higher prevalence rates generally observed at greater distances from the equator. This observation has led to investigations into the role of environmental factors, particularly vitamin D levels and sun exposure, in MS risk and progression (Ascherio & Munger, 2017).

2. Pathophysiology and Disease Course

The pathogenesis of MS involves an interaction of inflammatory processes, autoimmune reactions, and neurodegeneration (Reich et al., 2018). In MS, the immune system erroneously attacks myelin, causing disruptions in nerve signal transmission and forming lesions in the CNS. This autoimmune assault results in the characteristic plaques or lesions visible on neuroimaging, particularly in the white matter of the CNS (Lassmann et al., 2007).

At the cellular level, the pathological hallmarks of MS include inflammation, demyelination, axonal loss, and gliosis. The inflammatory process is characterised by the infiltration of activated T lymphocytes, B cells, and macrophages into the CNS. These immune cells, along with resident microglia, release pro-inflammatory cytokines, chemokines, and other mediators that contribute to myelin damage and neuronal injury (Compston & Coles, 2008).

The demyelination process in MS leads to the formation of acute inflammatory lesions, which can partially remyelinate over time. However, repeated episodes of inflammation and demyelination can result in chronic lesions characterised by significant axonal loss and glial scarring. This progressive neurodegeneration is thought to be the primary driver of irreversible disability in MS (Trapp & Nave, 2008).

2.1. Disease Course and Clinical Phenotypes

The disease course of MS varies substantially between individuals, and several patterns are recognised. Relapsing-remitting MS (RRMS) is the most common form and accounts for approximately 85% of initial MS diagnoses; it is marked by distinct episodes of new or worsening neurological symptoms, termed relapses, followed by periods of partial or complete recovery (Lublin et al., 2014). Many patients with RRMS eventually transition to secondary progressive MS (SPMS), in which neurologic function worsens steadily over time, with or without superimposed relapses (Wong et al., 2023); this transition typically occurs 10 to 20 years after the initial diagnosis of RRMS. Primary progressive MS (PPMS), which affects approximately 10% to 15% of patients, differs in that neurologic function worsens steadily from the onset of symptoms, without early relapses or remissions (Wong et al., 2023). Progressive-relapsing MS (PRMS) is a historical category that was removed in the 2013 phenotype revisions. Progression from onset with superimposed relapses is now described as PPMS with disease activity (Lublin et al., 2014).

The frequency and severity of relapses in RRMS have been associated with poorer recovery of neurological function, accelerated brain atrophy, and increased focal inflammatory activity (Kalincik et al., 2012). Understanding the factors influencing disease progression and the transition between these various forms of MS remains a key focus of ongoing research, with implications for both prognosis and treatment strategies.

3. Etiology and Risk Factors

The aetiology of MS is complex and multifactorial, involving an interaction between genetic susceptibility and environmental triggers. While the precise cause remains elusive, significant progress has been made in identifying factors that contribute to disease risk and progression.

3.1. Genetic Factors

Genetic studies have revealed a strong hereditary component in MS susceptibility. The risk of developing MS is significantly higher in first-degree relatives of affected individuals, with concordance rates in monozygotic twins ranging from 20% to 30% (Ebers, 1994). Genome-wide association studies (GWAS) have identified over 200 genetic variants associated with MS risk, many of which are involved in immune system regulation (International Multiple Sclerosis Genetics Consortium, 2019).

The strongest genetic association with MS is found in the Human Leukocyte Antigen (HLA) region, particularly the HLA-DRB1*15:01 allele, which confers a threefold increased risk of developing the disease (Hollenbach & Oksenberg, 2015). Other genes implicated in MS susceptibility include those encoding interleukin receptors (IL7R, IL2RA) and T-cell activation markers (CD58, CD6).

3.2. Environmental Factors

Several environmental exposures have been associated with an increased risk of developing MS. The most strongly implicated is infection with Epstein-Barr virus (EBV), particularly when it occurs later in life and results in infectious mononucleosis (Hassani et al., 2018; Cortese et al., 2024); reports of EBV-infected B cells in MS brain tissue have been proposed as a mechanistic link between viral infection and disease development, although these neuropathological findings remain contested. Low levels of vitamin D have likewise been associated with an increased risk of MS and with greater disease activity, an association that partly explains the latitude gradient observed in MS prevalence, with higher rates in regions farther from the equator (Ascherio & Munger, 2017). Cigarette smoking is a further recognised risk factor and has been associated with earlier conversion from a clinically isolated syndrome to clinically definite MS (Di Pauli et al., 2008), while childhood and adolescent obesity have been linked to an increased risk of developing MS, possibly through inflammatory mechanisms and altered vitamin D metabolism (Munger et al., 2013). More recently, attention has turned to the gut microbiome, whose importance in MS pathogenesis and progression has been highlighted by recent studies: alterations in gut microbial diversity correlate with disease activity, suggesting a potential avenue for therapeutic intervention (Ní Choileáin et al., 2020; Tremlett & Waubant, 2018).

3.3. Gene-Environment Interactions

The interplay between genetic susceptibility and environmental factors is increasingly recognised as essential in MS pathogenesis. For example, the effect of vitamin D deficiency on MS risk appears to be more pronounced in individuals carrying certain HLA risk alleles (Ramagopalan et al., 2009). Similarly, the impact of EBV infection on MS risk may be modulated by genetic factors.

4. Diagnosis and Biomarkers

The diagnosis of MS can be challenging due to the lack of a single, definitive diagnostic test and the variable presentation of symptoms (Krahn, 2013; Sandberg-Wollheim &

Olsson, 2013). The diagnostic process for multiple sclerosis (MS) often begins with the identification of a clinically isolated syndrome (CIS), an initial episode of neurological disturbance such as optic neuritis, transverse myelitis, or brainstem syndrome. These episodes last at least 24 hours and are not associated with infection or fever (Polman et al., 2011). CIS often reflects focal inflammation or demyelination within the central nervous system (CNS), detectable through magnetic resonance imaging (MRI) (Filippi et al., 2016). MRI is essential for identifying lesions in the CNS, assessing their dissemination in space and time, and supporting the diagnosis of MS (Thompson et al., 2018). Cerebrospinal fluid (CSF) analysis, which can detect oligoclonal bands, provides additional evidence for MS and helps rule out other neurological conditions (Polman et al., 2011). Evoked potentials, measuring sensory response delays, also support the diagnosis by identifying CNS dysfunction (Polman et al., 2011).

MS diagnosis requires integration of clinical presentation, MRI and, where appropriate, cerebrospinal fluid findings, with exclusion of better explanations. The 2017 McDonald criteria remain relevant to the interpretation of older studies (Thompson et al., 2018); the revised 2024 criteria were published in 2025 (Montalban et al., 2025). Early diagnostic assessment can support appropriate treatment, but a clinically isolated syndrome does not invariably progress to MS. Diagnostic criteria should be applied to the populations and clinical situations for which they were developed.

4.1. Diagnostic Criteria

The 2024 McDonald revisions provide a unified diagnostic framework across relapsing and progressive presentations. They add the optic nerve as a fifth anatomical location and incorporate the central vein sign, paramagnetic rim lesions and cerebrospinal fluid kappa free-light chains in specified circumstances. The complete criteria and their safeguards against misdiagnosis should guide application (Montalban et al., 2025).

Early external evaluations published in 2026 suggest that the 2024 criteria can increase diagnostic sensitivity and shorten time to diagnosis in cohorts assessed for suspected MS, but the balance between earlier diagnosis and preservation of specificity remains an important implementation issue. These studies support careful use of the new imaging, optic-nerve, and biomarker elements rather than automatic application outside the populations in which they have been evaluated (Abad-Inchaurrondo et al., 2026; Brownlee et al., 2026). Implementation guidance published in late 2025 describes practical adoption of the revised criteria, including the role of optic-nerve assessment with optical coherence tomography and visual evoked potentials (Moccia et al., 2025; Saidha et al., 2025).

4.2. Neuroimaging

Magnetic Resonance Imaging (MRI) is central to the diagnosis and monitoring of MS. MRI can visualise characteristic CNS lesions, providing essential information about disease activity, lesion load, and distribution (Haacke et al., 2009; Filippi et al., 2019). Typical MS lesions appear as hyperintense areas on T2-weighted and FLAIR sequences, while active inflammation can be detected through gadolinium enhancement on T1-weighted images.

Advanced MRI techniques, such as magnetization transfer imaging, diffusion tensor imaging, and spectroscopy, offer additional insights into tissue damage and repair processes in MS (Filippi & Rocca, 2011). These techniques allow for more sensitive detection of subtle changes in normal-appearing white and gray matter, providing a more detailed assessment of disease burden.

4.3. Cerebrospinal Fluid Analysis

Examination of cerebrospinal fluid (CSF) remains an important diagnostic tool in MS. The presence of oligoclonal bands (OCBs) in the CSF, not mirrored in serum, is a hallmark of MS and provides evidence of intrathecal immunoglobulin synthesis (Sandberg-Wollheim & Olsson, 2013). OCBs are present in more than 90% of MS patients and can provide valuable diagnostic information, particularly in cases where clinical and radiological findings are equivocal.

4.4. Biomarkers

The identification and validation of biomarkers for MS have been areas of intense research, with the greatest current interest focused on measures that can complement clinical assessment and MRI rather than replace them. Neurofilament light chain (NfL) is among the most extensively studied markers of neuroaxonal injury in MS. Higher concentrations in cerebrospinal fluid and serum are associated with inflammatory activity and future clinical or radiological events (Disanto et al., 2017). A 2025 systematic review of 75 studies found that higher serum NfL was consistently associated with relapse risk, MRI activity and disease progression, while a 2026 implementation-focused review emphasized longitudinal, age-adjusted interpretation alongside MRI and clinical findings rather than reliance on an isolated cut-off (Freedman et al., 2025;

Caminero & Fernández, 2026). Chitinase 3-like 1 (CHI3L1) has shown prognostic value in clinically isolated syndrome (Cantó et al., 2015), and blood or cerebrospinal-fluid microRNA profiles remain under investigation as candidate biomarkers (Gandhi, 2015). Optical coherence tomography is not a soluble biomarker, but retinal nerve-fibre-layer and ganglion-cell measurements provide complementary evidence of neuroaxonal injury (Petzold et al., 2010). In practice, biomarker interpretation requires attention to age, renal function, body composition, intercurrent neurological injury and assay platform, and should be integrated with the clinical and imaging context (Coker et al., 2025; Caminero & Fernández, 2026).

Two 2026 meta-analyses further support the association of blood biomarkers with disease activity and prognosis. Higher serum NfL was associated with subsequent clinical relapse, MRI activity, new T2 lesions, and confirmed worsening, while pooled analyses of NfL and GFAP indicated that their prognostic performance varies by phenotype and disease-activity pattern. These findings strengthen their role as adjunctive measures, but they do not justify replacing clinical assessment or MRI with a single laboratory threshold (Chen et al., 2026; Yang et al., 2026).

Additional 2026 syntheses refine the clinical interpretation of serum NfL. An updated meta-analysis of 68 studies confirmed higher blood NfL across major MS phenotypes, whereas a diagnostic-accuracy meta-analysis found only modest discrimination for inflammatory disease activity and concluded that sNfL is not sufficiently accurate as a stand-alone decision tool. Taken together, current evidence supports longitudinal and contextual use of NfL rather than an isolated universal cutoff (Ebrahimi et al., 2026; Santana et al., 2026).

4.5. Differential Diagnosis

The differential diagnosis of MS is broad and includes other inflammatory, infectious, and degenerative disorders of the CNS. Conditions that can mimic MS include neuromyelitis optica spectrum disorders, acute disseminated encephalomyelitis, systemic lupus erythematosus, Sjögren's syndrome, and various metabolic and genetic disorders (Weisfeld-Adams et al., 2015). Careful clinical evaluation, neuroimaging, and laboratory studies are essential to distinguish MS from these mimics.

5. Clinical Manifestations and Impact

The clinical manifestations of MS are unusually diverse, reflecting the variable distribution of lesions within the CNS. Motor involvement is frequent and may present as weakness, spasticity, impaired coordination, and gait disturbance, all of which can significantly affect mobility and independence. Sensory symptoms such as numbness, tingling, and altered sensation are often among the earliest manifestations of the disease, and pain syndromes, including neuropathic pain and trigeminal neuralgia, can also occur. Visual disturbance is similarly common: optic neuritis, characterised by pain and vision loss in one eye, is a frequent presenting symptom of MS, while double vision and nystagmus are also encountered. Fatigue, often described as an overwhelming sense of exhaustion disproportionate to physical exertion, is one of the most common and debilitating symptoms and affects up to 80% of patients (Román et al., 2022). Cognitive impairment is likewise common and debilitating, affecting up to 70% of patients across domains such as information processing speed, attention, executive function, memory, and learning, and it can significantly reduce quality of life and functional abilities even in the early stages of the disease (Pierson & Griffith, 2006). Bladder and bowel dysfunction are also frequent, with urinary urgency, frequency, and incontinence occurring alongside constipation and fecal incontinence, as is sexual dysfunction, which in both men and women may include decreased libido, erectile dysfunction, and altered genital sensation. Finally, emotional and psychiatric symptoms such as depression, anxiety, and mood swings are common in MS and can significantly impact quality of life (Feinstein et al., 2014).

The frequency of cognitive difficulty has made cognitive rehabilitation an important component of MS care. Cognitive training programs, including computer-based interventions and integrated rehabilitation approaches, have shown promise in improving cognitive performance and often target specific areas such as attention, working memory, and executive function (Menascu et al., 2021; Sharbafshaaer et al., 2022). Repeated working memory training has been associated with improvements in cognitive test scores and with enhanced neural efficiency (Aguirre et al., 2019; Covey et al., 2018), and low-cost options such as commercial video games have been used successfully to enhance sustained attention and executive functioning in MS (De Giglio et al., 2014). Alongside these approaches, compensatory strategies help patients adapt to cognitive deficits by utilising external aids and environmental modifications; typical examples include the use of memory aids such as calendars or smartphone applications to manage daily tasks, and the simplification of workspaces

together with reduced multitasking to improve focus and efficiency (Goverover et al., 2018). Pharmacological treatment has also been explored, although acetylcholinesterase inhibitors, commonly used in Alzheimer's disease, have been investigated in MS with inconclusive results. The effectiveness of cognitive rehabilitation ultimately depends on factors such as the specific cognitive domains targeted, the duration and intensity of the intervention, and individual patient characteristics; while significant progress has been made, more research is needed to optimise long-term outcomes and to integrate cognitive rehabilitation into broader MS care strategies (Covey et al., 2018).

5.1. Impact on Quality of Life

The impact of MS extends far beyond physical symptoms, affecting various aspects of an individual's life. The disease is associated with long-term disability and a reduced life expectancy (Rejdak et al., 2010; Bull, 2015). MS can significantly impact employment, with many patients experiencing reduced work capacity or early retirement due to disease-related disability (Kobelt et al., 2017).

Social relationships and family dynamics can be profoundly affected by MS. The unpredictable nature of the disease, coupled with the potential for progressive disability, can strain interpersonal relationships and lead to social isolation (Grytten & Måseide, 2006).

The economic burden of MS is substantial, encompassing direct medical costs, indirect costs related to lost productivity, and intangible costs associated with reduced quality of life. A comprehensive approach to care that addresses not only the physical manifestations of the disease but also its psychological, social, and economic dimensions is essential for optimising outcomes and quality of life for individuals with MS.

6. Treatment and Management

The management of MS has evolved significantly in recent decades, with an expanding array of disease-modifying therapies (DMTs) and a growing emphasis on thorough, multidisciplinary care. Treatment strategies aim to reduce inflammation, manage symptoms, slow disease progression, and enhance overall quality of life (Chisari et al., 2014).

6.1. Disease-Modifying Therapies

Disease-modifying therapies form the cornerstone of MS management, particularly for relapsing forms of the disease. These medications work to modulate the immune response, reduce the frequency and severity of relapses, and potentially slow the accumulation of disability (Hauser & Cree, 2020). The range of DMTs has expanded rapidly, with newer agents offering improved efficacy and novel mechanisms of action. Current FDA-approved DMTs for MS fall into three broad groups defined by their route of administration. Injectable therapies comprise the interferon beta preparations, such as Avonex, Rebif, and Plegridy, together with glatiramer acetate (Copaxone, Glatopa) and the subcutaneous anti-CD20 antibody ofatumumab. Oral therapies include the sphingosine-1-phosphate receptor modulators fingolimod, siponimod, ozanimod, and ponesimod, the fumarates dimethyl fumarate, diroximel fumarate, and monomethyl fumarate, teriflunomide, and cladribine. Infusion therapies comprise natalizumab, alemtuzumab, ocrelizumab, and ublituximab, with rituximab also used off-label.

The selection of appropriate therapy requires careful consideration of factors such as disease activity, patient characteristics, and potential side effects. The concept of escalation therapy, where treatment is intensified based on disease activity, versus induction therapy, where highly effective treatments are used from the outset, remains a subject of debate in the MS community (Comi et al., 2017). Individual agents, their central trial evidence and their adverse-effect profiles are described in Section 3.1.

6.2. Symptom Management

Symptom management is a cornerstone of care for individuals with multiple sclerosis (MS). This approach integrates pharmacological and non-pharmacological interventions to alleviate symptoms and improve patients' quality of life, and it addresses several domains in parallel.

Fatigue, one of the most prevalent and debilitating symptoms in MS, is usually approached on several fronts at once. Energy conservation techniques, in which patients are taught to prioritise tasks, schedule rest periods, and pace their activities, can help minimise fatigue, and structured exercise can also be considered, although intervention type, supervision, and individual tolerance all matter (Pilutti et al., 2013). Cognitive behavioural therapy can help patients address the psychological aspects of fatigue and develop coping strategies (Thomas et al., 2013; van Kessel et al., 2008). Amantadine and modafinil are sometimes prescribed, but a randomised crossover trial did not demonstrate superiority over placebo and reported more adverse events with the active drugs (Nourbakhsh et al., 2021).

Spasticity, characterised by involuntary muscle stiffness and spasms, can hinder mobility and daily functioning. Stretching and range-of-motion exercises may be incorporated into an individualized physical therapy plan addressing mobility and stiffness, with goals and response assessed for each patient, and oral agents such as baclofen and tizanidine are used in symptom management, their choice and dosing requiring individualized assessment of spasticity, adverse effects, and functional goals.

Pain in MS can be neuropathic, musculoskeletal, or a combination of both. In neuropathic pain, treatment selection depends on the pain mechanism, comorbidity, tolerability, and functional goals, and clinical response and adverse effects require reassessment over time.

Bladder dysfunction, including urinary urgency, frequency, and incontinence, is likewise common in MS. Assessment should distinguish storage from emptying dysfunction, and pharmacological options require individualized consideration of efficacy, retention risk, and adverse effects. In refractory cases, intradetrusor botulinum toxin injections can reduce bladder overactivity (Kalsi et al., 2007).

6.3. Rehabilitation and Exercise

Rehabilitation programs are an important part of managing the diverse symptoms and functional impairments associated with MS. These interventions aim to improve mobility, enhance cognitive function, and promote independence in daily activities (Chisari et al., 2014), and they rest on four principal components.

Physical therapy is the most established of these. Multiple systematic reviews and meta-analyses confirm its benefits in improving strength, balance, coordination, and mobility among individuals with MS, and supervised exercise programs have shown significant improvements in physical functioning, independence in daily activities, and overall quality of life. Within such programs, gait training enhances walking mobility and reduces the risk of falls (Snook & Motl, 2009), resistance training improves muscle strength and functional abilities (Latimer-Cheung et al., 2013), and coordination exercises are associated with better motor control and sensorimotor integration (Pilutti et al., 2013). Taken together, techniques such as gait training, resistance exercises, balance training, and coordination drills have demonstrated efficacy in clinical studies, and their incorporation helps improve functional performance, reduce disability, and enhance quality of life for individuals with MS.

Occupational therapy aims to enhance independence in activities of daily living through adaptive techniques and equipment (Yu & Mathiowetz, 2014), while speech and language therapy addresses the speech and swallowing difficulties that may occur in MS (Yorkston et al., 2003). Cognitive rehabilitation, the fourth component, employs various techniques to improve cognitive function or to develop compensatory strategies for cognitive deficits (Guimarães & Sá, 2012), an important consideration given that cognitive impairment affects a significant proportion of MS patients, manifesting in domains such as processing speed, attention, and executive functions (Mattioli et al., 2015).

The approaches used in cognitive rehabilitation fall into three broad groups. Structured cognitive training programs, whether computer-assisted or therapist-guided, target specific cognitive domains such as attention, memory, and executive function, and randomised controlled trials have demonstrated that such programs can improve cognitive performance and functional outcomes (Messinis et al., 2017). Compensatory strategies, including memory aids, organizational tools, and environmental modifications, help patients adapt to deficits and maintain daily functioning. Combined interventions that integrate physical activity, cognitive exercises, and psychological support have shown synergistic effects on cognitive and emotional well-being (Lee et al., 2017).

The evidence supporting cognitive rehabilitation is encouraging across several dimensions. Benefits have been observed in both relapsing-remitting and progressive MS and across varying degrees of impairment (Ruano et al., 2017). Cognitive gains are most marked immediately after intervention, and maintenance of these improvements often requires booster sessions and ongoing support (Lampit et al., 2019). Cognitive rehabilitation also has a positive impact on mood and quality of life, although results can vary based on the intervention type and patient characteristics (Sehanovic et al., 2020). Larger randomised controlled trials are nonetheless needed to explore long-term benefits and optimise intervention designs (Mayo et al., 2019), and combining cognitive rehabilitation with physical exercise or pharmacological treatments may further enhance outcomes (Sandroff et al., 2015). Through tailored training programs and compensatory strategies, cognitive rehabilitation addresses key challenges of the disease, improving cognitive performance and overall quality of life for individuals with MS (Mattioli et al., 2015; Ruano et al., 2017).

Exercise therapy has shown early findings in improving physical function, fatigue management, and overall well-being in individuals with MS. Regular physical activity has been associated with improvements in mobility, fatigue, and quality of life. Exercise modalities such as aerobic training, resistance training, and yoga have all demonstrated benefits in MS populations, with the choice of exercise often tailored to individual patient capabilities and preferences.

6.4. Emerging Therapies and Future Directions

MS treatment is moving quickly, and several lines of research stand out.

Among cell-based approaches, haematopoietic stem cell transplantation (HSCT) can produce substantial suppression of inflammatory disease activity in selected patients with highly active relapsing MS through immune ablation and reconstitution (Smith et al., 2021; Abbasi-Kangevari et al., 2019). Mesenchymal stem cells (MSCs) are being extensively studied for their neuroprotective and immunomodulatory properties and have shown promise in repairing damaged neural tissue and reducing inflammation in both preclinical and clinical studies (Abbasi-Kangevari et al., 2019; Jafarzadeh Bejargafshe et al., 2019), while human amniotic mesenchymal stem cells (hAMSCs) exhibit strong anti-inflammatory and immunomodulatory effects that make them a promising option for both relapsing-remitting and secondary progressive MS (Abbasi-Kangevari et al., 2019). Several practical considerations shape the translation of these approaches. MSCs appear to lose their ability to promote oligodendrocyte generation during remyelination as they age, which emphasizes the importance of patient-specific factors in therapy (Rivera et al., 2019), and the route of administration matters, since intrathecal injection of MSCs has been shown to be more effective than intravenous delivery in reducing neurodegeneration markers in progressive MS patients (Petrou et al., 2022). Adjunctive agents have also been explored, among them lithium chloride, a GSK3-β inhibitor found to promote neural stem cell proliferation and differentiation and thereby offering potential support for remyelination (Ghosouri et al., 2022; Zhang et al., 2018).

Remyelination strategies form a second line of investigation, and efforts are underway to develop therapies that can promote remyelination and repair of damaged neural tissue. Experimental compounds such as clemastine fumarate have demonstrated the ability to enhance remyelination in preclinical and early clinical trials (Green et al., 2017), although clinical interpretation should also consider the later disability-worsening signal in a small advanced-progressive-MS cohort; clemastine is not an established MS repair therapy (Kocot et al., 2025).

Neuroprotective strategies, which aim to reduce or prevent neuronal and axonal loss, represent a third direction. Lipoic acid, a natural antioxidant, has shown promise in slowing disease progression in secondary progressive MS (SPMS): a two-year randomised controlled trial demonstrated that daily oral 1,200 mg lipoic acid significantly reduced the rate of brain atrophy compared to placebo, with trends toward improvement in disability and quality of life measures (Spain et al., 2017). These neuroprotective effects may be mediated by its antioxidant properties, reduction of oxidative stress, and promotion of mitochondrial function (Jiang et al., 2013; Sanadgol et al., 2017). Finally, precision medicine approaches, including the development of biomarker-driven treatment algorithms and the use of advanced imaging techniques, aim to personalize MS therapy by optimising treatment selection based on individual patient characteristics and disease features (Comabella & Montalban, 2014).

6.5. Comprehensive Care and Patient-Centered Approaches

The multifactorial nature of MS necessitates a broad, patient-centered approach to care, which rests on several interdependent elements.

Multidisciplinary teams are essential for addressing the diverse and complex needs of MS patients. These teams typically include neurologists, MS specialist nurses, rehabilitation professionals (e.g., physical and occupational therapists), psychologists, and other healthcare providers. A collaborative approach enhances the quality of life for patients by providing individualized care, improving treatment adherence, and addressing physical, psychological, and social aspects of the disease (Hutchinson, 2016; Hobart et al., 2018). Effective multidisciplinary care requires seamless communication and coordination among team members to develop and implement personalised care plans. Additionally, integrating innovative models like home-based therapies and specialized MS clinics can further improve patient outcomes (Papeix et al., 2015; Skovgaard et al., 2012).

Empowering MS patients with knowledge about their condition and self-management strategies plays a critical role in optimising outcomes. Self-management involves managing symptoms such as fatigue, maintaining physical

activity, adhering to medication regimens, and monitoring changes in condition. Studies have shown that tailored self-management interventions, such as fatigue management programs and mobile health (m-health) tools, improve patients' self-efficacy and quality of life (Salimzadeh et al., 2019). For instance, digital tools like MS INFoRm enable patients to effectively reduce fatigue's impact on daily life. Furthermore, addressing psychosocial aspects and fostering adaptive coping strategies can enhance patients' ability to manage disease progression and maintain emotional well-being.

Comprehensive care must also address the psychosocial dimension of the disease, recognising and addressing the psychological and social impact of MS through counseling, support groups, and social services (Feinstein et al., 2014). Lifestyle modification is a further element, since stress management, exercise, dietary adjustments, and smoking cessation all contribute substantially to managing multiple sclerosis (MS) and its symptoms.

Stress Management:

Managing stress is central for individuals with MS, as it can significantly affect disease progression and symptom severity. Studies have shown that perceived stress and maladaptive coping strategies can negatively impact the physical and psychological well-being of people with MS (Agland et al., 2018). Mindfulness-based stress reduction has been found effective in reducing anxiety, depression, and overall stress levels in MS patients, contributing to improved quality of life (Kolahkaj & Zargar, 2015).

Exercise and Physical Activity:

Physical activity is an essential component of MS management, as it helps improve physical fitness, reduce disability, and enhance overall quality of life. Rehabilitation interventions incorporating exercise can alleviate fatigue and improve functional outcomes (Asano et al., 2014). However, many individuals with MS report lower levels of physical activity, so targeted interventions to promote adherence are needed (Stennett et al., 2019).

Dietary Modifications:

Dietary interventions focusing on reducing oxidative stress and inflammation may have potential benefits for MS management. Oxidative stress is a significant contributor to MS pathogenesis, and maintaining a balance between reactive oxygen species and antioxidant defenses is important for preventing further neurological damage (Pădureanu et al., 2019).

Smoking Cessation:

Smoking has been associated with an increased risk of developing MS and can exacerbate disease progression. Therefore, smoking cessation is a critical lifestyle modification for individuals with MS, contributing to better disease outcomes and reduced symptom severity (Frohman et al., 2011).

Digital Self-Management Tools:

Self-management strategies, including the use of mobile health (m-health) applications, provide MS patients with resources to manage their symptoms effectively. These tools can address fatigue, depression, and cognitive impairment, empowering patients to take an active role in their care (Salimzadeh et al., 2019; Giunti et al., 2017).

Shared decision-making (SDM) has become a cornerstone of patient-centered care for individuals with multiple sclerosis (MS). This approach involves a collaborative exchange of information between the healthcare provider and the patient, where the provider shares expertise and the patient communicates their values, preferences, and treatment goals (Kuusisto et al., 2022).

SDM is particularly well-suited to MS care, as the disease is highly heterogeneous and treatment options are diverse. Patients often express a strong desire to participate in their treatment decisions, making education about disease-modifying therapies (DMTs) and their associated benefits and risks essential (Bravo et al., 2015; Burtchell et al., 2022).

Effective SDM requires clear communication and tailored information. Healthcare providers should invite patients to engage in the decision-making process, educate them about available options, and elicit their preferences for treatment attributes, such as mode and frequency of administration and tolerance for risk (Ng et al., 2015; Kremer et al., 2021). Studies show that incorporating patient values and goals into treatment decisions improves adherence and satisfaction with care (Ben-Zacharia et al., 2023).

Despite its benefits, SDM faces challenges in implementation. The complexity of MS and the uncertainty surrounding disease progression can make decision-making difficult (Dennison et al., 2016; Doherr et al., 2017). Moreover, some patients prefer to rely on their healthcare provider for final treatment decisions, so shared decision-making has to be adapted to the individual.

Efforts to improve SDM include the development of tools such as decision aids and questionnaires designed to support the process and enhance patient engagement (Col et al., 2018; Álvarez-Rodríguez et al., 2022). These tools help ensure that patients are informed, empowered, and able to

make choices aligned with their values and goals, ultimately leading to better outcomes in MS care (Burtchell et al., 2022).

MS specialist nurses contribute to patient education, monitoring and coordination of care. Specific claims about improvements in adherence or quality of life require comparative evidence.

MS specialist nurses are skilled in assessing and managing various symptoms of the disease, including fatigue, motor dysfunction, cognitive impairments, and pain. They implement strategies and nursing interventions designed to alleviate these symptoms and improve the well-being of patients (MEEHAN & Doody, 2020).

Care coordination should define professional responsibilities and routes for communication, monitoring and referral.

Multiple Sclerosis is a complex autoimmune disease with diverse clinical presentations, intricate pathophysiology, and variable prognosis. Significant advances in our understanding of MS pathogenesis, coupled with the development of increasingly effective therapies, have transformed MS management over the past few decades. Early diagnosis, accurate disease monitoring, and personalised treatment approaches are important for optimising outcomes and enhancing quality of life for individuals with MS.

Ongoing research is refining disease mechanisms, therapeutic targets, and strategies for neuroprotection and repair. Advanced imaging, validated biomarkers, and precision-medicine methods may improve diagnosis, prognosis, and treatment selection when their added clinical value is demonstrated prospectively.

Despite these advances, significant problems remain. The progressive forms of MS, in particular, continue to pose therapeutic challenges, and strategies to prevent or reverse accumulated disability are urgently needed. Additionally, addressing the long-term economic and societal impact of MS requires ongoing efforts in health policy and resource allocation.

Ultimately, the goal of MS research and clinical practice is to develop interventions that can halt or reverse disease progression, restore function, and significantly improve the lives of those affected by this complex neurological condition. Achieving this goal will require continued collaboration between researchers, clinicians, patients, and advocacy groups, as well as sustained investment in basic and clinical research.

Future MS care will depend on better control of progression, more reliable markers of treatment response, and therapies that address neurodegeneration and repair. These advances should be judged by reproducible effects on outcomes that matter to patients rather than by mechanistic plausibility alone.

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1.2. EPIDEMIOLOGY OF MS: PREVALENCE AND INCIDENCE GLOBALLY

1. Introduction

Estimates of MS prevalence and incidence depend on case ascertainment, diagnostic criteria, population structure, and access to neurological care. These factors help explain why reported rates vary between regions and over time. Figure 1.2.1 outlines the principal epidemiological features discussed below.

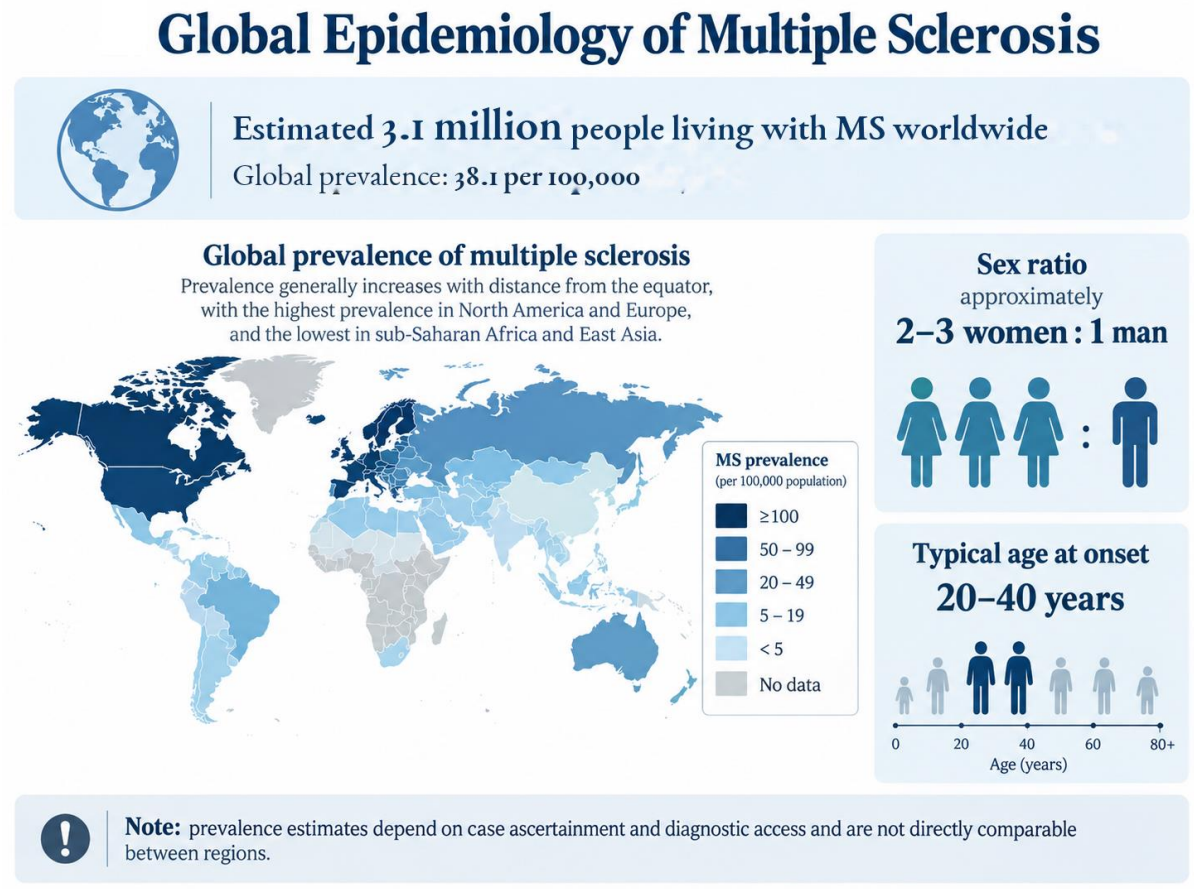


Figure 1.2.1. Global epidemiology of multiple sclerosis. Prevalence estimates depend on case ascertainment and diagnostic access and are not directly comparable between regions.

2. Global Prevalence and Incidence Trends

The recorded prevalence of MS has increased over recent decades, a pattern influenced by improved case ascertainment, longer survival, population change, and possibly changes in disease occurrence. The Atlas of MS 2026, based on data gathered for its 2024 update, estimates that approximately 3.1 million people are living with MS worldwide, corresponding to a global prevalence of 38.1 per 100,000; the previous Atlas estimate was 2.8 million in 2020 (Multiple Sclerosis International Federation [MSIF], 2026; King et al., 2026; Walton et al., 2020). Because surveillance methods, diagnostic access, and population denominators differ between settings and over time, the rise in recorded case numbers should not by itself be interpreted as evidence of an equivalent increase in individual biological risk.

The Global Burden of Disease (GBD) study has been instrumental in documenting the rising prevalence of MS. The GBD 2016 Multiple Sclerosis Collaborators reported a substantial increase in MS prevalence between 1990 and 2016, with over 2.2 million individuals living with MS in 2016 (Wallin et al., 2019). This upward trajectory is a reason for continued research to understand the factors driving this trend and to develop effective management strategies.

Incidence rates of MS have also been the subject of numerous studies, with varying results across different regions. While some areas have reported relatively stable incidence rates, others have observed increases over time. For

instance, a study in Bavaria, Germany, found a slight increase in MS incidence from 2006 to 2015, with rates rising from 10.0 to 12.6 per 100,000 person-years (Daltrozzo et al., 2018).

3. Regional Variations in MS Epidemiology

Regional differences in reported MS frequency reflect both disease distribution and the ability of health systems to identify and record cases.

3.1. North America

North America consistently reports some of the highest prevalence rates of MS globally. In the United States, recent estimates suggest a prevalence of approximately 309 per 100,000 population, translating to nearly 1 million adults living with MS (Wallin et al., 2019). Canada has reported similarly high rates, with some studies indicating a prevalence of over 300 per 100,000 in certain provinces.

The high prevalence in North America is attributed to a combination of genetic susceptibility, environmental factors, and advanced diagnostic capabilities. Studies have also highlighted significant variations within the region, with higher rates typically observed in northern states and provinces compared to southern areas, supporting the latitude gradient hypothesis in MS distribution.

3.2. Europe

Europe, like North America, is characterised by high MS prevalence rates, although significant variations exist between countries and even within national borders. Nordic countries, in particular, have reported some of the highest prevalence rates worldwide. For example, in Norway, a dramatic increase in MS prevalence has been observed over five decades, with rates rising from 20 to 203 per 100,000 inhabitants between 1961 and 2014 (Grytten et al., 2015).

In the United Kingdom, a large observational study revealed not only high prevalence rates but also significant comorbidities associated with MS. The study found that following MS diagnosis, common comorbidities included infections in 80% of cases and depression in 46% of cases (Jick et al., 2015). These findings show the complex health challenges faced by individuals with MS and the need for multidisciplinary care approaches.

Southern European countries generally report lower prevalence rates compared to their northern counterparts, although recent studies have shown increasing trends. In Catalonia, Spain, researchers documented an increase in MS prevalence over a 17-year period, from 58.6 per 100,000 in 1991 to 91.2 per 100,000 in 2008 (Otero-Romero et al., 2012). This trend highlights the dynamic nature of MS epidemiology even within regions traditionally considered to have moderate prevalence rates.

3.3. Eastern Europe and Russia

The epidemiology of MS in Eastern Europe and Russia has gained increasing attention in recent years, revealing patterns that both align with and diverge from those observed in Western Europe. In the Russian Federation, a 30-year study revealed a consistent increase in both prevalence and incidence of MS (Boyko & Melnikov, 2020). The prevalence of MS in Russia has increased from 30-70 per 100,000 in the 1990s to 40-100 per 100,000 in recent years, with significant regional variations observed across the vast territory of the country.

Other Eastern European countries have reported similar trends of increasing prevalence, although rates generally remain lower than those observed in Western Europe. The reasons for these differences are not fully understood but may involve a combination of genetic factors, environmental influences, and variations in diagnostic practices and healthcare access.

3.4. Middle East and North Africa

While traditionally considered low-prevalence regions, parts of the Middle East and North Africa have reported rising rates of MS in recent years, challenging previous assumptions about the disease's distribution. In Iran, for example, the estimated prevalence of MS is 112.0 per 100,000 individuals, with an incidence of 5.2 per 100,000 (Eskandarieh et al., 2018). This increase is not unique to Iran but has been observed in other countries in the region as well.

The rising prevalence in these areas has prompted researchers to investigate potential contributing factors, including changes in lifestyle, environmental exposures, and improved diagnostic capabilities. Additionally, the high consanguinity rates in some Middle Eastern populations have provided unique opportunities to study genetic factors contributing to MS susceptibility.

3.5. Sub-Saharan Africa

Data on MS prevalence and incidence in Sub-Saharan Africa remain limited compared to other regions, presenting a significant gap in our global understanding of MS epidemiology. The available studies suggest generally low prevalence rates, particularly in equatorial regions. However, it's important to note that these low rates may partially reflect

underdiagnosis due to limited healthcare resources and competing health priorities in many African countries.

Recent efforts to improve MS diagnosis and care in Africa have led to increased recognition of the disease, particularly in urban areas and among populations with higher socioeconomic status. More detailed epidemiological studies are needed in the region to accurately assess the true burden of MS and its impact on African populations.

3.6. Asia

The epidemiology of MS in Asia presents a complex and varied picture, with significant differences observed between countries and ethnic groups. Generally, Asian populations have been considered to have lower MS prevalence compared to Western countries, but recent studies have challenged this notion, revealing increasing rates in several Asian nations.

In Japan, for instance, the prevalence of MS has been reported to have increased significantly over the past few decades, from about 2-4 per 100,000 in the 1950s to approximately 10-15 per 100,000 in recent years. Similar trends have been observed in other East Asian countries, including South Korea and parts of China.

South Asian countries, including India, have traditionally reported low MS prevalence rates. However, improved diagnostic capabilities and increased awareness have led to more frequent recognition of MS cases in recent years. The unique genetic makeup of South Asian populations, combined with rapidly changing environmental and lifestyle factors, presents an important area for future MS epidemiological research.

3.7. Latin America and the Caribbean

Studies focusing on Latin America and the Caribbean have highlighted the variability in MS incidence and prevalence within this diverse region. While generally lower than rates observed in North America and Europe, MS prevalence in Latin America has shown an increasing trend in recent years (Cristiano & Rojas, 2017).

A systematic review of MS epidemiology in Latin America and the Caribbean revealed prevalence rates ranging from 0.75 to 38.2 per 100,000 inhabitants, with the highest rates observed in Cuba and the lowest in Ecuador (Cristiano et al., 2012). These variations argue for attention to geographical, genetic and environmental factors in disease epidemiology and the need for more thorough studies in regions traditionally underrepresented in MS research.

3.8. Oceania

Australia and New Zealand report high MS prevalence rates, comparable to those seen in North America and Northern Europe. In Australia, recent estimates suggest a prevalence of around 95.5 per 100,000 population, with higher rates observed in the southern states compared to the north, consistent with the latitude gradient observed globally (Walton et al., 2020).

New Zealand, particularly its South Island, has reported some of the highest MS prevalence rates worldwide, with figures exceeding 130 per 100,000 in some studies. The high rates in these countries, combined with their unique geographical isolation, make them important locations for studying both genetic and environmental factors contributing to MS risk.

4. Factors Influencing MS Epidemiology

The observed variations in MS prevalence and incidence across different regions have led researchers to investigate potential factors influencing these patterns. Several key factors have become significant contributors to MS epidemiology:

4.1. Genetic Factors

Genetic predisposition is a key determinant of MS susceptibility. Population studies have revealed higher rates of MS in certain genetic populations, such as those of Northern European descent. The interaction between genetic factors and environmental influences continues to be a focus of research in understanding MS epidemiology.

Specific genetic variations, particularly in the Human Leukocyte Antigen (HLA) complex, have been strongly associated with MS risk. The HLA-DRB1*15:01 allele, for example, is the strongest genetic risk factor identified for MS, increasing the risk of developing the disease by about threefold. However, genetic factors alone do not fully explain the observed epidemiological patterns, demonstrating the importance of gene-environment interactions in MS pathogenesis.

4.2. Environmental Factors

Environmental factors have been increasingly recognised as important contributors to MS risk, and several influences have been studied extensively in relation to MS epidemiology. The most consistent of these findings is the latitude gradient, with higher prevalence rates generally observed at greater distances from the equator; this pattern has been attributed in part to differences in sunlight

exposure and consequent vitamin D levels, since vitamin D is known to have immunomodulatory effects and lower levels have been associated with increased MS risk. Closely related to sunlight exposure, vitamin D deficiency has itself been implicated as a risk factor for MS: population studies have shown inverse correlations between serum vitamin D levels and MS risk, and some interventional studies have explored the potential of vitamin D supplementation in MS prevention and management. The role of Epstein-Barr virus (EBV) infection in MS pathogenesis has likewise been a subject of intense research, since nearly all individuals with MS show evidence of prior EBV infection compared to about 90% of the general population; the exact mechanisms linking EBV to MS remain under investigation, but theories include molecular mimicry and altered immune responses following infection. Cigarette smoking has been consistently associated with increased MS risk and worse disease outcomes, with studies showing that smoking can increase the risk of developing MS by up to 50% and may also accelerate disease progression, while obesity, particularly in childhood and adolescence, has been linked to increased MS risk through mechanisms that may involve altered vitamin D metabolism, increased inflammation, and hormonal factors. Emerging research has also focused on the role of diet and the gut microbiome in MS risk and progression, and some studies have suggested that Western diets high in saturated fats and low in fibre may increase MS risk, possibly through effects on inflammation and the gut microbiome.

4.3. Socioeconomic Factors

Socioeconomic factors may influence MS epidemiology through various mechanisms, including access to healthcare, diagnostic capabilities, and lifestyle factors. The generally higher prevalence rates observed in developed countries may partly reflect these socioeconomic influences, although the relationship is complex and not fully understood.

Healthcare access and quality can significantly impact MS diagnosis and reporting, potentially leading to underestimation of prevalence in regions with limited healthcare resources. Additionally, socioeconomic status may influence exposure to certain environmental risk factors and access to protective factors, further complicating the epidemiological picture.

5. Climate Change and MS Epidemiology

An emerging area of research in MS epidemiology is the potential impact of climate change on disease patterns. As global temperatures rise and weather patterns shift, traditional latitude gradients in MS prevalence may be altered. Changes in UV exposure, vitamin D synthesis, and other environmental factors linked to climate change could have far-reaching effects on MS risk and distribution in the coming decades.

6. Gender Disparities in MS Epidemiology

A notable aspect of MS epidemiology is the gender disparity observed in disease prevalence. Globally, MS affects women more frequently than men, with a female-to-male ratio typically around 2-3:1, although this ratio can vary across different populations and has changed over time (Walton et al., 2020).

The increasing female preponderance in MS has been documented in various studies. For instance, research conducted in Isfahan, Iran, revealed a growing female predominance in MS cases over time, with the female-to-male ratio increasing from 2.0 in 2006 to 3.36 in 2014 (Maghzi et al., 2010). This trend is not unique to Iran but has been observed in multiple populations worldwide.

The reasons for this gender disparity are not fully understood but likely involve an interaction of genetic, hormonal, and environmental factors, and several hypotheses have been advanced to explain the female preponderance in MS. Hormonal influences are prominent among them: sex hormones, particularly estrogen, have been implicated in modulating MS risk and disease course, and the protective effects of pregnancy on relapse rates together with the increased risk of relapses postpartum support the role of hormonal factors. Genetic factors may also contribute, since the presence of two X chromosomes in females may increase autoimmune susceptibility and certain MS risk genes may exert stronger effects in females. Differential exposure to environmental risk factors between males and females, such as vitamin D levels, smoking habits, and occupational exposures, may further contribute to the observed disparity, as may sex-specific epigenetic modifications that influence gene expression patterns relevant to MS risk and progression.

Understanding the mechanisms underlying the gender disparity in MS is key for developing targeted prevention strategies and personalised treatment approaches.

7. Temporal Trends in MS Epidemiology

Longitudinal studies have provided valuable insights into the changing patterns of MS epidemiology over time. A consistent trend observed across many regions is the

increasing prevalence of MS over the past several decades, and this increase has been attributed to a number of factors. Improved diagnostic techniques are among the most important: advances in neuroimageing, particularly magnetic resonance imaging (MRI), have led to earlier and more accurate MS diagnoses, and the introduction and refinement of diagnostic criteria such as the McDonald criteria have also contributed to increased case identification. Increased survival is a further contributor, since improved treatment options and overall healthcare have led to increased life expectancy for individuals with MS, and the introduction of disease-modifying therapies has significantly altered the natural history of the disease for many patients. Some studies suggest a genuine increase in incidence as well, although distinguishing this from improved detection remains challenging; changing environmental exposures and lifestyle factors may contribute to a true increase in disease occurrence. Changing demographics also play a part, as aging populations in many countries may contribute to increasing prevalence given that the cumulative risk of developing the disease increases with age, and greater public and professional awareness of MS may lead to increased healthcare-seeking behaviour and improved case ascertainment.

The dynamic nature of MS epidemiology is further illustrated by studies identifying spatial and temporal clusters of MS in specific locations. These clusters suggest the potential influence of localised environmental or lifestyle factors on MS risk (Otero-Romero et al., 2012).

8. MS Epidemiology in Special Populations

8.1. Pediatric MS

While MS is typically diagnosed in young adults, pediatric-onset MS (defined as onset before age 18) has gained increased recognition in recent years. The prevalence of pediatric MS varies globally but is generally estimated to account for 2-5% of all MS cases. Understanding the epidemiology of pediatric MS is essential for early diagnosis and appropriate management in this vulnerable population.

Studies have shown that pediatric MS may have a more aggressive course than adult-onset MS, with higher relapse rates and potentially faster accumulation of disability. The female preponderance observed in adult MS is less pronounced in pediatric cases, particularly in very young children, suggesting age-related differences in disease susceptibility.

8.2. Elderly-Onset MS

At the other end of the age spectrum, elderly-onset MS (typically defined as onset after age 50) presents unique epidemiological and clinical challenges. The prevalence of elderly-onset MS has been increasing, partly due to improved diagnostic capabilities and increased life expectancy. This subgroup of MS patients often experiences more rapid disability progression and may be at higher risk for comorbidities, complicating disease management.

8.3. Ethnic and Racial Variations

MS epidemiology shows significant variations across different ethnic and racial groups, providing important insights into genetic and environmental risk factors. African Americans in the United States have been found to have a lower overall risk of developing MS compared to White Americans; however, when African Americans do develop MS, they often experience more severe disease courses with faster disability progression. Hispanic populations generally show lower MS prevalence rates compared to non-Hispanic White populations, although these rates have been increasing in recent years, and Asian populations, particularly those of East Asian descent, have traditionally had lower prevalence rates that now appear to be rising, possibly due to westernization of lifestyles and environmental changes. Indigenous populations in various parts of the world often show distinct epidemiological patterns; the Maori population in New Zealand, for instance, has a lower MS prevalence compared to the non-Maori population, despite living at similar latitudes.

These ethnic and racial variations in MS epidemiology highlight the interaction between genetic susceptibility and environmental factors in disease risk and progression.

9. Challenges in MS Epidemiology Research

While significant progress has been made in understanding MS epidemiology, several challenges persist. Diagnostic variability is among the most fundamental of these: differences in diagnostic criteria and healthcare access across regions can lead to variability in reported prevalence and incidence rates, and the evolution of diagnostic criteria over time further complicates longitudinal comparisons. The introduction of the McDonald criteria and their subsequent revisions has allowed for earlier diagnosis but may in itself affect prevalence and incidence estimates, making comparisons of epidemiological data across different periods difficult to interpret. A second major limitation is the concentration of data in North America and Europe, with

limited information available from many developing countries; this data gap potentially skews our understanding of global MS patterns and limits the generalizability of findings. The heterogeneity of MS presents a further difficulty, since its diverse clinical presentations and courses make epidemiological studies challenging, particularly in capturing the full spectrum of the disease, and the recognition of different MS phenotypes, namely relapsing-remitting, primary progressive, and secondary progressive disease, adds complexity to epidemiological analyses. Environmental complexity compounds the problem, as the complexity of environmental influences on MS risk makes it difficult to isolate and quantify individual contributing factors, and the long latency period between exposure to risk factors and disease onset complicates these analyses further. The increasing recognition of comorbidities in MS patients adds another layer of complexity, since understanding how comorbid conditions interact with MS risk and progression is central for broad disease management. Finally, while migration studies have provided valuable insights into environmental influences on MS risk, conducting and interpreting these studies presents numerous challenges of its own, including selection bias and the interaction of genetic and environmental factors.

10. Implications for Healthcare and Research

The changing picture of MS epidemiology has significant implications for healthcare systems and research priorities. The increasing prevalence of MS necessitates appropriate allocation of healthcare resources to meet the growing demand for MS-related services, including specialized neurology care, rehabilitation services, and access to disease-modifying therapies. Understanding regional variations and risk factors can inform the development of targeted prevention strategies, particularly in high-risk populations, and may include public health initiatives focused on modifiable risk factors such as vitamin D supplementation, smoking cessation, and obesity prevention. Because MS epidemiology is inherently global in nature, international collaboration in research efforts is essential for a better understanding of disease patterns and risk factors, and initiatives such as the Atlas of MS provide valuable platforms for collating and comparing global data. Insights from epidemiological studies also contribute to the development of personalised approaches to MS prevention and treatment that take into account individual risk profiles based on genetic, environmental, and lifestyle factors, and they can inform public health initiatives aimed at modifying environmental risk factors and promoting protective behaviours, including strategies to increase vitamin D intake in high-risk populations or programs to reduce exposure to environmental toxins. The changing picture of MS epidemiology further necessitates ongoing education for healthcare professionals to ensure timely diagnosis and appropriate management, particularly in regions where MS was previously considered rare, and it supports the development of targeted educational materials and support programs for patients and their families that address the specific needs of different demographic groups.

11. Future Directions in MS Epidemiology Research

Several priorities for future epidemiological research follow from the work reviewed here. Efforts to improve data collection and to standardize diagnostic criteria in underrepresented regions will be important for developing a truly global understanding of the disease, and this includes initiatives to enhance MS surveillance in developing countries and rural areas. Continued investment in long-term cohort studies is likewise essential for tracking changes in MS epidemiology over time and identifying emerging trends and risk factors, as is further research into the interaction between genetic susceptibility and environmental factors, including studies on epigenetic modifications and how they may mediate environmental influences on MS risk. As global climate patterns shift, research into the potential impacts of climate change on MS epidemiology will become increasingly important, encompassing changing UV exposure patterns, migration trends, and alterations in infectious disease prevalence. The role of the gut microbiome in MS pathogenesis represents another emerging priority, and large-scale epidemiological studies incorporating microbiome analysis could provide valuable insights into this complex relationship, while the incorporation of advanced neuroimaging techniques into epidemiological studies may allow for earlier detection of MS and a better understanding of disease progression patterns across different populations. Leveraging big data analytics and artificial intelligence could help identify subtle patterns and risk factors in MS epidemiology that may not be apparent through traditional research methods, and further investigation into the causes of health disparities in MS, including differences in disease course and outcomes among various ethnic and socioeconomic groups, is important for developing equitable care strategies.

12. Conclusion

The epidemiology of Multiple Sclerosis presents a complex and dynamic picture, characterised by significant regional variations and changing patterns over time. The global trend of increasing MS prevalence points to the growing importance of this neurological disorder as a public health concern. While substantial progress has been made in mapping the epidemiology of MS, many questions remain unanswered.

The interplay between genetic susceptibility and environmental factors in MS risk remains a key area for further investigation. Longitudinal studies tracking changes in MS epidemiology over time, coupled with advances in genetic and environmental research, may help disentangle the complex aetiology of this disease.

As our knowledge continues to evolve, so too must our approaches to addressing the global burden of Multiple Sclerosis. The integration of epidemiological insights into clinical practice, public health initiatives, and research priorities will be key for improving outcomes for individuals with MS worldwide. By maintaining a global perspective and leveraging interdisciplinary collaborations, we can continue to make strides in understanding and managing this challenging neurological condition, working towards a future where the impact of MS is significantly reduced.

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1.3. PATHOPHYSIOLOGY AND SYMPTOMS OF MULTIPLE SCLEROSIS

1. Introduction

MS is one of the most common neurological disorders affecting young adults, with a global prevalence of approximately 3.1 million people (King et al., 2026). The disease typically occurs between the ages of 20 and 40, with a peak incidence around age 30. Women are disproportionately affected, with a female-to-male ratio of approximately 3:1 in most populations (Rejdak et al., 2010). See Figure 1.3.1.

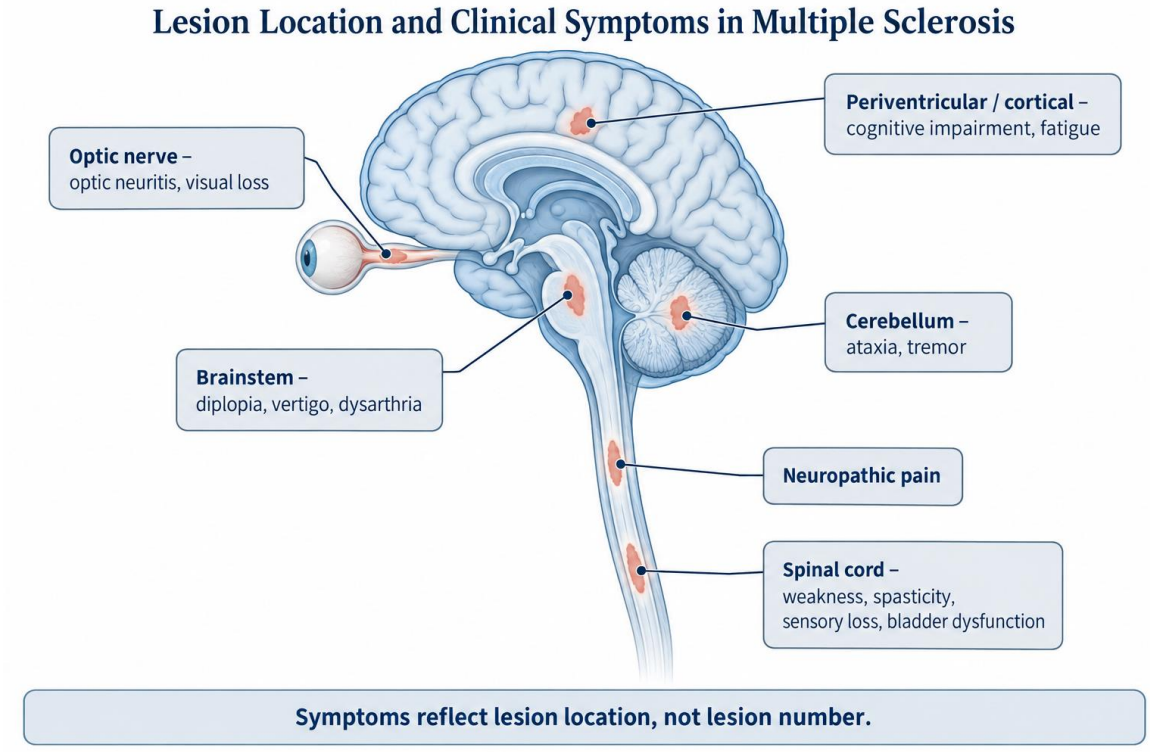


Figure 1.3.1. Lesion location and clinical symptoms in multiple sclerosis. The clinical picture reflects where lesions arise rather than how many there are.

The aetiology of MS is multifactorial, involving an interaction of genetic susceptibility and environmental factors. While the exact cause remains unknown, research has identified several risk factors, including Epstein-Barr virus infection, vitamin D deficiency, smoking, and certain genetic variations, particularly in the HLA region (Ascherio & Munger, 2017).

2. Pathophysiology of Multiple Sclerosis

The pathophysiology of MS is heterogeneous, involving several interconnected processes that contribute to the characteristic damage observed in the CNS. Understanding these mechanisms is essential for developing targeted therapies and improving patient outcomes.

2.1. Inflammatory Processes

At the core of MS pathophysiology is an aberrant immune response directed against components of the CNS, particularly the myelin sheath. Autoreactive T cells, and in particular CD4+ T helper cells and CD8+ cytotoxic T cells, are closely involved in MS pathogenesis. These cells become activated in the periphery, possibly due to molecular mimicry with environmental antigens, and then cross the blood-brain barrier to enter the CNS, where they recognize myelin antigens presented by antigen-presenting cells, leading to their reactivation and the release of pro-inflammatory cytokines (Lassmann, 2005). While MS has traditionally been considered a T cell-mediated disease, the role of B cells in its pathogenesis has gained increasing recognition. B cells contribute to MS pathology through several mechanisms: the production of autoantibodies against myelin and other CNS components, antigen presentation to T cells, the production of both pro-inflammatory cytokines such as TNF-α and IL-6 and regulatory cytokines such as IL-10, and the formation of ectopic lymphoid follicles in the meninges, which may contribute to cortical pathology in progressive MS (Hauser et al., 2008). The innate immune system is activated as well, and

microglia and macrophages play significant roles in MS pathology: these cells can contribute to tissue damage through the production of pro-inflammatory cytokines, reactive oxygen species, and proteases, although they may also have protective functions, such as debris clearance and the promotion of remyelination (Lassmann, 2005).

2.2. Demyelination

The hallmark of MS is the destruction of myelin sheaths surrounding axons in the CNS, and multiple mechanisms contribute to this myelin damage, including direct attack by cytotoxic T cells, antibody-mediated damage, phagocytosis by activated macrophages and microglia, and oxidative stress-induced damage to oligodendrocytes. The demyelinated areas that result, known as plaques or lesions, form in various regions of the CNS, disrupting neural conduction and contributing to neurological symptoms; these plaques can be classified as active, chronic active, or inactive based on their inflammatory activity and degree of demyelination (Lassmann et al., 2007). Demyelination is not uniform in character. Lucchinetti et al. described four distinct patterns of demyelination in MS lesions: Pattern I, which is T cell and macrophage-mediated; Pattern II, which is antibody and complement-mediated; Pattern III, characterised by distal oligodendrogliopathy with preferential loss of myelin-associated glycoprotein; and Pattern IV, a rare form of primary oligodendrocyte degeneration.

These patterns may have implications for treatment response and prognosis (Lucchinetti et al., 2000).

2.3. Axonal Loss and Neurodegeneration

While initially considered secondary to demyelination, axonal loss and neurodegeneration are now recognised as early and ongoing processes in MS. Acute axonal injury occurs when inflammatory mediators and oxidative stress damage axons directly, even in the early stages of the disease, and axonal transection can be observed in active MS lesions, leading to Wallerian degeneration (Trapp & Nave, 2008). Over the longer term, persistent demyelination and the loss of trophic support from oligodendrocytes contribute to chronic neuronal and axonal degeneration, a process thought to be a major driver of disability progression, particularly in progressive forms of MS (Trapp & Nave, 2008). The progressive loss of neurons and axons ultimately produces measurable brain atrophy, which correlates with disability progression in MS patients; atrophy can be observed early in the disease course and accelerates over time, particularly in progressive MS (De Stefano et al., 2014).

2.4. Remyelination and Repair

The CNS has some capacity for repair following demyelination, although this process is often incomplete in MS. Oligodendrocyte precursor cells (OPCs) are present throughout the adult CNS and can differentiate into mature oligodendrocytes capable of remyelinating damaged axons. The efficiency of remyelination, however, is affected by various factors, including the age of the patient, disease duration, lesion location, and the presence of inhibitory factors in the lesion environment. In chronic MS lesions, remyelination often fails despite the presence of OPCs, suggesting that the lesion environment becomes non-permissive for repair over time (Franklin, 2002).

2.5. Oxidative Stress and Mitochondrial Dysfunction

Emerging evidence highlights the roles of oxidative stress and mitochondrial dysfunction in MS pathophysiology. Inflammatory processes and activated immune cells generate reactive oxygen and nitrogen species that contribute to tissue damage, and this oxidative stress, together with other factors, can impair mitochondrial function, leading to energy deficits that further contribute to neurodegeneration (Blagov et al., 2022). Increased iron deposition in the CNS, particularly in deep gray matter structures, may compound these effects by contributing to oxidative stress and neurodegeneration in MS (Hametner et al., 2013).

3. Disease Course and Clinical Subtypes

The pathophysiological processes in MS manifest in various clinical courses, which are categorized into several subtypes:

3.1. Relapsing-Remitting MS (RRMS)

RRMS is the most common form of MS, characterised by clearly defined attacks of new or increasing neurologic symptoms followed by periods of partial or complete recovery (remissions) (Lublin et al., 2014). Its key features follow from this pattern: periods of active inflammation and demyelination correspond to clinical relapses, while during remission some degree of remyelination and recovery may occur, although often incompletely. Despite these periods of remission, cumulative damage to the CNS can occur over time, and a significant proportion of RRMS patients eventually transition to a progressive course, typically after 10 to 20 years.

3.2. Secondary Progressive MS (SPMS)

Many patients with RRMS eventually transition to SPMS, characterised by a steady worsening of neurologic function over time, with or without superimposed relapses (Lublin et al., 2014). In this phase, disability accumulates independently of relapses, and although acute inflammatory lesions become fewer, smoldering inflammation continues. Neurodegeneration accelerates, with an increased rate of brain atrophy compared to RRMS, and cognitive decline is often more prominent than in the relapsing-remitting phase.

3.3. Primary Progressive MS (PPMS)

PPMS affects approximately 10-15% of MS patients and is characterised by steadily worsening neurologic function from the onset of symptoms, without early relapses or remissions (Lublin et al., 2014). Its onset is gradual, with slow, continuous worsening of neurological function from the beginning of the disease, and it is accompanied by less inflammatory activity, with fewer acute inflammatory lesions than in RRMS. Spinal cord pathology is often more prominent than in other MS subtypes, and the age of onset is typically later, with most patients diagnosed in their 40s or 50s.

3.4. Clinically Isolated Syndrome (CIS)

CIS refers to the first episode of neurologic symptoms caused by inflammation or demyelination in the CNS, and it may or may not progress to clinically definite MS (Lublin et al., 2014). Two considerations are particularly important here: the presence of MRI lesions at the time of CIS increases the risk of developing MS, and early treatment in high-risk CIS patients may delay conversion to clinically definite disease.

3.5. Radiologically Isolated Syndrome (RIS)

RIS refers to the incidental discovery of lesions suggestive of MS on MRI in the absence of clinical symptoms (Lublin et al., 2014). Regular follow-up is central to detecting the development of clinical symptoms, and certain MRI features and demographic factors may increase the risk of progression to clinical MS.

4. Symptoms of Multiple Sclerosis

The symptoms of MS are diverse and can affect multiple aspects of a patient's physical, cognitive, and psychological well-being. The wide array of symptoms reflects the variable distribution of lesions within the CNS and the interaction of pathophysiological processes.

4.1. Physical Symptoms

Motor symptoms are among the most prominent physical manifestations of MS. Weakness often presents as heaviness or clumsiness in one or more limbs and can result from damage to the corticospinal tracts or other motor pathways in the CNS. Spasticity, an increase in muscle tone leading to stiffness and spasm, is caused by damage to the descending motor pathways and can significantly impact mobility and quality of life. Ataxia, or impaired coordination and balance, typically results from cerebellar or sensory pathway involvement, while gait disturbances, often described as unsteady or wobbly gait, may arise from a combination of weakness, spasticity, ataxia, and sensory deficits. These motor symptoms are common in MS and can significantly impact mobility and independence in daily activities (Ben-Zacharia, 2011), and their severity and progression can vary widely among patients and may fluctuate over time.

Sensory symptoms are equally characteristic. Numbness and tingling are often among the earliest symptoms, typically affecting the limbs or the trunk, and these paresthesias result from demyelination of sensory pathways. Pain may be acute, as in trigeminal neuralgia, or chronic and neuropathic in character; central neuropathic pain is a significant issue in MS, affecting around 30% of individuals with the condition (Falah et al., 2011). Lhermitte's sign, an electric shock-like sensation running down the spine upon neck flexion, is indicative of cervical spinal cord involvement. Sensory symptoms can be particularly distressing for patients and may significantly impact quality of life, and the management of neuropathic pain in MS remains challenging, often requiring a multimodal approach.

Visual symptoms are similarly frequent. Optic neuritis, an inflammation of the optic nerve causing pain and vision loss, is often one of the earliest manifestations of MS and can lead to temporary or permanent visual deficits. Diplopia, or double vision due to impaired eye movement coordination, often results from brainstem lesions affecting ocular motor pathways, while nystagmus, an involuntary eye movement affecting visual stability, is typically due to cerebellar or brainstem involvement. Visual disturbances are common in MS and can be among the first symptoms experienced by patients (Simpson et al., 2020), and their proper assessment and management are important for maintaining quality of life and preventing complications.

Bladder and bowel dysfunction affect many patients. Urinary urgency and frequency, often accompanied by incomplete emptying, results from disruption of the neural pathways controlling bladder function; constipation is

common owing to reduced gut motility and mobility issues, and fecal incontinence, while less common, significantly impacts quality of life. These symptoms can significantly affect daily life and social interactions (Ben-Zacharia, 2011), and their proper management is essential to prevent complications such as urinary tract infections and to maintain quality of life.

Sexual dysfunction is a further common manifestation. Erectile dysfunction is common in men with MS and results from both neurological and psychological factors, decreased libido affects both men and women and is often multifactorial in origin, and altered genital sensation may impair sexual satisfaction. Sexual dysfunctions are common and distressing symptoms of MS, highlighting the pervasive impact of the disease on quality of life (Balsamo et al., 2017), and addressing sexual health concerns is an important aspect of integrated MS care.

Fatigue is one of the most common and debilitating symptoms of MS and is often described as an overwhelming sense of exhaustion disproportionate to activity level. It is a prevalent and challenging symptom, affecting a significant proportion of patients (Román et al., 2022), and its pathophysiology is complex and likely multifactorial, involving both central and peripheral mechanisms.

Speech and swallowing difficulties complete the physical picture. Dysarthria, or slurred and slow speech due to impaired muscle control, often results from brainstem or cerebellar involvement, while dysphagia, difficulty swallowing, can result from brainstem lesions or generalized weakness of the oropharyngeal muscles and may carry a risk of aspiration. These symptoms can impact communication and nutrition, further complicating MS management (Simpson et al., 2020), and early identification and intervention are important to prevent complications and maintain quality of life.

4.2. Cognitive Symptoms

Cognitive impairment is a common symptom of MS, affecting individuals with both relapsing-remitting and primary progressive forms of the disease (Rodrigues et al., 2011). Slowed information processing speed is often one of the earliest and most common cognitive deficits and can impact various aspects of daily life and work performance. Difficulties with attention and concentration, especially in complex or distracting environments, may affect task completion and social interactions. Memory is frequently involved in two respects: short-term memory problems with the encoding and retrieval of recent information and working memory difficulties in manipulating information held in short-term storage; such memory deficits can significantly impact daily functioning and quality of life for MS patients. Executive function may also be impaired, with difficulties in planning, organizing, problem-solving, and decision-making, higher-order functions that are key for independent living and work performance. Verbal fluency may be reduced, producing difficulties in word-finding and fluent language production that affect communication and social interactions, and visuospatial processing may be challenged, with difficulties in spatial perception and navigation that can impact activities such as driving and reading maps. Cognitive fatigue is a prevalent and debilitating symptom in MS, with limited effective treatment options currently available (Cercignani et al., 2021), and the impact of cognitive symptoms on daily life and quality of life is often underestimated, requiring careful assessment and management.

4.3. Psychological and Psychiatric Symptoms

MS can have significant psychological impacts, both as a direct result of CNS pathology and as a consequence of living with a chronic, unpredictable disease. Depression shows a higher prevalence in MS patients compared to the general population, affecting up to 50% of patients at some point in their disease course (Kano et al., 2012), and it may arise from both neurobiological factors, such as inflammatory processes and CNS damage, and psychosocial factors related to living with a chronic illness. Anxiety often coexists with depression and may be exacerbated by the unpredictable nature of MS; anxiety disorders in MS patients may include generalized anxiety disorder, panic disorder, and social anxiety. Emotional lability, consisting of rapid and unpredictable mood swings and sometimes referred to as pseudobulbar affect, can result from damage to neural pathways involved in emotional regulation. Cognitive fatigue, a mental exhaustion that can significantly impact daily functioning and quality of life, is often reported as one of the most disabling aspects of MS. Stress acts both as a potential trigger for symptom exacerbation and as a consequence of living with MS, and chronic stress can have detrimental effects on physical and mental health alike. Social isolation, arising from physical limitations, cognitive changes, and psychological factors, can further exacerbate depression and anxiety, creating a vicious cycle. The burden of psychosocial factors on worsening MS symptoms has been explored, revealing categories such as psychosocial tensions, stress, agitation, and stigmatization that can exacerbate the disease experience (Pourhaji et al., 2023), and

addressing these psychological and psychiatric symptoms is essential for holistic MS care and for improving overall quality of life.

4.4. Less Common and Atypical Symptoms

MS can present with a variety of less common or atypical symptoms that may pose diagnostic challenges. Sudden sensorineural hearing loss, although rare, can occur in MS, potentially due to demyelination of the auditory pathways. Seizures occur in a small percentage of MS patients and are more common in those with cortical lesions; their prevalence in MS is estimated to be two to three times higher than in the general population. Headaches, including migraine-like headache, are more prevalent in MS patients, although the relationship between MS and headaches is complex and not fully understood. Tremors can be disabling and difficult to treat in some patients and may be postural, intention, or resting in type, involving the head, trunk, or limbs. Paroxysmal symptoms, brief and recurring episodes of neurological dysfunction such as tonic spasms or paresthesias, are thought to result from ephaptic transmission in partially demyelinated axons. Uhthoff's phenomenon, a temporary worsening of symptoms with increased body temperature, is due to temperature-dependent conduction block in demyelinated axons. Finally, cognitive-motor interference describes difficulty in performing cognitive and motor tasks simultaneously, beyond what would be expected from the individual deficits, a problem that can have significant implications for daily activities and safety.

5. Factors Influencing Symptom Presentation

The presentation and severity of MS symptoms can be influenced by various factors:

5.1. Lesion Location:

The specific areas of the CNS affected by demyelination and neurodegeneration largely determine the nature of symptoms experienced. For example, optic nerve lesions lead to visual symptoms, while spinal cord lesions often result in motor and sensory deficits in the limbs.

5.2. Disease Course:

Symptom patterns may differ between relapsing and progressive forms of MS. Relapsing forms often have more distinct attacks with periods of recovery, while progressive forms show gradual worsening of symptoms over time.

5.3. Disease Duration:

Cumulative damage over time can lead to more persistent and severe symptoms. This is particularly evident in the transition from relapsing-remitting to secondary progressive MS.

5.4. Environmental Factors:

Heat, stress, and fatigue can temporarily exacerbate symptoms. This is known as pseudo-exacerbation and does not represent new disease activity.

5.5. Comorbidities:

The presence of other health conditions can interact with and potentially worsen MS symptoms. Common comorbidities in MS include depression, anxiety, hypertension, and hyperlipidemia.

5.6. Treatment Effects:

Disease-modifying therapies and symptomatic treatments can influence the manifestation and severity of symptoms. While these treatments aim to reduce disease activity and manage symptoms, they can also have side effects that may mimic or exacerbate MS symptoms.

5.7. Lifestyle Factors:

Diet, exercise, sleep patterns, and stress management can all influence symptom severity and overall well-being in MS patients.

6. Diagnostic Challenges

The diverse and often non-specific nature of MS symptoms can pose significant diagnostic challenges:

6.1. Mimicking Conditions:

Many symptoms of MS can be similar to those of other neurological or systemic disorders, necessitating careful differential diagnosis. Conditions that can mimic MS include neuromyelitis optica spectrum disorders, acute disseminated encephalomyelitis, and systemic lupus erythematosus.

6.2. Variable Presentation:

The wide range of possible symptoms and their fluctuating nature can make initial diagnosis challenging, particularly in cases with atypical presentations. This variability can lead to delayed diagnosis and treatment.

6.3. Pediatric MS:

Diagnosing MS in children presents unique challenges, as symptoms may differ from adult presentations and must

be distinguished from other pediatric neurological conditions (Carbonell et al., 2013). The initial presentation of MS in children often involves optic nerve involvement and cerebellar signs and symptoms (Etemadifar et al., 2016).

6.4. Psychiatric Presentations:

In some cases, psychiatric symptoms may be the initial or predominant manifestation of MS, potentially leading to misdiagnosis or delayed diagnosis (Algin et al., 2010). This makes the importance clear of considering MS in patients presenting with new-onset psychiatric symptoms, particularly when accompanied by neurological signs.

6.5. Subclinical Disease:

The concept of radiologically isolated syndrome (RIS) demonstrates the potential for MS pathology to exist without overt clinical symptoms, raising questions about optimal management and monitoring strategies. The management of RIS remains controversial, with ongoing debate about whether to initiate treatment in asymptomatic individuals.

6.6. Evolving Diagnostic Criteria:

Diagnostic criteria have evolved. The 2017 framework was revised by the 2024 McDonald criteria, published in 2025; historical studies must be interpreted using the criteria applied at enrolment (Montalban et al., 2025).

7. Impact on Quality of Life

The diverse symptomatology of MS can have profound effects on patients' quality of life:

7.1. Physical Functioning:

Motor symptoms and fatigue can significantly impact mobility and independence in daily activities. This can lead to reduced participation in work, leisure activities, and social interactions.

7.2. Cognitive Impact:

Cognitive symptoms can affect work performance, social interactions, and overall functional capacity. The "invisible" nature of cognitive symptoms can sometimes lead to misunderstandings and lack of appropriate support.

7.3. Emotional Well-being:

Psychological symptoms and the unpredictable nature of MS can lead to significant emotional distress and reduced quality of life. The burden of living with a chronic, potentially disabling condition can take a toll on mental health.

7.4. Social Relationships:

Physical limitations, cognitive changes, and psychological factors can strain personal and professional relationships. Social isolation is a common concern for many MS patients.

7.5. Employment:

MS symptoms can impact job performance and may lead to reduced work capacity or early retirement. Workplace accommodations and vocational rehabilitation can be important for maintaining employment.

7.6. Financial Burden:

The direct and indirect costs associated with managing MS symptoms can place significant financial strain on patients and their families. This includes medical expenses, lost productivity, and potential need for caregiving support.

7.7. Sexual Health and Relationships:

Sexual dysfunction and changes in intimate relationships can significantly impact quality of life for MS patients and their partners.

7.8. Independence and Self-image:

The progressive nature of MS can lead to increased dependence on others and changes in self-perception, which can be psychologically challenging for many patients.

8. Management Approaches

Given the complex and heterogeneous nature of MS symptoms, management typically requires a thorough, multidisciplinary approach:

8.1 Disease-Modifying Therapies:

Aimed at reducing disease activity and potentially slowing symptom progression. These include injectable, oral, and infusion therapies that modulate or suppress the immune system.

8.2 Symptomatic Treatments:

Targeted pharmacological and non-pharmacological interventions for specific symptoms (e.g., antispasmodics for spasticity, antidepressants for mood disorders, medications for fatigue).

8.3 Rehabilitation:

Physical therapy, occupational therapy, and speech therapy to address functional impairments and improve quality of life.

8.4 Cognitive Rehabilitation:

Strategies to improve cognitive function or develop compensatory techniques. This may include cognitive training exercises, memory aids, and environmental modifications.

8.5 Psychological Support:

Counseling, psychotherapy, and support groups to address the psychological impact of MS. Cognitive-behavioural therapy has shown particular benefit for managing depression and anxiety in MS patients.

8.6 Lifestyle Modifications:

Strategies for managing fatigue, adapting to limitations, and maintaining overall health. This includes exercise programs tailored to individual capabilities, dietary modifications, and stress management techniques.

8.7 Assistive Devices:

Mobility aids, cognitive aids, and adaptive technologies to support independence and function. These can range from simple tools like canes and walkers to more advanced technologies like voice-activated home control systems.

8.8 Complementary Therapies:

Interventions such as mindfulness, yoga, and acupuncture may provide additional symptom relief for some patients. While evidence for these approaches is often limited, many patients find them helpful as part of a multimodal management plan.

8.9 Self-Management Programs:

The interactive web-based program MSmonitor has been developed to aid in self-management and multidisciplinary care for individuals with MS, providing a general overview of symptoms and their subjective valuation (Jongen et al., 2016). Such programs empower patients to take an active role in their care and can improve communication with healthcare providers.

8.10 Emerging Therapies:

Research into novel treatments for MS is ongoing, including potential neuroprotective and remyelinating therapies. Stem cell therapies, particularly autologous haematopoietic stem cell transplantation, have shown promise for some patients with highly active relapsing MS.

9. Conclusion

Multiple Sclerosis is a complex neurological condition characterised by a diverse array of symptoms that can significantly impact patients' physical, cognitive, and psychological well-being. The pathophysiology of MS involves intricate processes of inflammation, demyelination, and neurodegeneration, resulting in a wide spectrum of clinical manifestations. Understanding the complexity of MS symptoms is central for accurate diagnosis, effective management, and improving quality of life for individuals living with this challenging condition.

Further work on MS pathophysiology and symptomatology may refine targeted therapy and comprehensive management. Priorities include individualized symptom management, imaging methods for earlier detection and monitoring, and neuroprotective or regenerative strategies directed at mechanisms of irreversible injury.

The complexity of MS symptoms calls for patient-centred, multidisciplinary care, tailored to the individual needs and experiences of each person living with MS. By continuing to expand our knowledge of MS pathophysiology and symptomatology, we can work towards improved outcomes and enhanced quality of life for the millions of individuals affected by this challenging neurological condition worldwide.

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1.4. GENETIC AND ENVIRONMENTAL RISK FACTORS

1. Introduction

MS risk reflects the interaction of genetic susceptibility with environmental exposures. Figure 1.4.1 sets out the factors considered below and their proposed relationships.

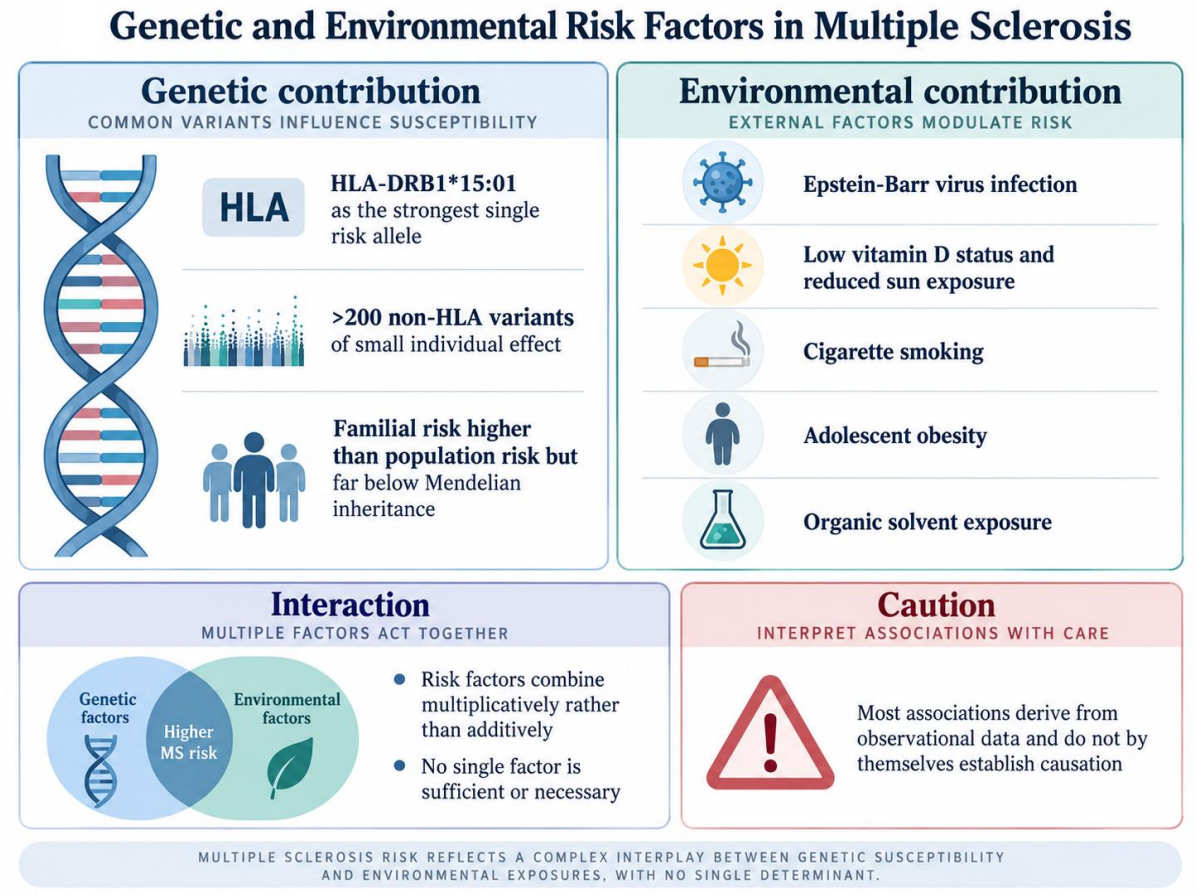


Figure 1.4.1. Genetic and environmental risk factors in multiple sclerosis. Most of these associations derive from observational data and do not by themselves establish causation.

1.1. Historical Perspective

The concept of genetic and environmental factors contributing to MS is not new. Early observations of familial clustering and geographical variations in MS prevalence led researchers to hypothesize about the role of both hereditary and environmental influences. In the late 19th century, Jean-Martin Charcot, who provided the first detailed description of MS, noted the potential influence of environmental factors such as stress and infection on disease onset and progression.

1.2. Evolution of Research

Over the past century, research into the genetic and environmental risk factors for MS has evolved dramatically. Between the 1930s and the 1960s, early twin studies and familial aggregation studies provided the first evidence for a genetic component in MS susceptibility. The 1970s and 1980s brought a significant breakthrough with the discovery of the association between MS and certain HLA alleles. From 2007 onward, the advent of genome-wide association studies (GWAS) led to the identification of numerous non-HLA genetic risk factors, and more recently the integration of genetic and environmental data, together with advances in epigenetics and microbiome research, has produced a broader understanding of MS aetiology.

2. Genetic Risk Factors

2.1. Human Leukocyte Antigen (HLA) System

The human leukocyte antigen (HLA) system matters for the genetic susceptibility to MS. HLA genes, particularly

those in the class II region, have been consistently associated with MS risk across various populations (Hollenbach & Oksenberg, 2015).

2.1.1 HLA-DRB1*15:01 Allele

The HLA-DRB1*15:01 allele is the strongest genetic risk factor for MS, increasing the risk of developing the disease by approximately three-fold (Hollenbach & Oksenberg, 2015).

Several findings define its contribution. The allele is present in about 60% of MS patients compared to 30% of healthy controls in European populations (Sawcer et al., 2011), and the odds ratio for MS risk associated with it is approximately 3.1 in these populations (International Multiple Sclerosis Genetics Consortium, 2011). Functionally, the allele is thought to influence MS risk by altering antigen presentation and T-cell activation, potentially leading to increased autoimmune responses against myelin components (Patsopoulos et al., 2013).

2.1.2 Other HLA Alleles

While HLA-DRB1*15:01 is the most well-established genetic risk factor, other HLA alleles have also been implicated in MS susceptibility. HLA-A*02:01 has been associated with a protective effect against MS, with an odds ratio of approximately 0.73 in European populations (Moutsianas et al., 2015), whereas HLA-DRB1*03:01 has been associated with increased MS risk, particularly in Southern European populations (Gourraud et al., 2012), and HLA-DRB1*13:03 with increased risk in some populations, with an odds ratio of approximately 2.4 (Moutsianas et al., 2015).

2.1.3 HLA Gene Interactions

The effects of HLA genes on MS risk are not simply additive, and complex interactions between different HLA alleles can modulate disease susceptibility. Epistatic interactions between alleles can result in either increased or decreased MS risk: carriage of HLA-DRB1*15:01 together with HLA-DRB1*08:01, for example, confers a higher risk than either allele alone, whereas HLA-DRB1*14 carried on the other chromosome markedly attenuates the risk associated with HLA-DRB1*15:01 (Lincoln et al., 2009). The risk associated with HLA-DRB1*15:01 can also be modified by other HLA alleles located on the same haplotype, in cis, or on the opposite chromosome, in trans (Dyment et al., 2004).

2.1.4 Population Differences

The influence of HLA genes on MS risk varies across different ethnic groups, a reminder of how much genetic background contributes to susceptibility. HLA-DRB1*15:01 is the primary risk allele in most European populations, with an odds ratio of approximately 3.1 (International Multiple Sclerosis Genetics Consortium, 2011). It is also a risk factor in African Americans, although its effect is somewhat weaker, with an odds ratio of approximately 1.9 (Isobe et al., 2013). In some Asian populations, other alleles such as HLA-DRB1*04:05 may play a more significant role in MS risk (Yoshimura et al., 2012).

2.2. Non-HLA Genetic Risk Factors

Genome-wide association studies (GWAS) have identified numerous non-HLA genetic variants associated with MS risk. These genes are primarily involved in immune system regulation, particularly T-cell function and cytokine signalling pathways.

2.2.1 Key Non-HLA Genes

Among the most consistently replicated non-HLA genetic associations with MS are variants in IL7R, IL2RA, TNFRSF1A, and CD58. IL7R encodes the IL-7 receptor α chain, which is important for T-cell development and homeostasis, and its rs6897932 variant has been associated with MS risk with an odds ratio of approximately 1.18 (Gregory et al., 2007). IL2RA encodes the α chain of the IL-2 receptor, which is important for T-cell activation and regulatory T-cell function, and multiple variants in this gene have been associated with MS risk, with odds ratios ranging from 1.12 to 1.25 (International Multiple Sclerosis Genetics Consortium, 2011). TNFRSF1A encodes a receptor for TNF-α, a key pro-inflammatory cytokine, and its rs1800693 variant carries an odds ratio of approximately 1.12 (De Jager et al., 2009). CD58 encodes a cell adhesion molecule involved in T-cell activation, and its rs2300747 variant has an odds ratio of approximately 0.82, indicating a protective effect (De Jager et al., 2009).

2.2.2 Functional Categories of MS Risk Genes

Non-HLA genes associated with MS risk can be broadly categorized based on their functional roles. Genes such as IL7R, IL2RA, and CD6 are involved in T-cell activation and differentiation processes, highlighting the central role of T cells in MS pathogenesis (International Multiple Sclerosis Genetics Consortium, 2019). Genes encoding cytokines or their receptors, including TNFRSF1A, IL12A, and IL22RA2, confirm the part played by inflammatory

processes in MS (Sawcer et al., 2011), while genes encoding co-stimulatory molecules such as CD80 and CD86 play a role in modulating T-cell responses and have likewise been implicated in MS risk (International Multiple Sclerosis Genetics Consortium, 2019). A fourth group comprises genes involved in vitamin D metabolism and signalling, such as CYP27B1 and CYP24A1, whose association with MS risk supports the link between vitamin D and disease susceptibility (Sawcer et al., 2011).

2.2.3 Polygenic Nature of MS Risk

The genetic architecture of MS risk is highly polygenic, with each individual variant contributing only a small effect to overall disease risk. Although individual non-HLA genetic variants typically have modest effect sizes, with odds ratios generally between 1.1 and 1.3, the cumulative effect of multiple risk alleles can substantially influence an individual's overall genetic risk for MS (International Multiple Sclerosis Genetics Consortium, 2019). On this basis, researchers have developed polygenic risk scores that combine information from multiple genetic variants to estimate an individual's overall genetic risk, and these scores have shown promise in predicting MS risk and potentially disease course (De Jager et al., 2009).

2.2.4 Rare Variants

While GWAS have primarily identified common genetic variants associated with MS risk, research into rare variants is ongoing, and studies using exome sequencing have identified rare variants in genes such as TYK2 and GALC that may contribute to MS risk in some individuals (Dyment et al., 2012).

2.3. Epigenetic Factors

Epigenetic modifications, which can be influenced by both genetic and environmental factors, are increasingly recognised as important contributors to MS pathogenesis.

2.3.1 DNA Methylation

DNA methylation is an epigenetic modification that can alter gene expression without changing the underlying DNA sequence. Studies have observed global DNA hypomethylation in peripheral blood mononuclear cells of MS patients compared to healthy controls (Calabrese et al., 2012) and altered methylation patterns have been described in specific genes relevant to MS pathogenesis, including HLA-DRB1, IL17A, and FOXP3 (Maltby et al., 2015). Because DNA methylation patterns can change over time and in response to environmental factors, they may contribute to the variable course of MS (Webb & Guerau-de-Arellano, 2017).

2.3.2 Histone Modifications

Histone modifications govern gene expression and have been implicated in MS pathogenesis. Altered histone acetylation patterns have been observed in MS patients, particularly in genes involved in inflammatory responses and myelin repair (Pedre et al., 2011), while changes in histone methylation have been associated with MS risk and disease progression, affecting genes involved in T-cell differentiation and function (Huynh & Casaccia, 2013).

2.3.3 microRNAs

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. Several miRNAs, including miR-155, miR-326, and miR-146a, have been found to be dysregulated in MS patients and in animal models of the disease (Jagot & Davoust, 2016). These dysregulated miRNAs can affect various aspects of MS pathogenesis, including T-cell activation, B-cell function, and blood-brain barrier integrity (Keller et al., 2014), and circulating miRNAs are being investigated as potential biomarkers for MS diagnosis, prognosis, and treatment response (Gandhi et al., 2015).

3. Environmental Risk Factors

Environmental factors are central to MS pathogenesis, interacting with genetic susceptibility to influence disease risk and progression.

3.1. Infections

Infectious agents, particularly viruses, have long been suspected as potential triggers for MS.

3.1.1 Epstein-Barr Virus (EBV)

EBV infection has been consistently associated with increased MS risk. Nearly all MS patients show evidence of prior EBV infection, compared to about 90% of the general population (Ascherio & Munger, 2015), and a history of infectious mononucleosis, caused by EBV, increases the risk of a subsequent MS diagnosis by about two- to threefold (Handel et al., 2010). Mechanistically, EBV may contribute to MS pathogenesis through molecular mimicry, bystander activation of autoreactive T cells, or by promoting a pro-inflammatory environment (Pender & Burrows, 2014). More recently, Lanz et al. (2022) reported antibodies from clonally expanded B cells in the cerebrospinal fluid of people with MS that recognize both EBV EBNA1 and the glial protein GlialCAM; these findings support

investigation of molecular mimicry, although the cited study did not establish that EBV-infected B cells were detected in MS brain tissue.

3.1.2 Human Herpesvirus 6 (HHV-6)

HHV-6, and particularly HHV-6A, has also been implicated in MS pathogenesis. HHV-6A has been associated with axonal injury preceding the clinical onset of MS (Grut et al., 2024), the virus has been detected in oligodendrocytes within MS lesions, suggesting a potential role in demyelination (Leibovitch & Jacobson, 2014), and HHV-6 can alter the expression of myelin genes and modulate immune responses in ways that may contribute to MS pathogenesis (Álvarez-Lafuente et al., 2008).

3.1.3 Other Infectious Agents

Several other infectious agents have been investigated in relation to MS risk. While initial studies suggested a possible association between Chlamydia pneumoniae infection and MS, subsequent research has yielded conflicting results (Pawate & Sriram, 2010). The activation of human endogenous retroviruses (HERVs), particularly HERV-W, has been observed in MS patients and may contribute to neuroinflammation and demyelination (Perron & Lang, 2010).

3.1.4 Hygiene Hypothesis

The hygiene hypothesis suggests that early-life exposure to diverse microbial antigens may protect against MS, while reduced exposure may increase risk. Epidemiologically, MS prevalence tends to be lower in regions with higher infectious burden and poorer sanitation (Fleming & Cook, 2006), and the immunological basis proposed for this is that early-life infections help shape the immune system, potentially reducing the risk of autoimmune responses later in life (Okada et al., 2010). The data are not uniform, however: while some studies support the hygiene hypothesis in MS, others have found no clear association, which highlights the complex relationship between infections and MS risk (Conradi et al., 2011).

3.2. Vitamin D Deficiency

Vitamin D deficiency has attracted attention as a significant environmental risk factor for MS:

3.2.1 Epidemiological Evidence

MS prevalence generally increases with distance from the equator, correlating with reduced sunlight exposure and vitamin D production (Ascherio & Munger, 2017). Some studies have additionally observed a higher proportion of MS patients born in spring months, potentially related to maternal vitamin D levels during pregnancy (Willer et al., 2005).

3.2.2 Serum Vitamin D Levels

Low serum levels of 25-hydroxyvitamin D are associated with an increased risk of MS and with greater disease activity, and a dose-response relationship has been observed, with higher vitamin D levels associated with lower MS risk (Munger et al., 2006).

3.2.3 Mechanisms of Action

Vitamin D may influence MS risk and progression through several mechanisms. It has immunomodulatory effects, including the promotion of regulatory T cells and the suppression of pro-inflammatory cytokines (Cantorna et al., 2015), and it can regulate the expression of numerous genes involved in immune function and myelin maintenance (Pierrot-Deseilligny & Souberbielle, 2017).

3.2.4 Vitamin D Supplementation

Some studies suggest that vitamin D supplementation may reduce the risk of developing MS, particularly when started early in life (Munger et al., 2017). Evidence on disease modification is mixed, although some studies indicate that supplementation in MS patients may reduce relapse rates and slow disease progression (Pierrot-Deseilligny & Souberbielle, 2017). The optimal dose of vitamin D for MS prevention and treatment remains unclear, and ongoing clinical trials are investigating various supplementation strategies (Bhargava et al., 2014).

3.3. Smoking

Cigarette smoking is a well-established environmental risk factor for MS:

3.3.1 Risk Association

Smoking increases the risk of developing MS by approximately 50% (Zhang et al., 2016). The risk appears to be dose-dependent, with heavier smokers at greater risk (Hedström et al., 2009), and some studies suggest that the effect of smoking on MS risk may be stronger in men than in women (Sundström & Nyström, 2008).

3.3.2 Impact on Disease Course

Smoking has been associated with faster disease progression and earlier transition to secondary progressive MS (Manouchehrinia et al., 2013), and current smokers may have a higher risk of relapses compared to non-smokers (Pittas et al., 2009). On imaging, smoking has been

associated with increased brain atrophy and lesion load in MS patients (Zivadinov et al., 2009).

3.3.3 Mechanisms of Action

Several mechanisms have been proposed to explain the link between smoking and MS risk. Cigarette smoke contains numerous oxidants that can contribute to oxidative stress and tissue damage in the CNS (Pryor et al., 1998), and smoking can alter immune function in ways that potentially promote pro-inflammatory responses (Arnson et al., 2010).

3.3.4 Passive Smoking

Exposure to second-hand smoke during childhood may increase the risk of developing MS later in life (Mikaeloff et al., 2007), and passive smoking in adulthood has also been associated with increased MS risk, although the effect appears to be weaker than that of active smoking (Hedström et al., 2011).

3.4. Obesity

Obesity, particularly during adolescence and young adulthood, has been identified as a risk factor for MS:

3.4.1 Risk Association

A high body mass index (BMI) during adolescence is associated with a two- to three-fold increased risk of developing MS (Munger et al., 2009). The association between obesity and MS risk appears to be stronger in females than in males (Hedström et al., 2012), and obesity in early life seems to be more strongly associated with MS risk than obesity in adulthood (Langer-Gould et al., 2013).

3.4.2 Mechanisms of Action

Several mechanisms have been proposed to explain the link between obesity and MS risk. Adipose tissue produces pro-inflammatory adipokines, which may contribute to systemic inflammation and autoimmune processes (Versini et al., 2014); obesity is associated with lower vitamin D levels, potentially exacerbating MS risk (Vimaleswaran et al., 2013); and leptin, an adipokine elevated in obesity, can promote pro-inflammatory T-cell responses (Matarese et al., 2008).

3.4.3 Impact on Disease Course

Some studies suggest that obesity may be associated with a more severe disease course and increased disability progression in MS patients (Tettey et al., 2014), and obesity may also affect the efficacy of some MS treatments, potentially due to altered drug metabolism or immune function (Kvistad et al., 2015).

3.5. Environmental Pollutants

Exposure to various environmental pollutants has been investigated as a potential risk factor for MS:

3.5.1 Air Pollution

Exposure to particulate matter (PM2.5) has been associated with an increased risk of MS in some studies (Bergamaschi et al., 2021), and living in areas with high levels of traffic-related air pollution may likewise increase MS risk (Angelici et al., 2016). Air pollutants may contribute to MS risk through oxidative stress, systemic inflammation, and disruption of the blood-brain barrier (Mousavi et al., 2019).

3.5.2 Organic Solvents

Occupational exposure to organic solvents has been linked to an increased risk of MS in some studies, with solvents such as trichloroethylene, perchloroethylene, and benzene specifically implicated (Landtblom et al., 1996). These agents may contribute to MS risk through neurotoxicity, immune system dysregulation, and oxidative stress (Riise et al., 2002).

3.5.3 Heavy Metals

Some studies have suggested a potential link between mercury exposure and MS risk, although the evidence remains inconclusive (Aminzadeh & Etminan, 2007), and occupational exposure to lead has been associated with increased MS risk in some studies, possibly due to its neurotoxic effects.

3.6. Stress

The role of stress in MS remains controversial, but some evidence suggests that stress may influence disease onset and exacerbations:

3.6.1 Psychological Stress

Psychological stress has been associated with an increased risk of MS exacerbations in some studies (Mohr et al., 2004).

3.6.2 Physiological Mechanisms

The hypothalamic-pituitary-adrenal (HPA) axis, which regulates stress responses, may be dysregulated in MS patients, and chronic stress may contribute to immune system dysfunction and increased inflammation, potentially exacerbating MS symptoms (Heesen et al., 2007).

3.6.3 Stress Management

Some studies suggest that stress management interventions may help reduce MS symptoms and improve quality of life (Artemiadis et al., 2011), although the subjective nature of stress and the difficulty of measuring its effects pose ongoing challenges for research in this area (Briones-Buixassa et al., 2015).

4. Gene-Environment Interactions

The complex aetiology of MS likely involves interactions between genetic susceptibility and environmental exposures. Some examples of gene-environment interactions in MS include:

4.1. HLA Genes and Smoking

The risk of MS associated with smoking is significantly higher in individuals carrying certain HLA risk alleles, particularly HLA-DRB1*15:01 (Hedström et al., 2011), and smoking may trigger MS in genetically susceptible individuals through mechanisms involving antigen presentation and T-cell activation (Hedström et al., 2011). This interaction appears to be dose-dependent, with heavier smokers showing a stronger gene-environment interaction (Hedström et al., 2009).

4.2. Vitamin D Receptor Gene and Vitamin D Levels

Polymorphisms in the vitamin D receptor (VDR) gene may modify the relationship between vitamin D levels and MS risk (Smolders et al., 2008), since certain VDR genotypes may alter the efficiency of vitamin D signalling and thereby influence its immunomodulatory effects in MS (Orton et al., 2008). This interaction between VDR genotype and vitamin D levels may in turn have implications for vitamin D supplementation strategies in MS prevention and treatment (Smolders et al., 2008).

4.3. Epstein-Barr Virus and HLA Genes

The risk of developing MS following EBV infection may be influenced by HLA genotype, with certain alleles conferring greater susceptibility to virus-induced autoimmunity (Sundqvist et al., 2012). The interaction between EBV and HLA risk alleles may involve molecular mimicry, in which viral antigens share structural similarities with myelin components (Lünemann et al., 2008), and HLA genotype may also influence the nature and intensity of the immune response to EBV, potentially affecting MS risk (Nielsen et al., 2009).

4.4. Obesity and Genetic Risk Factors

Variants in the FTO gene, which is associated with obesity, may interact with MS risk genes to modify disease susceptibility (Gianfrancesco & Barcellos, 2016), and obesity-related genetic variants involved in inflammatory pathways may likewise interact with MS risk genes in ways that potentially exacerbate disease risk (Gianfrancesco et al., 2017).

4.5. Environmental Pollutants and Detoxification Genes

Polymorphisms in glutathione S-transferase genes, which are involved in detoxification processes, may modify the risk of MS associated with exposure to environmental toxins (Stavropoulou et al., 2007), and genetic variations in cytochrome P450 enzymes may influence individual susceptibility to the MS risk associated with organic solvent exposure (Landtblom et al., 1996).

5. Epigenetic Mechanisms in MS

The principal epigenetic processes implicated in MS include:

5.1. DNA Methylation

Studies have observed global DNA hypomethylation in peripheral blood mononuclear cells of MS patients compared to healthy controls (Calabrese et al., 2012), and altered DNA methylation patterns have been described in specific genes relevant to MS pathogenesis, including HLA-DRB1, IL17A, and FOXP3 (Maltby et al., 2015). Environmental factors such as smoking and vitamin D deficiency may themselves influence DNA methylation profiles, potentially contributing to MS risk (Marabita et al., 2017).

5.2. Histone Modifications

Altered histone acetylation patterns have been observed in MS patients, particularly in genes involved in inflammatory responses and myelin repair (Pedre et al., 2011), while changes in histone methylation have been associated with MS risk and disease progression, affecting genes involved in T-cell differentiation and function (Huynh & Casaccia, 2013). The therapeutic relevance of these mechanisms is suggested by histone deacetylase inhibitors, which have shown promise as potential therapeutic agents in MS which in turn supports a role for epigenetic regulation in pathogenesis (Camelo et al., 2005).

5.3. microRNAs

Several microRNAs, including miR-155, miR-326, and miR-146a, have been found to be dysregulated in MS patients and in animal models of the disease (Jagot & Davoust, 2016). These dysregulated miRNAs can affect various aspects of MS pathogenesis, including T-cell activation, B-cell function, and blood-brain barrier integrity (Keller et al., 2014), and circulating miRNAs are being investigated as potential biomarkers for MS diagnosis, prognosis, and treatment response (Gandhi et al., 2015).

6. Protective Factors

While much research has focused on risk factors for MS, some factors have been associated with a reduced risk of developing the disease:

6.1. Helicobacter pylori Infection

Some studies have suggested that H. pylori infection may be protective against MS, particularly in females (Pedrini et al., 2015). This protective effect may be related to the immunomodulatory properties of H. pylori infection, which potentially promote regulatory T-cell responses (Cook et al., 2015).

6.2. Parasitic Infections

Parasitic infections, and particularly helminth infections, have been associated with a reduced risk of MS in some populations (Correale & Farez, 2007). Helminths may exert protective effects through the induction of regulatory T cells and anti-inflammatory cytokines (Fleming, 2013), and the observation of these potential protective effects has led to investigations into the therapeutic use of helminth-derived molecules in MS treatment (Correale & Farez, 2011).

6.3. Moderate Alcohol Consumption

Some studies have suggested that moderate alcohol consumption may be associated with a reduced risk of MS, although the relationship remains controversial (Hedström et al., 2014). Any protective effect may be related to the anti-inflammatory properties of moderate consumption or to its effects on lipid metabolism (Andersen et al., 2019). These observations must be interpreted with caution, however, since the observed association may be confounded by other lifestyle factors, and excessive alcohol consumption is not recommended (Massa et al., 2013).

6.4. Physical Activity

Regular physical activity, particularly during adolescence and young adulthood, has been associated with a reduced risk of MS in some studies (Olsson et al., 2017). Physical activity may exert protective effects through modulation of immune function, reduction of inflammation, and promotion of neuroprotective factors (Dalgas & Stenager, 2012), and beyond potentially reducing MS risk it may also have beneficial effects on disease course and quality of life in MS patients (Motl & Pilutti, 2012).

7. Implications for Prevention and Treatment

Understanding the interaction between genetic and environmental risk factors in MS has important implications for disease prevention and treatment:

7.1. Risk Stratification

Integration of genetic and environmental risk factors could lead to the development of more accurate predictive models for MS risk (Olsson et al., 2017), and identifying high-risk individuals on the basis of genetic and environmental profiles may allow for targeted prevention strategies, such as lifestyle modifications or early intervention.

7.2. Personalized Medicine

Knowledge of an individual's genetic risk profile and environmental exposures may help guide treatment decisions and predict disease courses, and because genetic factors may influence response to MS therapies, pharmacogenomic information could allow for more personalised treatment approaches (Grossman et al., 2017).

7.3. Modifiable Risk Factors

Addressing modifiable environmental risk factors, such as smoking cessation, maintaining a healthy BMI, ensuring adequate vitamin D levels, and regular physical activity, may help reduce MS risk and potentially slow disease progression (Olsson et al., 2017). At a broader level, population-level interventions targeting modifiable risk factors could reduce the overall burden of MS (Ascherio & Munger, 2017).

7.4. Novel Therapeutic Approaches

Understanding the role of epigenetic modifications in MS may lead to the development of new therapeutic strategies targeting these mechanisms (Vakhitov et al., 2020), while emerging research on the role of the gut microbiome in MS may lead to novel therapeutic approaches involving microbiome modulation (Berer et al., 2017). Insights into the

mechanisms of neurodegeneration in MS may likewise inform the development of neuroprotective therapies (Correale et al., 2017).

8. Future Directions

As our understanding of the genetic and environmental risk factors in MS continues to evolve, several key areas for future research emerge:

8.1 Large-scale, Longitudinal Studies

Long-term studies following individuals from early life through adulthood are needed to better understand the temporal relationships between environmental exposures and MS risk. Integration of genomic, epigenomic, transcriptomic, and metabolomic data in large cohorts may provide a more full understanding of MS pathogenesis (Hauser et al., 2013), and the inclusion of diverse populations in genetic and environmental studies is important both to understand population-specific risk factors and to improve the generalizability of findings (Isobe et al., 2016).

8.2 Advanced Genomic and Epigenomic Analyses

Whole-genome and whole-exome sequencing studies may identify rare genetic variants contributing to MS risk (Zuk et al., 2014), and studies investigating the functional consequences of MS-associated genetic variants are needed to elucidate their role in disease pathogenesis (Farh et al., 2015). The application of single-cell sequencing technologies may further provide insights into cell-specific contributions to MS pathology (Schafflick et al., 2020).

8.3 Microbiome Research

Further investigation into the role of the gut microbiome in MS risk and progression may provide new avenues for prevention and treatment (Berer et al., 2017), and elucidation of the mechanisms by which gut microbiota influence MS pathogenesis is needed in order to develop targeted interventions (Cekanaviciute et al., 2017). Microbiome-based therapies, such as probiotics or fecal microbiota transplantation, also warrant exploration in MS management (Mestre et al., 2018).

8.4 Precision Medicine Approaches

The identification of biomarkers for disease activity, progression, and treatment response would help guide personalised treatment strategies (Comabella & Montalban, 2014), and the development of personalised risk prediction models incorporating genetic, environmental, and clinical factors could improve MS management and outcomes. Genetic and molecular insights may also be leveraged to identify existing drugs that could be repurposed for MS treatment (Khanna, 2012).

8.5 Environmental Exposure Assessment

More accurate and detailed methods for assessing environmental exposures are needed, including wearable devices and biomarkers of exposure (Turner et al., 2017), together with investigation of the critical periods of susceptibility to environmental risk factors throughout the lifespan (Goodin, 2024). Large-scale studies designed specifically to detect gene-environment interactions, and able to account for the complexity of both genetic and environmental factors, will be essential.

8.6 Novel Therapeutic Targets

Therapies aimed at promoting remyelination and neural repair in MS represent one priority (Plemel et al., 2017), as does the identification of neuroprotective agents that can slow or prevent neurodegeneration (Correale et al., 2017). New approaches to modulating the immune system, such as regulatory T-cell therapies or targeted cytokine interventions, form a third line of development (Comi et al., 2017).

8.7 Artificial Intelligence and Machine Learning

Machine learning algorithms may be applied to develop more accurate predictive models for MS risk, progression, and treatment response (Zhao et al., 2017), and artificial intelligence is increasingly used in the analysis of MRI and other imaging data to improve MS diagnosis and monitoring (Yoo et al., 2018). AI and machine learning approaches may also accelerate the discovery of new therapeutic targets and compounds for MS treatment (Stokes et al., 2020).

8.8 Epigenetic Therapeutics

Therapies that can specifically target epigenetic modifications implicated in MS pathogenesis represent a promising direction (Vakhitov et al., 2020), as do combination therapies that address both genetic and epigenetic factors in MS (Küçükali et al., 2015). A further question concerns how individual genetic and environmental profiles may influence response to epigenetic therapies (Huynh & Casaccia, 2013).

9. Conclusion

Multiple sclerosis is a complex disease influenced by a multitude of genetic and environmental factors. The interaction between these factors contributes to the heterogeneity of MS in terms of disease onset, progression, and clinical

manifestations. Recent advances in genomics, epigenetics, and environmental exposure assessment have significantly enhanced our understanding of MS pathogenesis, but many questions remain unanswered.

The identification of numerous genetic risk factors, particularly within the HLA region and immune-related genes, has provided valuable insights into the underlying mechanisms of MS. However, genetic factors alone do not fully explain MS risk, highlighting the key role of environmental influences. Environmental factors such as EBV infection, vitamin D deficiency, smoking, and obesity have been consistently associated with MS risk, although the precise mechanisms by which they contribute to disease development remain to be fully elucidated.

The complex interactions between genetic and environmental factors in MS call for integrated research approaches. Large-scale, longitudinal studies incorporating genomic, epigenomic, and environmental data are essential to unravel the complex aetiology of MS. Such thorough studies may lead to the development of more accurate risk prediction models and personalised prevention strategies.

Advances in our understanding of MS pathogenesis have already begun to translate into clinical practice, with the development of new therapeutic approaches and personalised treatment strategies. However, significant obstacles persist in preventing MS onset and halting disease progression. Future research directions, including microbiome studies, precision medicine approaches, and novel therapeutic targets, may further improve MS management and outcomes.

Better definition of genetic and environmental risk may improve stratification, prevention research, and treatment selection, but these applications require prospective validation. Translation into routine care depends on reproducible associations, clinically useful effect sizes, and studies showing that risk-informed decisions improve outcomes.

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1.5. CURRENT TREATMENT METHODS FOR MULTIPLE SCLEROSIS AND THEIR LIMITATIONS: A COMPREHENSIVE ANALYSIS

1. Introduction

Multiple Sclerosis (MS) is a complex, chronic autoimmune disorder that affects the central nervous system (CNS), characterised by inflammation, demyelination, and neurodegeneration. It is one of the most common neurological disorders affecting young adults, with a global prevalence of approximately 3.1 million people (King et al., 2026). The disease typically occurs between the ages of 20 and 40, significantly impacting individuals during their most productive years. See Figure 1.5.1.

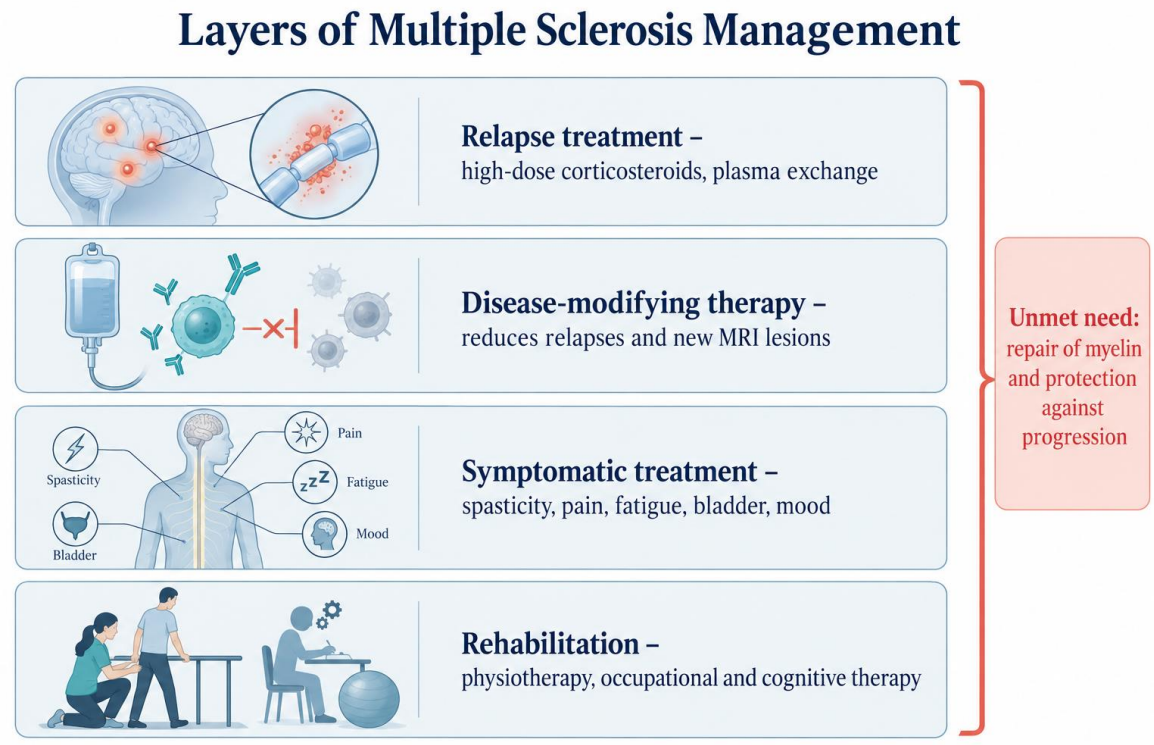


Figure 1.5.1. Layers of multiple sclerosis management and the remaining unmet need: repair of myelin and protection against progression.

The aetiology of MS involves an interaction between genetic susceptibility and environmental factors. Recent genome-wide association studies have identified over 200 genetic variants associated with MS risk, primarily involving genes related to immune function (International Multiple Sclerosis Genetics Consortium, 2019). Environmental factors such as vitamin D deficiency, Epstein-Barr virus infection, smoking, and obesity have also been implicated in MS risk (Ascherio & Munger, 2017).

The pathophysiology of MS is characterised by the infiltration of autoreactive lymphocytes into the CNS, leading to inflammation, demyelination, and ultimately axonal loss. This process results in the formation of sclerotic plaques in the white and gray matter of the brain and spinal cord, disrupting neural signalling and causing a wide array of neurological symptoms (Compston & Coles, 2008).

The clinical course of MS is highly variable, and several phenotypes are recognised. Relapsing-remitting MS (RRMS) is characterised by clearly defined attacks followed by partial or complete recovery. Secondary progressive MS (SPMS) begins as RRMS and is followed by gradual worsening, with or without relapses. Primary progressive MS (PPMS) involves gradual worsening of neurologic function from disease onset without early relapses, while progressive-relapsing MS (PRMS) described progressive disease from onset accompanied by clear acute relapses, with or without full recovery; since the 2013 revision of the phenotype descriptors, such cases are classified as primary progressive MS with activity.

Current treatment seeks to prevent relapses, limit disability accumulation, and manage symptoms. The following discussion distinguishes these goals, because success in

controlling inflammatory activity does not establish reversal of neurological damage.

2. Disease-Modifying Therapies (DMTs)

Disease-Modifying Therapies (DMTs) form the cornerstone of MS treatment, aiming to modulate the immune system in order to reduce relapse frequency, limit the accumulation of new lesions and slow disability accrual. They fall broadly into first-generation injectable agents (interferon beta and glatiramer acetate), second-generation agents with greater efficacy but more demanding monitoring (natalizumab, fingolimod), and later oral and monoclonal-antibody therapies (dimethyl fumarate, teriflunomide, ocrelizumab and related agents). Because none of these agents halts progression in all patients, and because each carries its own risk profile, their limitations provide the clinical rationale for the investigational cell- and vesicle-based approaches discussed in this book. A detailed, agent-by-agent account of mechanisms, decisive trials, adverse effects and practical limitations is given in Section 3.1, and the resulting safety and adherence problems are analysed in Section 3.3; the present section therefore does not repeat that material.

3. Symptomatic Treatments

While DMTs aim to modify the disease course, symptomatic treatment remains central to daily function and quality of life. Management is usually organized around the major symptom domains described below:

3.1. Spasticity Management

Spasticity affects up to 80% of MS patients and can significantly impair mobility and quality of life (Otero-Romero et al., 2016).

3.1.1. Baclofen

Baclofen is a GABA-B receptor agonist that can be administered orally or intrathecally. It is effective in moderate to severe spasticity, with up to 35% improvement in spasticity scores, but it may cause sedation, weakness, and cognitive impairment, especially at higher doses. Tolerance may develop with continued use, and abrupt discontinuation can lead to a withdrawal syndrome.

3.1.2. Tizanidine

Tizanidine is an α2-adrenergic agonist that is particularly effective in treating painful spasms, with efficacy similar to that of baclofen. Dry mouth, sedation, and hypotension are its principal side effects, but it may have less impact on muscle strength than baclofen.

3.1.3. Gabapentin

Gabapentin, a calcium channel modulator and GABA analogue, is moderately effective for spasticity and offers additional benefits for neuropathic pain. Sedation, dizziness, and peripheral edema are its main side effects.

3.1.4. Cannabinoids

Cannabinoids act through activation of cannabinoid receptors and are available as nabiximols (Sativex), an oromucosal spray. They produce a moderate reduction in spasticity, particularly in patients with an inadequate response to other treatments, although they are not approved in all countries and carry potential cognitive side effects.

3.1.5. Botulinum Toxin Injections

Botulinum toxin injections inhibit acetylcholine release at neuromuscular junctions and are highly effective in focal spasticity. Their use requires repeated injections, and antibody formation is a potential problem with repeated administration.

3.1.6. Physical Therapy and Rehabilitation

Physical therapy and rehabilitation form an important component of spasticity management. Regular stretching can help maintain muscle length and reduce spasticity, targeted strength training can improve muscle function and control, and hydrotherapy provides resistance while reducing the impact of gravity. Studies have shown improvements in both spasticity scores and functional outcomes with these approaches, although they require ongoing commitment from patients and their effects may be temporary without consistent practice.

3.2. Fatigue Management

Fatigue affects up to 90% of MS patients and is often described as one of the most disabling symptoms (Krupp, 2003).

3.2.1. Amantadine

Amantadine exerts dopaminergic and noradrenergic effects. In the randomised TRIUMPHANT-MS trial it did not outperform placebo for overall MS fatigue (Nourbakhsh et al., 2021). Its side effects include insomnia, anxiety, and, rarely, hallucinations, and its effect size is generally small, with not all patients responding.

3.2.2. Modafinil

Modafinil is a wakefulness-promoting agent whose exact mechanism remains unclear. Wakefulness and fatigue are distinct outcomes, and modafinil did not outperform placebo for overall MS fatigue in TRIUMPHANT-MS (Nourbakhsh et al., 2021). Headache, nausea, and anxiety are its common side effects, and results across clinical trials have been mixed, with some studies showing no significant benefit over placebo.

3.2.3. Methylphenidate

Methylphenidate is a stimulant that increases dopamine and norepinephrine. It did not outperform placebo for overall MS fatigue in TRIUMPHANT-MS, and adverse events were more frequent with the active drugs (Nourbakhsh et al., 2021). It may cause insomnia and anxiety and carries a potential for abuse; long-term data in MS are limited, and there are concerns about cardiovascular side effects.

3.2.4. Non-pharmacological Approaches

Non-pharmacological approaches are important in fatigue management. Cognitive behavioural therapy can address coping and energy-management strategies, regular aerobic exercise may improve fatigue and overall fitness, and cooling strategies can help patients with heat sensitivity. These approaches require active participation and should be tailored to individual tolerance and goals.

3.3. Cognitive Dysfunction Management

Cognitive impairment affects 40-70% of MS patients and can significantly impact daily functioning and quality of life (Chiaravalloti & DeLuca, 2008).

3.3.1. Acetylcholinesterase Inhibitors

Acetylcholinesterase inhibitors such as donepezil and rivastigmine increase acetylcholine levels in the brain. They may have positive effects on memory and attention, but results are mixed, and their side effects include nausea, diarrhea, and insomnia. Benefits are generally modest, and not all patients respond.

3.3.2. Memantine

Memantine, an NMDA receptor antagonist, has shown improvements in cognitive function in some studies, although results are inconsistent. Dizziness, headache, and confusion are its main side effects, and the evidence specifically in the MS population remains limited.

3.3.3. Cognitive Rehabilitation

Cognitive rehabilitation employs compensatory strategy training, cognitive exercises, and computer-assisted cognitive rehabilitation, and can improve specific cognitive domains and daily functioning. Typical strategies include the use of external memory aids such as calendars and smartphone reminders, organizational techniques for managing complex tasks, and attention training exercises. Its effects may not generalize to all cognitive domains, and it requires ongoing practice.

3.3.4. Computer-Based Cognitive Training

Computer-based cognitive training programs such as BrainHQ, Lumosity, and CogniFit have shown improvements in trained cognitive tasks in some studies. Whether these gains transfer to real-world functioning is debated, and benefits may not persist without continued training.

3.4. Depression and Anxiety Management

Depression affects up to 50% of MS patients, while anxiety disorders are present in up to 36% (Marrie et al., 2015).

3.4.1. Selective Serotonin Reuptake Inhibitors (SSRIs)

Selective serotonin reuptake inhibitors such as fluoxetine, sertraline, and escitalopram are effective in treating depression in MS, with response rates similar to those in the general population. Sexual dysfunction, gastrointestinal symptoms, and a potential increase in bleeding risk are the main side effects, and these agents may have beneficial effects on fatigue and cognitive function in some patients.

3.4.2. Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs)

Serotonin-norepinephrine reuptake inhibitors such as venlafaxine and duloxetine are effective for both depression and anxiety and may offer additional benefits for neuropathic pain. Nausea, dry mouth, and increased blood pressure are their principal side effects, and duloxetine may be particularly useful in patients with comorbid neuropathic pain.

3.4.3. Cognitive Behavioral Therapy (CBT)

Cognitive behavioural therapy focuses on identifying and changing negative thought patterns and behaviours, and numerous studies show its effectiveness in reducing depression and anxiety symptoms in MS. It carries no medication side effects and provides long-term coping skills, but

it requires active participation, and access to trained therapists may be limited.

3.4.4. Mindfulness-Based Interventions

Mindfulness-based interventions, including Mindfulness-Based Stress Reduction (MBSR) and Mindfulness-Based Cognitive Therapy (MBCT), have growing evidence for benefits in reducing depression, anxiety, and fatigue in MS. They can be delivered in group settings and promote overall well-being, although they require regular practice and may not be suitable for all patients.

4. Emerging and Experimental Therapies

While current treatments have significantly improved outcomes for many MS patients, there remains a need for more effective therapies, particularly for progressive forms of the disease. Several areas of research are currently being pursued.

4.1. Remyelination Strategies

4.1.1. Anti-LINGO-1 Antibodies

LINGO-1 is a protein that inhibits oligodendrocyte precursor cell maturation and myelin production, and opicinumab is the anti-LINGO-1 antibody studied in this context. Phase II trials have been completed and showed some improvement in optic nerve conduction velocity after optic neuritis, but the agent failed to meet primary endpoints in MS trials. Further research is needed to identify optimal patient populations and dosing strategies.

4.1.2. Clemastine Fumarate

Clemastine fumarate is an antimuscarinic compound that promotes oligodendrocyte differentiation. Phase II trials have been completed and showed modest improvement in optic nerve conduction velocity in chronic optic neuropathy. As a repurposed drug it has a long-established general safety profile, although a disability-worsening signal was later reported in a small cohort with progressive MS, and optimal dosing and long-term effects are still to be determined.

4.1.3. Biotin (MD1003)

High-dose biotin (MD1003) may enhance myelin production and increase energy production in demyelinated axons. Clinical trial results have been mixed: initial studies showed some promise in progressive MS, but the compound failed to meet primary endpoints in a larger phase III trial. Further research is needed to understand which subgroups might benefit.

4.2. Neuroprotective Strategies

4.2.1. Ibudilast

Ibudilast is a phosphodiesterase inhibitor with anti-inflammatory and neuroprotective properties. Phase II trials have been completed and showed potential to slow brain atrophy in progressive MS, although its side effect profile, which includes headache and gastrointestinal symptoms, may limit its use.

4.2.2. Simvastatin

Simvastatin is an HMG-CoA reductase inhibitor with potential neuroprotective and anti-inflammatory effects. Phase II results suggested slower brain atrophy, but the subsequent phase III trial in secondary progressive MS (MS-STAT2, published in 2025) did not show a slowing of disability progression. Its well-known safety profile and low cost had made it an attractive candidate for repurposing.

4.2.3. Oxidative Stress Reduction

Strategies aimed at reducing oxidative stress, including lipoic acid and epigallocatechin-3-gallate (green tea extract), rely on antioxidant properties that may help reduce neurodegeneration. These agents remain in early-phase clinical trials, and a central challenge is determining optimal dosing and delivery methods to achieve effects within the CNS.

4.3. Cell-Based Therapies

4.3.1. Mesenchymal Stem Cell Transplantation

Mesenchymal stem cell transplantation is being investigated for its potential immunomodulatory and neuroprotective effects. Multiple phase I/II trials have been completed or are ongoing, and some studies show promising preliminary effects on inflammation and disability. Determining the optimal cell source, dosing, and administration route remains a key challenge.

4.3.2. Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation aims to “reset” the immune system through high-dose chemotherapy followed by stem cell rescue. Multiple studies have been completed and some are ongoing, with early findings in highly active relapsing MS and some patients achieving long-term remission. The procedure is associated with significant risks, which makes optimal patient selection important.

4.4. Targeting B Cells

4.4.1. Ofatumumab

Ofatumumab is a fully human anti-CD20 monoclonal antibody that has been approved for relapsing forms of MS. Its subcutaneous administration is a notable advantage, since it potentially allows for self-administration at home.

4.4.2. Ublituximab

Ublituximab is a glycoengineered anti-CD20 monoclonal antibody that was approved by the US FDA in 2022 for relapsing forms of MS after the ULTIMATE I and II phase III trials. Its glycoengineering enhances antibody-dependent cellular cytotoxicity and permits a shorter infusion time.

4.5. Biomarkers and Personalized Medicine

4.5.1. Serum Neurofilament Light Chain (sNfL)

Serum neurofilament light chain (sNfL) is being evaluated in multiple studies as a biomarker for axonal damage and treatment response. Its potential impact lies in allowing more personalised treatment decisions and monitoring.

4.5.2. MRI-Based Biomarkers

MRI-based biomarkers, including brain atrophy measures and novel imaging techniques such as the magnetization transfer ratio, could provide more sensitive measures of disease progression and treatment response. Standardisation across centres and integration into clinical practice remain the principal challenges.

5. Challenges and Future Directions

Despite significant advances in MS treatment, several difficulties remain:

5.1. Progressive MS

Current DMTs have limited efficacy in progressive forms of MS, and there is a clear need for therapies that target neurodegeneration and promote repair.

5.2. Personalized Medicine

Strategies are needed to match individual patients with optimal treatments, which will require integrating genetic, imaging, and biomarker data into treatment decisions.

5.3. Long-term Safety and Efficacy

Long-term outcomes of newer therapies must continue to be monitored, and efficacy must be balanced against safety concerns, especially regarding opportunistic infections and malignancy risks.

5.4. Combination Therapies

The potential synergistic effects of combining different treatment modalities remain to be explored, and designing and interpreting combination therapy trials presents its own challenges.

5.5. Improving Quality of Life

More effective symptomatic treatments are needed, together with the integration of multidisciplinary care approaches that include rehabilitation and psychological support.

5.6. Access and Cost

Ensuring access to high-cost therapies across different healthcare systems remains a pressing concern, as does the development of cost-effective treatment strategies.

6. Conclusion

The range of MS treatments has evolved dramatically over the past three decades, with numerous therapeutic options now available. While these treatments have significantly improved outcomes for many patients, especially those with relapsing forms of MS, important questions remain open. The complex and heterogeneous nature of MS necessitates a varied approach to treatment, combining disease-modifying therapies with symptomatic management and comprehensive care.

Future directions in MS research and treatment will likely focus on developing more effective therapies for progressive MS, refining personalised medicine approaches, exploring combination therapies, and improving long-term outcomes and quality of life for all individuals living with MS. A deeper grasp of MS pathophysiology should, in time, yield treatments that are both more effective and better targeted.

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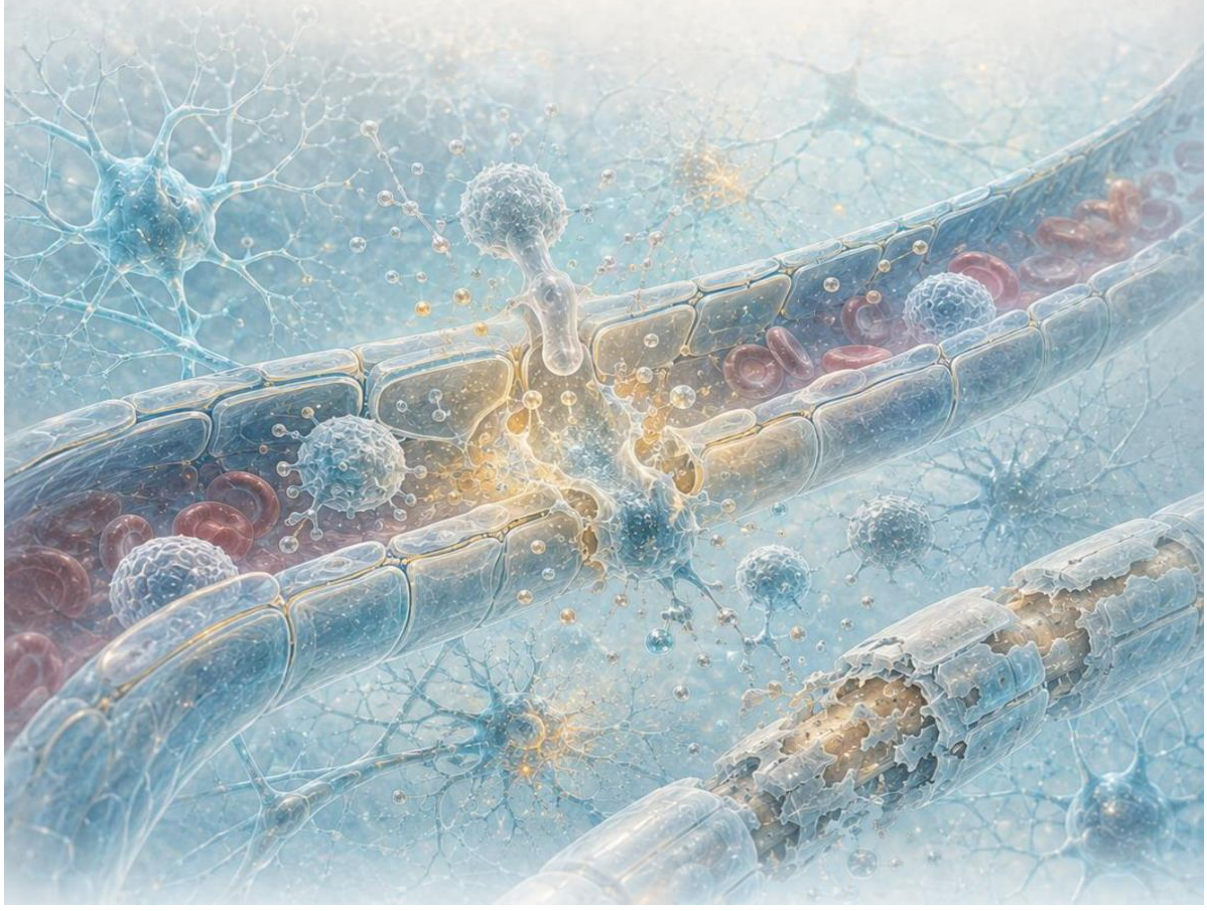
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CHAPTER 2

PATHOPYSIOLOGY OF MULTIPLE SCLEROSIS



2.1. IMMUNE SYSTEM DYSFUNCTION IN MS

Immune dysfunction in MS involves interactions between peripheral immune cells and the CNS (Fathallah et al., 2023; Oukka & Bettelli, 2018). These processes contribute to demyelination, axonal injury, and neuronal loss (Ma et al., 2023), with consequences for daily function and quality of life (Browne et al., 2014). Figure 2.1.1 follows the sequence from peripheral activation to compartmentalised CNS inflammation.

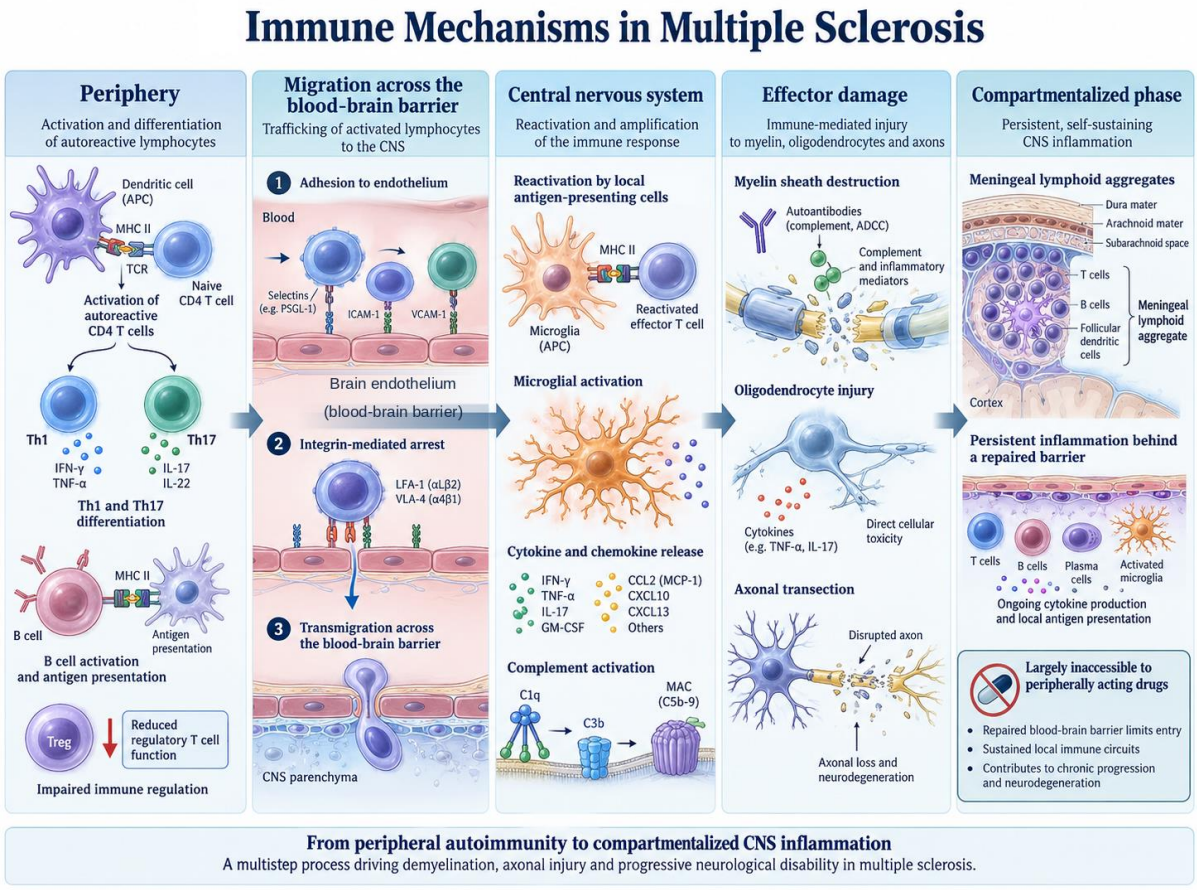


Figure 2.1.1. Immune mechanisms in multiple sclerosis, from peripheral activation of autoreactive lymphocytes to compartmentalised inflammation behind a repaired blood-brain barrier.

1. Immune System Dysregulation in MS

1.1. Overview of Immune System Involvement

At the core of MS pathogenesis lies a profound dysfunction of the immune system. In healthy individuals, the immune system serves as a protective mechanism against pathogens and foreign entities. However, in MS, this system erroneously targets components of the CNS, particularly the myelin sheath surrounding nerve fibres (Awad & Stüve, 2010). This autoimmune attack initiates a complex series of events that culminate in the characteristic pathological features of MS. See Figure 2.1.2.

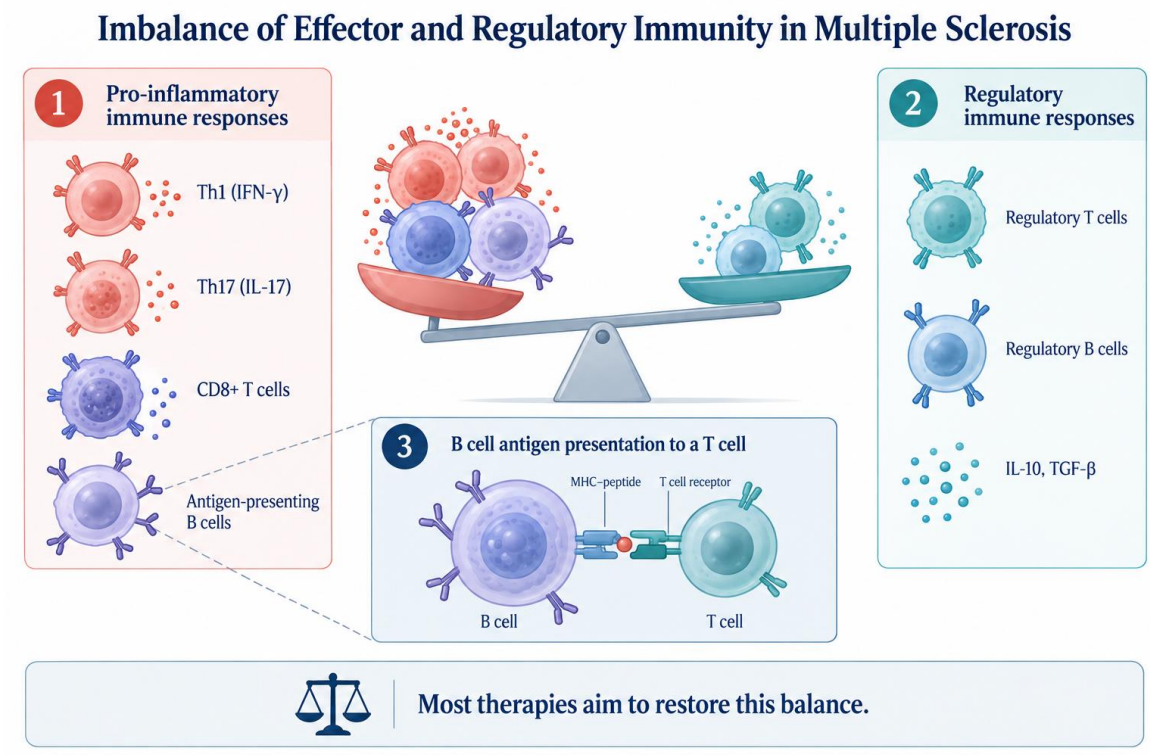


Figure 2.1.2. Imbalance between effector and regulatory immunity in multiple sclerosis, including antigen presentation by B cells to T cells.

The immune-mediated inflammation in MS involves various cellular components of both the innate and adaptive immune systems. The interaction between these immune cells and CNS resident cells creates a hostile environment that promotes ongoing tissue damage and impairs repair mechanisms (Dendrou et al., 2015).

1.2. T Cell-Mediated Pathology

T cells, particularly CD4+ and CD8+ T cells, contribute substantially to orchestrating the inflammatory response in MS (Fletcher et al., 2010). These autoreactive T cells, which have escaped central tolerance mechanisms in the thymus, become activated in the periphery and subsequently infiltrate the CNS by crossing the blood-brain barrier (BBB) (Dendrou et al., 2015).

1.2.1 CD4+ T Cells

CD4+ T helper cells are key players in MS pathogenesis, and several subsets have been implicated in the disease process. Th1 cells produce pro-inflammatory cytokines such as interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α), which contribute to the activation of macrophages and microglia and thereby to further inflammation and tissue damage (Korn et al., 2009). Th17 cells, characterised by their production of interleukin-17 (IL-17), have been found to be particularly pathogenic in MS, since IL-17 promotes BBB disruption, recruits neutrophils, and enhances the pro-inflammatory functions of other immune cells (Kebir et al., 2007). Th9 cells, a more recently described subset, produce IL-9 and have been shown to enhance the function of other pathogenic T cell subsets in MS (Elyaman et al., 2009), while T follicular helper (Tfh) cells support B cell function and contribute to the formation of ectopic lymphoid structures in the meninges of MS patients, promoting sustained inflammation (Mitsdoerffer & Peters, 2016).

1.2.2 CD8+ T Cells

While less studied than their CD4+ counterparts, CD8+ T cells are abundant in MS lesions and can directly target neurons and oligodendrocytes expressing MHC class I molecules. These cells contribute to tissue damage through cytotoxic mechanisms and the release of pro-inflammatory mediators (Friese & Fugger, 2009).

1.2.3 Regulatory T Cells (Tregs)

Regulatory T cells are central to maintaining immune homeostasis and preventing autoimmunity. In MS, both the frequency and function of Tregs have been reported to be impaired, contributing to the loss of immune tolerance to CNS antigens (Viglietta et al., 2004).

1.3. B Cell Involvement

B cells, another key component of the adaptive immune system, are closely involved in MS pathogenesis. These cells contribute to the disease process through various mechanisms:

1.3.1 Antibody Production

B cells produce autoantibodies that can target myelin and other CNS components. The presence of oligoclonal bands in the cerebrospinal fluid of MS patients, which are indicative of intrathecal antibody production, indicates the involvement of B cells in the disease (Link & Huang, 2006). These autoantibodies can contribute to tissue damage through complement activation and antibody-dependent cell-mediated cytotoxicity.

1.3.2 Antigen Presentation

B cells serve as efficient antigen-presenting cells (APCs), playing an essential role in the activation and reactivation of pathogenic T cells. This function is particularly significant in the later stages of multiple sclerosis (MS) and in secondary progressive MS. Fraussen et al. (2014) reviewed evidence that the humoral autoimmune response in MS is not limited to myelin but also targets various central nervous system cells, including neurons, oligodendrocytes, and astrocytes, reflecting the complexity and heterogeneity of MS pathogenesis.

The antigen presentation process begins when B cells internalize antigens through their B cell receptors (BCRs). These internalized antigens are processed and presented on the B cell surface bound to major histocompatibility complex (MHC) molecules. This presentation is essential for the activation of T cells, as it provides the necessary signals for T cell receptor (TCR) engagement.□ □In MS, B cells have been shown to express higher levels of costimulatory molecules such as CD80 and CD86, which are central for effective T cell activation.

Furthermore, B cells can influence T cell polarisation by secreting cytokines that modulate the immune response. For instance, B cells can produce pro-inflammatory cytokines like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), which promote the differentiation of T helper 1 (Th1) and T helper 17 (Th17) cells, both implicated in MS pathogenesis. Conversely, regulatory B cells (Bregs) can secrete interleukin-10 (IL-10), a cytokine with anti-inflammatory properties, highlighting the dual role of B cells in modulating immune responses.

The involvement of B cells in antigen presentation and their interaction with T cells suggest that targeting B cell functions could be a therapeutic strategy in MS. Indeed, treatments such as rituximab, a monoclonal antibody that depletes CD20+ B cells, have shown efficacy in reducing disease activity in MS patients, further emphasizing the significance of B cells in the disease's immunopathology.

1.3.3 Cytokine Production

B cells produce both pro-inflammatory (e.g., TNF-α, IL-6) and anti-inflammatory (e.g., IL-10) cytokines, influencing the overall inflammatory milieu in MS (Li et al., 2015).

1.3.4 Formation of Ectopic Lymphoid Structures

In some MS patients, B cells contribute to the formation of ectopic lymphoid follicles in the meninges. These structures serve as sites of sustained inflammation and have been associated with more severe cortical pathology and accelerated disease progression (Magliozzi et al., 2007).

1.4. Innate Immune System Involvement

The innate immune system, particularly microglia and macrophages, matters for amplifying and sustaining the inflammatory response in MS.

1.4.1 Microglia

As the resident immune cells of the CNS, microglia are key players in MS pathology. Upon activation they release pro-inflammatory mediators, including cytokines, chemokines, and reactive oxygen species, present antigens to T cells and thereby perpetuate the adaptive immune response, and phagocytose myelin debris, an action that can have both beneficial and detrimental effects (Voet et al., 2019).

The dual role of microglia in both injury and repair mechanisms highlights the complex nature of immune responses in MS (Galloway et al., 2022).

1.4.2 Macrophages

Infiltrating macrophages derived from blood monocytes contribute significantly to tissue damage in MS. These cells produce pro-inflammatory cytokines and reactive oxygen species, phagocytose myelin and thereby contribute to demyelination, and present antigens to T cells, further activating the adaptive immune response (Boven et al., 2006).

1.4.3 Dendritic Cells

Dendritic cells (DCs) drive the initiation and shaping of the adaptive immune response in MS. They present

antigens to naive T cells and promote their differentiation into pathogenic effector cells, produce cytokines that influence the type of T cell response generated, and migrate across the BBB, facilitating the entry of other immune cells into the CNS (Ifergan et al., 2008).

1.4.4 Natural Killer Cells

The role of natural killer (NK) cells in MS is complex and not fully understood. Some studies suggest a protective role for certain NK cell subsets, while others implicate NK cells in promoting inflammation and tissue damage (Gross et al., 2016).

2. Demyelination and Axonal Damage

2.1. Mechanisms of Demyelination

The immune-mediated attack on the myelin sheath results in widespread demyelination throughout the CNS. This process not only disrupts the saltatory conduction of nerve impulses but also leaves axons vulnerable to further damage (Franklin & Ffrench-Constant, 2008). Several mechanisms contribute to demyelination in MS:

2.1.1 Autoantibody-Mediated Demyelination

Autoantibodies targeting myelin components can lead to demyelination through two principal routes: complement activation, which results in the formation of the membrane attack complex and lysis of oligodendrocytes, and antibody-dependent cell-mediated cytotoxicity, in which antibody-coated oligodendrocytes are targeted by immune cells (Lucchinetti et al., 2000).

2.1.2 T Cell-Mediated Demyelination

T cells contribute to demyelination through direct cytotoxic effects on oligodendrocytes, particularly in the case of CD8+ T cells, through the release of pro-inflammatory cytokines that activate other immune cells and directly damage oligodendrocytes, and through the recruitment of other immune cells, such as macrophages, that phagocytose myelin (Sospedra & Martin, 2005).

2.1.3 Macrophage and Microglia-Mediated Demyelination

Activated macrophages and microglia contribute to demyelination by phagocytosing myelin and by producing pro-inflammatory cytokines and reactive oxygen species that damage oligodendrocytes. Hendrickx et al. (2014) demonstrated that myelin isolated from the normal-appearing white matter of MS patients is phagocytosed more efficiently by THP-1 macrophages and primary human microglia compared to control myelin, which suggests that intrinsic alterations in MS myelin may prime phagocytic cells, accelerating demyelination even in seemingly unaffected areas (Hendrickx et al., 2014).

2.2. Axonal Damage and Neurodegeneration

While demyelination is a hallmark of MS, axonal damage and neurodegeneration are increasingly recognised as major contributors to permanent neurological disability in MS patients (Trapp & Nave, 2008). Several factors contribute to axonal injury:

2.2.1 Loss of Trophic Support

Demyelination results in the loss of trophic support normally provided by oligodendrocytes to axons. This can lead to axonal degeneration over time (Nave, 2010).

2.2.2 Energy Failure

Demyelinated axons require increased energy to maintain ion homeostasis and conduct action potentials. This increased energy demand, coupled with mitochondrial dysfunction, can lead to energy failure and subsequent axonal degeneration (Witte et al., 2014).

2.2.3 Glutamate Excitotoxicity

Elevated levels of glutamate in MS lesions can lead to excitotoxicity, causing axonal injury and neuronal death (Pitt et al., 2000).

2.2.4 Direct Immune-Mediated Damage

Immune cells, particularly CD8+ T cells and macrophages, can directly target and damage axons through the release of cytotoxic molecules and proteases (Neumann et al., 2002).

2.3. Grey Matter Pathology

Recent research has highlighted the significance of grey matter pathology in MS, challenging the traditional view of MS as primarily a white matter disease. Cortical demyelination and neuronal loss in grey matter regions contribute significantly to cognitive impairment and disease progression in MS patients (Geurts et al., 2012). Grey matter pathology in MS includes:

2.3.1 Cortical Lesions

These can be classified into different types based on their location and extent, with some being entirely intracortical and others extending from the white matter or meninges (Bø et al., 2003).

2.3.2 Neuronal Loss

Significant neuronal loss has been observed in both cortical and deep grey matter structures in MS patients, contributing to brain atrophy and cognitive decline (Magliozzi et al., 2010).

2.3.3 Synaptic Dysfunction

Alterations in synaptic density and function have been reported in MS, potentially contributing to cognitive impairment and other neurological deficits (Mandolesi et al., 2015).

3. Blood-Brain Barrier Disruption

The integrity of the blood-brain barrier (BBB) is central to maintaining CNS homeostasis and protecting it from potentially harmful substances and cells. In MS, however, the BBB becomes compromised, allowing for increased infiltration of immune cells into the CNS (Minagar & Alexander, 2003). This breakdown of the BBB is an early event in MS pathogenesis and contributes significantly to the initiation and propagation of the inflammatory response.

3.1. Mechanisms of BBB Disruption

Several factors contribute to BBB disruption in MS:

3.1.1 Pro-inflammatory Cytokines

Cytokines such as TNF-α, IL-1β, and IFN-γ can directly affect tight junction proteins and increase BBB permeability (Ortiz et al., 2014).

3.1.2 Matrix Metalloproteinases (MMPs)

MMPs, produced by both immune cells and CNS resident cells, can degrade components of the BBB, facilitating immune cell infiltration (Rosenberg, 2002).

3.1.3 Reactive Oxygen Species (ROS)

Oxidative stress can damage BBB endothelial cells and disrupt tight junctions, increasing BBB permeability (van Horssen et al., 2011).

3.1.4 Altered Expression of Adhesion Molecules

Upregulation of adhesion molecules on endothelial cells facilitates the adhesion and transmigration of immune cells across the BBB (Engelhardt & Ransohoff, 2012).

3.2. Consequences of BBB Disruption

BBB breakdown in MS has several important consequences:

3.2.1 Increased Immune Cell Infiltration

The compromised BBB allows for enhanced entry of peripheral immune cells into the CNS, exacerbating inflammation and tissue damage.

3.2.2 Edema Formation

Increased BBB permeability can lead to vasogenic edema, contributing to tissue swelling and neurological deficits.

3.2.3 Entry of Potentially Harmful Molecules

The disrupted BBB may allow the entry of serum proteins and other potentially harmful molecules into the CNS, further contributing to tissue damage (Knowland et al., 2014).

4. Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress is essential to the pathophysiology of MS, contributing to both inflammation and neurodegeneration. The excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by activated immune cells leads to oxidative damage of lipids, proteins, and nucleic acids within the CNS (Haider et al., 2011).

4.1. Sources of Oxidative Stress in MS

4.1.1 Activated Immune Cells

Macrophages, microglia, and other immune cells produce large amounts of ROS and RNS as part of their inflammatory response (Fischer et al., 2012).

4.1.2 Mitochondrial Dysfunction

Dysfunctional mitochondria can become a significant source of ROS, creating a vicious cycle of oxidative damage and energy failure (Witte et al., 2014).

4.1.3 Iron Accumulation

Increased iron deposition in MS lesions can promote the formation of highly reactive hydroxyl radicals through the Fenton reaction (Hametner et al., 2013).

4.2. Consequences of Oxidative Stress

4.2.1 Lipid Peroxidation

Oxidative damage to lipids in myelin and cell membranes can contribute to demyelination and cellular dysfunction (Gonsette, 2008).

4.2.2 Protein Oxidation

Oxidative modification of proteins can lead to loss of function and accumulation of damaged proteins, contributing to cellular stress and death (Griffiths et al., 2017).

4.2.3 DNA Damage

Oxidative damage to DNA can lead to mutations and cellular dysfunction, potentially contributing to neurodegeneration (Vladimirova et al., 1998).

4.3. Mitochondrial Dysfunction in MS

Mitochondrial dysfunction has been investigated as a critical factor in MS pathogenesis, particularly in the context of axonal degeneration and neuronal loss (Witte et al., 2014). Several aspects of mitochondrial dysfunction in MS have been identified:

4.3.1 Impaired Energy Production

Damage to mitochondrial DNA and proteins can lead to decreased ATP production, compromising cellular energy metabolism (Campbell et al., 2011).

4.3.2 Altered Mitochondrial Dynamics

Changes in mitochondrial fusion, fission, and transport have been observed in MS, potentially contributing to energy deficits in axons and neurons (Witte et al., 2014).

4.3.3 Mitochondrial DNA Damage

Increased levels of mitochondrial DNA deletions have been found in neurons within MS lesions, potentially contributing to respiratory chain deficiency and energy failure (Campbell et al., 2011)

4.3.4 Calcium Dysregulation

Mitochondrial dysfunction can lead to impaired calcium buffering, contributing to excitotoxicity and cellular stress in MS (Su et al., 2009). This dysregulation can trigger apoptotic pathways and exacerbate neuronal damage.

4.4. Therapeutic Implications of Oxidative Stress and Mitochondrial Dysfunction

Understanding the role of oxidative stress and mitochondrial dysfunction in MS has led to the exploration of novel therapeutic approaches:

4.4.1 Antioxidant Therapies

Various antioxidant compounds, such as alpha-lipoic acid and N-acetylcysteine, have shown promise in preclinical studies and are being investigated for their potential neuroprotective effects in MS (Carvalho et al., 2014).

4.4.2 Mitochondrial-Targeted Therapies

Compounds that specifically target mitochondria, such as MitoQ and SS-31, are being studied for their potential to improve mitochondrial function and reduce oxidative stress in MS (Witte et al., 2014).

5. Remyelination and Repair Mechanisms

Despite the extensive damage caused by the immune attack, the CNS possesses some capacity for repair and regeneration. Remyelination, the process by which new myelin sheaths are formed around demyelinated axons, is an important aspect of this repair mechanism (Franklin & Ffrench-Constant, 2017).

5.1. Oligodendrocyte Precursor Cells (OPCs)

OPCs contribute substantially to remyelination:

5.1.1 Recruitment

Following demyelination, OPCs are recruited to the site of injury through the action of chemokines and growth factors (Kotter et al., 2011).

5.1.2 Proliferation

Once at the lesion site, OPCs proliferate in response to various mitogenic factors, including PDGF and FGF-2 (Murtie et al., 2005).

5.1.3 Differentiation

OPCs differentiate into mature oligodendrocytes capable of producing new myelin sheaths. This process is regulated by an interaction of transcription factors and signalling pathways (Fancy et al., 2011).

5.2. Factors Influencing Remyelination Efficiency

The efficiency of remyelination often declines as the disease progresses, contributing to the accumulation of neurological deficits over time (Chamberlain et al., 2016). Several factors influence remyelination efficiency:

5.2.1 Age

Remyelination efficiency decreases with age, possibly due to changes in OPC function and the CNS microenvironment (Sim et al., 2002).

5.2.2 Chronic Inflammation

Persistent inflammation in MS lesions can create an environment that is inhibitory to remyelination (Lampron et al., 2015).

5.2.3 Axonal Health

The health and integrity of demyelinated axons influence their ability to be remyelinated (Franklin & Ffrench-Constant, 2017).

5.2.4 Presence of Inhibitory Factors

Various molecules, including chondroitin sulfate proteoglycans and myelin debris, can inhibit OPC differentiation and remyelination (Lau et al., 2013).

5.3. Promoting Remyelination as a Therapeutic Strategy

Enhancing remyelination has developed into an investigational therapeutic approach in MS:

5.3.1 Clemastine

This antihistamine has been shown to promote OPC differentiation and enhance remyelination in preclinical models and a small clinical trial (Green et al., 2017).

5.3.2 Anti-LINGO-1 Antibodies

LINGO-1 is a negative regulator of OPC differentiation. Antibodies targeting LINGO-1 have shown promise in promoting remyelination in animal models (Mi et al., 2013), but the anti-LINGO-1 antibody opicinumab did not meet its primary endpoints in phase II clinical trials.

5.3.3 RXR Agonists

Retinoid X receptor (RXR) agonists have been shown to enhance OPC differentiation and remyelination in preclinical studies (Huang et al., 2011).

6. Genetic and Environmental Factors

While the exact aetiology of MS remains elusive, it is widely accepted that both genetic and environmental factors contribute to disease susceptibility and progression.

6.1. Genetic Factors

6.1.1 HLA Associations

The strongest genetic association with MS is with the HLA-DRB1*15:01 allele, which confers a three-fold increased risk of developing the disease (International Multiple Sclerosis Genetics Consortium, 2019).

6.1.2 Non-HLA Genes

Genome-wide association studies have identified over 200 genetic variants associated with MS risk, many of which are related to immune function (International Multiple Sclerosis Genetics Consortium, 2019).

6.1.3 Epigenetic Factors

Epigenetic modifications, such as DNA methylation and histone modifications, have been implicated in MS pathogenesis and may mediate gene-environment interactions (Koch et al., 2013).

6.2. Environmental Factors

Several environmental factors have been associated with MS risk and progression:

6.2.1 Vitamin D Deficiency

Low levels of vitamin D have been associated with increased MS risk and disease activity (Sintzel et al., 2018).

6.2.2 Epstein-Barr Virus (EBV) Infection

EBV infection, particularly if occurring later in life, is associated with an increased risk of developing MS (Ascherio & Munger, 2007).

6.2.3 Smoking

Cigarette smoking has been linked to an increased risk of developing MS and more rapid disease progression (Hedström et al., 2009).

6.2.4 Obesity

Childhood and adolescent obesity have been associated with an increased risk of developing MS (Munger et al., 2013).

6.3. Gene-Environment Interactions

The interaction between genetic and environmental factors highlights the heterogeneous nature of MS etiology:

6.3.1 HLA-Vitamin D Interaction

Some studies have suggested that vitamin D may interact with HLA-DRB1*15:01 to influence MS risk (Ramagopalan et al., 2009).

6.3.2 Smoking-Genetic Interactions

Smoking has been shown to interact with certain genetic variants to increase MS risk beyond the effect of either factor alone (Hedström et al., 2011).

7. Emerging Concepts in MS Pathophysiology

Recent research has clarified several aspects of MS pathophysiology:

7.1. Gut Microbiome

The gut microbiome has come to be seen as a potential factor in MS pathogenesis:

7.1.1 Altered Microbiome Composition

Studies have shown differences in gut microbiome composition between MS patients and healthy controls (Jangi et al., 2016).

7.1.2 Microbiome-Immune Interactions

The gut microbiome can influence both innate and adaptive immune responses, potentially modulating MS disease activity (Cekanaviciute et al., 2017).

7.2. Vascular Factors

Vascular abnormalities have been proposed to play a role in MS pathogenesis:

7.2.1 Chronic Cerebrospinal Venous Insufficiency (CCSVI)

While controversial, some researchers have suggested that impaired venous drainage from the CNS may contribute to MS pathology (Zamboni et al., 2009).

7.2.2 Vascular Comorbidities

Cardiovascular risk factors and comorbidities have been associated with increased MS progression and worse outcomes (Marrie et al., 2010).

7.3. Glymphatic System

The recently discovered glymphatic system, which facilitates waste clearance from the CNS, may be impaired in MS, potentially contributing to the accumulation of toxic substances and neurodegeneration (Louveau et al., 2017).

8. Therapeutic Implications and Future Directions

Understanding the intricate pathophysiology of MS has provided a basis for the development of various disease-modifying therapies (DMTs) and has highlighted potential avenues for future therapeutic interventions.

8.1 Current Therapeutic Approaches

8.1.1 Immunomodulatory Therapies

These therapies aim to modulate the immune response to reduce inflammation and prevent relapses. Examples include interferon-beta, glatiramer acetate, and dimethyl fumarate (Tintore et al., 2019).

8.1.2 Immunosuppressive Therapies

More potent immunosuppressive drugs, such as fingolimod, natalizumab, and alemtuzumab, target specific aspects of the immune response to reduce disease activity (Derfuss et al., 2020).

8.1.3 B Cell-Targeted Therapies

The success of B cell-depleting therapies, such as rituximab and ocrelizumab, has highlighted the important role of B cells in MS pathogenesis (Hauser et al., 2017).

8.2 Emerging Therapeutic Strategies

8.2.1 Neuroprotective Approaches

Therapies aimed at protecting neurons and axons from damage are being explored, including antioxidants, ion channel blockers, and compounds targeting mitochondrial function (Kawachi & Lassmann, 2017).

8.2.2 Remyelination-Promoting Therapies

As discussed earlier, various approaches to enhance remyelination are under investigation, including clemastine, anti-LINGO-1 antibodies, and RXR agonists (Plemel et al., 2017).

8.2.3 Cell-Based Therapies

Stem cell transplantation, including haematopoietic stem cell transplantation and mesenchymal stem cell therapy, is being explored for its potential to reset the immune system and promote repair (Scolding et al., 2017).

8.2.4 Combination Therapies

Given the complex nature of MS pathophysiology, combination therapies targeting multiple aspects of the disease process may prove more effective than single-agent approaches (Ontaneda et al., 2017).

8.3 Personalized Medicine in MS

Advances in our understanding of MS pathophysiology are supporting the development of more personalised treatment approaches:

8.3.1 Biomarker Development

Ongoing research is focused on identifying biomarkers that can predict disease course, treatment response, and prognosis, allowing for more tailored therapeutic strategies (Housley et al., 2015).

8.3.2 Pharmacogenomics

The study of genetic variations influencing drug response may help in selecting the most appropriate therapy for individual patients (Grossman et al., 2017).

8.3.3 Advanced Imaging Techniques

Novel imaging modalities, such as advanced MRI techniques and PET imaging, are being developed to better monitor disease activity and treatment response (Filippi et

al., 2019). Selecting appropriate microglial markers is critical for pathological assessments. Hendrickx et al. (2017) emphasized that HLA-DR and CD68 are superior for tracking immune activation, while Iba1 is more suited for structural analyses, guiding biomarker selection in MS research (Hendrickx et al., 2017).

9. Conclusion

The pathophysiology of multiple sclerosis is an interaction of immune dysregulation, demyelination, axonal damage, oxidative stress, and impaired repair mechanisms within the central nervous system. The intricate involvement of various immune cell types, coupled with the breakdown of the blood-brain barrier, mitochondrial dysfunction, and the influence of genetic and environmental factors, creates a challenging environment for effective treatment.

Therapeutic strategies aimed at halting progression and promoting repair have developed alongside this understanding of pathogenesis. Future research directions should focus on integrating our knowledge of immune dysfunction with emerging insights into neurodegenerative processes and repair mechanisms. This holistic approach will be important in developing multidisciplinary treatment strategies that not only modulate the immune response but also protect neurons, promote regeneration, and address the heterogeneity of the disease.

MS research continues to refine understanding of disease mechanisms and therapeutic targets. Progress in basic science, clinical investigation, imaging, biomarkers, and computational methods may support more effective treatment strategies, but translation requires reproducible evidence from appropriately designed studies. Continued integration of mechanistic and clinical research is therefore important for improving outcomes for people with multiple sclerosis.

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2.2. MECHANISMS OF DEMYELINATION AND AXONAL DAMAGE IN MULTIPLE SCLEROSIS

1. Introduction

Multiple sclerosis (MS) is a complex, chronic autoimmune disorder characterised by inflammation, demyelination, and neurodegeneration within the central nervous system (CNS) (Fathallah et al., 2023). The pathophysiology of MS involves intricate interactions between the immune system and the CNS, resulting in a cascade of events that ultimately lead to myelin destruction, axonal damage, and neuronal loss (Ma et al., 2023). This thorough review aims to elucidate the complex mechanisms underlying demyelination and axonal damage in MS, highlighting the interaction between immune-mediated processes and neurodegenerative changes. The stages of this process, from acute demyelination to irreversible axonal loss, are shown in Figure 2.2.1.

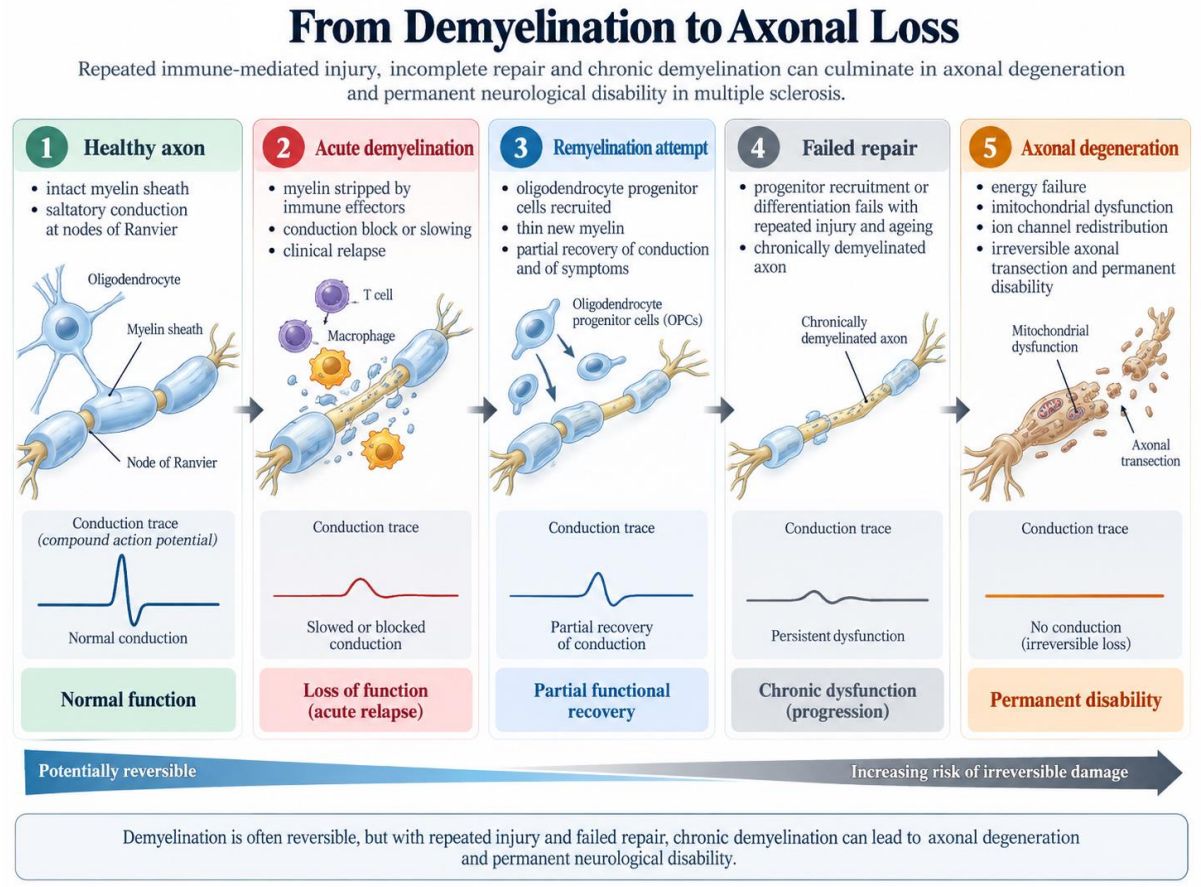


Figure 2.2.1. From demyelination to axonal loss. Conduction traces are schematic and illustrate the functional consequence of each stage rather than measured values.

2. Immune-Mediated Demyelination

2.1. T Cell-Mediated Mechanisms

The immune-mediated attack on myelin in MS is primarily orchestrated by autoreactive T cells that have escaped central tolerance mechanisms (Fletcher et al., 2010). These T cells, particularly CD4+ and CD8+ subsets, play key roles in initiating and perpetuating the inflammatory response in MS lesions (Sellebjerg & Sørensen, 2015). See Figure 2.2.2.

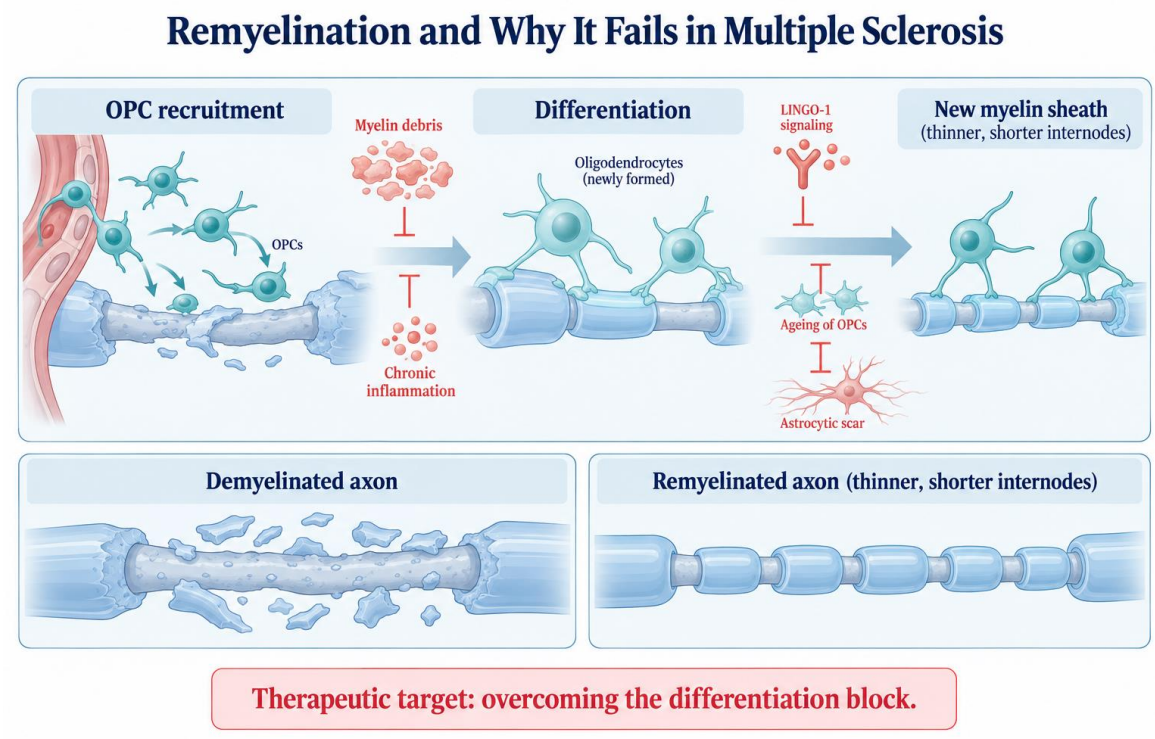


Figure 2.2.2. Remyelination and the factors that limit it in multiple sclerosis. Newly formed myelin is thinner and has shorter internodes than the original sheath.

2.1.1 Th1 Cells

Th1 cells produce pro-inflammatory cytokines such as interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α), which activate macrophages and microglia, leading to further inflammation and tissue damage (Korn et al., 2009). The production of IFN-γ by Th1 cells has been shown to enhance the expression of MHC class II molecules on antigen-presenting cells, further amplifying the immune response (Wijdeven et al., 2018).

Recent studies have also implicated Th1 cells in the disruption of the blood-brain barrier (BBB) through the production of matrix metalloproteinases (MMPs), particularly MMP-9 (Mirshafiey et al., 2014). This BBB disruption facilitates the entry of additional immune cells into the CNS, exacerbating the inflammatory process.

2.1.2 Th17 Cells

Characterised by their production of interleukin-17 (IL-17), Th17 cells have been found to be particularly pathogenic in MS. IL-17 promotes BBB disruption, neutrophil recruitment, and enhances the pro-inflammatory functions of other immune cells (Kebir et al., 2007; Balasa et al., 2020).

Recent research has uncovered additional mechanisms by which Th17 cells contribute to MS pathogenesis. For instance, Th17 cells have been shown to produce granulocyte-macrophage colony-stimulating factors (GM-CSF), which is a key determinant of the activation and recruitment of myeloid cells to the CNS (Rasouli et al., 2015).

2.1.3 CD8+ T Cells

While historically less studied than their CD4+ counterparts, CD8+ T cells are abundant in MS lesions and can directly target neurons and oligodendrocytes expressing MHC class I molecules. These cells contribute to tissue damage through cytotoxic mechanisms and the release of pro-inflammatory mediators (Friese & Fugger, 2009).

Recent advances in single-cell sequencing technologies have provided new insights into the heterogeneity and function of CD8+ T cells in MS. A study by Ostkamp et al. (2022) used a single-cell analysis framework to characterize cerebrospinal fluid leukocytes and their tissue of origin in MS, identifying distinct T cell populations. These findings suggest a more nuanced role for CD8+ T cells in MS pathogenesis, with some subsets potentially contributing to tissue damage while others may play a regulatory role.

2.1.4 T Regulatory Cells (Tregs)

T regulatory cells (Tregs) are closely involved in maintaining immune tolerance by suppressing autoreactive

immune responses. In multiple sclerosis (MS), dysfunction of Tregs has been implicated in disease pathogenesis. Studies have demonstrated that MS patients exhibit impaired suppressive function of $CD4^{+}CD25^{high}$ Tregs, contributing to the breakdown of self-tolerance and promoting autoimmune responses. For instance, Viglietta et al. (2004) reported a significant decrease in the suppressive function of these regulatory T cells in MS patients compared to healthy controls.

Environmental factors also influence Treg function in MS. Vitamin D deficiency has been associated with reduced Treg numbers and impaired suppressive capacity, suggesting that adequate vitamin D levels are important for Treg-mediated immune regulation. Smolders et al. (2008) found that lower levels of vitamin D metabolites correlated with higher relapse rates and increased disability in MS patients.

Additionally, gut microbiota dysbiosis has been linked to Treg dysfunction in MS. Alterations in the gut microbiome can affect Treg development and function, further contributing to immune dysregulation in MS. Cekanaviciute et al. (2017) demonstrated that gut bacteria from MS patients modulate human T cells and exacerbate symptoms in mouse models, highlighting the interaction between the gut microbiome and immune responses in MS.

These findings show the complexity of Treg dysfunction in MS, involving both intrinsic cellular defects and extrinsic environmental factors.

2.2. B Cell Involvement

B cells contribute to MS pathogenesis through multiple mechanisms:

2.2.1 Antibody Production

B cells produce autoantibodies that target myelin and other CNS components. The presence of oligoclonal bands in the cerebrospinal fluid of MS patients reinforces the involvement of B cells in the disease (Link & Huang, 2006). These autoantibodies can contribute to tissue damage through complement activation and antibody-dependent cell-mediated cytotoxicity (Fraussen et al., 2014).

Recent research has identified novel autoantigens recognised by B cells in MS, including proteins involved in axonal transport and synaptic function (Ayoglu et al., 2016). This expanded repertoire of autoantigens suggests that B cell-mediated responses may target a broader range of CNS structures than previously thought, potentially contributing to both white and gray matter pathology in MS.

2.2.2 Antigen Presentation

B cells drive multiple sclerosis (MS) not only as producers of antibodies but also as potent antigen-presenting cells (APCs). These cells express major histocompatibility complex (MHC) class II molecules and co-stimulatory ligands, enabling them to activate and reactivate brain-homing autoreactive CD4+ T cells. This immunological function is particularly relevant during the later phases of disease progression, such as in secondary progressive MS, where compartmentalised inflammation within the CNS becomes a dominant pathological mechanism (Li et al., 2018).

Advanced immunological studies using in vitro and ex vivo models have further demonstrated the ability of memory B cells to directly stimulate T cells, thus contributing to epitope spreading and chronic CNS inflammation. These findings support the therapeutic targeting of B cells in MS, not solely for antibody reduction but also to limit their role in T cell modulation and CNS immune surveillance (Jelcic et al., 2018).

2.2.3 Cytokine Production

B cells produce both pro-inflammatory (e.g., TNF-α, IL-6) and anti-inflammatory (e.g., IL-10) cytokines, influencing the overall inflammatory milieu in MS (Li et al., 2018). Recent studies have identified a subset of B cells, termed "regulatory B cells" (Bregs), that produce IL-10 and have immunosuppressive functions (Matsushita et al., 2010). The balance between pro-inflammatory and regulatory B cell subsets may determine disease activity and progression in MS.

2.2.4 Formation of Ectopic Lymphoid Structures

In a subset of patients with multiple sclerosis (MS), particularly those with a progressive disease course, B cells are essential to the formation of ectopic lymphoid follicles (ELFs) within the meninges. These tertiary lymphoid structures, which resemble germinal centres, contain B cells, T cells, follicular dendritic cells, and plasma cells, forming a highly organized immune microenvironment within the central nervous system (CNS) (Magliozzi et al., 2007).

These ELFs are thought to act as local hubs of antigen presentation and immune activation, contributing to sustained inflammation and demyelination in adjacent cortical areas. Indeed, their presence has been strongly correlated with subpial cortical demyelination, neuronal loss,

and more aggressive disease progression, particularly in secondary progressive MS.

Advances in ultra-high field MRI (7 Tesla) have enabled researchers to detect meningeal contrast enhancement and leptomeningeal inflammation in vivo, which is considered a radiological surrogate for these ectopic follicles. These findings provide a non-invasive window into CNS immune pathology, offering potential biomarkers for disease progression and a target for B-cell–directed therapies.

2.3. Innate Immune System Involvement

The innate immune system, particularly microglia and macrophages, contributes substantially to amplifying and sustaining the inflammatory response in MS:

2.3.1 Microglia

As the resident immune cells of the CNS, microglia are key players in MS pathology. Upon activation, microglia can release pro-inflammatory mediators, present antigens to T cells, and phagocytose myelin debris (Voet et al., 2019). The dual role of microglia in both injury and repair mechanisms highlights the complex nature of immune responses in MS (Galloway et al., 2022).

Recent studies have revealed that microglia exhibit significant heterogeneity in MS lesions, with distinct subpopulations associated with different stages of lesion evolution (Masuda et al., 2019). Single-cell transcriptomics has identified a unique microglial signature associated with chronic active lesions, characterised by increased lipid metabolism and phagocytic activity (Absinta et al., 2019). This suggests that targeting specific microglial subsets may offer new therapeutic opportunities for MS.

2.3.2 Macrophages

Infiltrating macrophages derived from blood monocytes contribute significantly to tissue damage in MS. These cells produce pro-inflammatory cytokines, phagocytose myelin, and present antigens to T cells, further activating the adaptive immune response (Boven et al., 2006).

Recent research has highlighted the importance of macrophage polarisation in MS pathogenesis. While classically activated (M1) macrophages promote inflammation and tissue damage, alternatively activated (M2) macrophages may have neuroprotective and pro-regenerative functions. The balance between these macrophage phenotypes may influence lesion evolution and repair processes in MS.

2.3.3 Dendritic Cells

Dendritic cells (DCs) are key determinants in initiating and shaping the adaptive immune response in multiple sclerosis (MS). They are involved in presenting antigens to naive T cells, secreting pro-inflammatory cytokines, and migrating across the blood-brain barrier (BBB), thereby facilitating the entry of other immune cells into the central nervous system (Ifergan et al., 2008).

Recent studies have identified a subset of DCs, termed "tolerogenic DCs," which possess the potential to induce regulatory T cells and promote immune tolerance. These cells are characterised by low expression of co-stimulatory molecules and high production of anti-inflammatory cytokines, such as IL-10 and TGF-β. Strategies to exploit the immunomodulatory properties of these DCs are being explored as potential therapeutic approaches for MS, with early clinical trials indicating their safety and tolerability (Mansilla et al., 2023).

3. Mechanisms of Axonal Damage

While demyelination is a hallmark of MS, axonal damage and neurodegeneration are increasingly recognised as major contributors to permanent neurological disability in MS patients (Trapp & Nave, 2008). Several mechanisms contribute to axonal injury in MS:

3.1. Direct Immune-Mediated Damage

3.1.1 T Cell-Mediated Axonal Injury

CD8+ T cells can directly target and damage axons through the release of cytotoxic molecules such as perforin and granzymes (Neumann et al., 2002). Recent studies have shown that CD8+ T cells can form immunological synapses with neurons, leading to focal axonal degeneration even in the absence of demyelination (Siffrin et al., 2010).

3.1.2 Antibody-Mediated Axonal Damage

Autoantibodies targeting axonal antigens can lead to complement-mediated injury and antibody-dependent cell-mediated cytotoxicity. Mathey et al. (2007) demonstrated that antibodies against neurofascin can induce axonal damage in animal models, which suggests that such antibodies may contribute to MS pathogenesis.

Recent studies have identified autoantibodies against neurofascin-155 (NF155) and contactin-1 (CNTN1) in subsets of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and other demyelinating diseases. These antibodies are associated with distinct clinical

features and may contribute to disease mechanisms through disruption of the paranodal axoglial junctions. For instance, anti-CNTN1 antibodies have been shown to induce axoglial dysjunction, leading to impaired nerve conduction (Appeltshauser et al., 2023).

While the presence of these autoantibodies is more established in peripheral demyelinating conditions, their role in central nervous system demyelination, including MS, is an area of ongoing research. The identification of such antibodies in MS patients suggests a broader spectrum of antibody-mediated mechanisms contributing to axonal damage and disease progression.

3.2. Indirect Mechanisms of Axonal Damage

3.2.1 Loss of Trophic Support

Demyelination results in the loss of trophic support normally provided by oligodendrocytes to axons. This can lead to axonal degeneration over time (Nave, 2010). Studies have identified specific trophic factors, such as ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), that are essential for axonal survival in the context of demyelination (Stankoff et al., 2002). These findings suggest potential therapeutic targets for neuroprotection in MS.

3.2.2 Energy Failure

Demyelinated axons require increased energy to maintain ion homeostasis and conduct action potentials. This heightened energy demand, coupled with mitochondrial dysfunction, can lead to energy failure and subsequent axonal degeneration (Witte et al., 2014).

Advanced imaging techniques, such as in vivo two-photon microscopy, have provided new insights into the dynamics of energy failure in demyelinated axons. Misgeld et al. (2007) developed methods for imaging axonal transport of mitochondria in vivo, which later work used to show that mitochondrial transport is impaired in models of neuroinflammation. Such imaging approaches help to clarify the mechanisms underlying energy failure in multiple sclerosis.

3.2.3 Glutamate Excitotoxicity

Elevated levels of glutamate in MS lesions can lead to excitotoxicity, causing axonal injury and neuronal death (Pitt et al., 2003). Recent research has implicated specific glutamate receptor subtypes, particularly NMDA receptors, in mediating axonal injury in MS (Kostic et al., 2013). These findings have prompted the exploration of glutamate receptor antagonists as potential neuroprotective agents in MS clinical trials.

3.2.4 Oxidative Stress

Oxidative stress is closely involved in the pathogenesis of multiple sclerosis (MS), contributing to axonal degeneration and neuronal loss. Activated immune cells, such as microglia and macrophages, produce reactive oxygen and nitrogen species (ROS and RNS), leading to oxidative damage of axonal proteins, lipids, and DNA. Haider et al. (2011) demonstrated that active MS lesions exhibit profound oxidative injury, with elevated levels of oxidized DNA and lipid peroxidation products, particularly in oligodendrocytes and neurons.

Furthermore, Adamczyk and Adamczyk-Sowa (2016) highlighted the role of oxidative stress in both the initiation and progression of MS. Their review emphasized that oxidative stress not only initiates inflammatory processes in the acute phase but also sustains neurodegeneration in the chronic phase. They also discussed the potential of antioxidant therapies in mitigating oxidative damage and improving clinical outcomes in MS patients.

3.3. Mitochondrial Dysfunction

Mitochondrial dysfunction has become a critical factor in MS pathogenesis, particularly in the context of axonal degeneration and neuronal loss (Witte et al., 2014). Several aspects of mitochondrial dysfunction in MS have been identified:

3.3.1 Impaired Energy Production

Damage to mitochondrial DNA and proteins can lead to decreased ATP production, compromising cellular energy metabolism (Campbell et al., 2011). Recent studies using high-resolution respirometry have revealed stage-specific alterations in mitochondrial respiratory chain function in different MS lesion types (Mahad et al., 2009). On this evidence, tailored mitochondrial-targeted therapies may be necessary for different stages of MS progression.

3.3.2 Altered Mitochondrial Dynamics

Changes in mitochondrial fusion, fission, and transport have been observed in MS, potentially contributing to energy deficits in axons and neurons (Witte et al., 2014). Advanced live-cell imaging techniques have revealed impaired mitochondrial transport along demyelinated axons, leading to focal energy deficits and axonal vulnerability (Sorbara et al., 2014). Proper mitochondrial distribution is therefore part of what keeps axons healthy in MS.

3.3.3 Mitochondrial DNA Damage

Mitochondrial dysfunction has attracted attention as a central player in the pathogenesis of multiple sclerosis (MS), particularly in the progressive forms of the disease. One of the most compelling findings in this context is the accumulation of mitochondrial DNA (mtDNA) deletions within neurons residing in chronic active MS lesions. According to Campbell et al. (2011), these deletions are notably enriched in the gray matter of the central nervous system and are strongly associated with respiratory chain deficiencies.

The study by Campbell and colleagues employed laser capture microdissection to isolate single neurons from postmortem MS brain tissue and revealed that affected neurons harbored high levels of mtDNA deletions. These deletions impair the function of respiratory chain complexes, most prominently complex IV, leading to diminished ATP production and increased vulnerability to energy failure. This bioenergetic deficit may contribute to axonal degeneration, which is a major determinant of irreversible neurological disability in MS.

Moreover, the findings suggest that mitochondrial pathology is not merely a byproduct of inflammation but may play a primary role in neurodegeneration. The progressive accumulation of mtDNA damage in MS neurons could reflect an intrinsic vulnerability of mitochondria to oxidative stress and a diminished capacity for mtDNA maintenance and repair. These observations support the hypothesis that targeting mitochondrial dysfunction, particularly mtDNA integrity, could offer novel therapeutic strategies aimed at neuroprotection in MS.

4. The Interplay Between Demyelination and Axonal Damage

The relationship between demyelination and axonal damage in MS is complex and bidirectional:

4.1. Demyelination-Induced Axonal Vulnerability

Demyelinated axons are more susceptible to injury due to increased energy demands, loss of trophic support, and exposure to inflammatory mediators (Franklin & Ffrench-Constant, 2008). Recent studies have revealed that demyelinated axons exhibit altered ion channel distributions, leading to increased susceptibility to calcium-mediated injury. For instance, Kornek et al. (2001) demonstrated an upregulation of N-type voltage-gated calcium channels in dystrophic axons within demyelinating lesions, suggesting a mechanism for calcium influx contributing to axonal degeneration. Additionally, Witte et al. (2019) identified that nanoscale ruptures in the axonal plasma membrane allow extracellular calcium entry, driving axon degeneration in a model of multiple sclerosis. What follows from this is that targeting specific ion channels and membrane integrity may offer neuroprotective benefits in MS.

4.2. Axonal Damage-Induced Demyelination

While demyelination is a hallmark of multiple sclerosis (MS), evidence suggests that primary axonal injury can also precipitate secondary demyelination. Trapp and Stys (2009) introduced the concept of "virtual hypoxia," wherein demyelinated axons experience energy deficits due to impaired mitochondrial function, leading to axonal degeneration and subsequent loss of axon-derived trophic support essential for oligodendrocyte survival and myelin maintenance.

Axon-derived factors, notably brain-derived neurotrophic factor (BDNF) and neuregulin-1, play critical roles in sustaining myelin integrity. BDNF, through its interaction with the TrkB receptor on oligodendrocytes, promotes myelination and supports oligodendrocyte survival (Fletcher et al., 2018). Neuregulin-1 influences myelination by modulating oligodendrocyte differentiation and enhancing activity-dependent myelination processes (Lundgaard et al., 2013).

Preserving axonal health thus appears necessary to maintain myelin integrity and prevent progressive neurodegeneration in MS.

4.3. Chronic Neurodegeneration

The ongoing cycle of demyelination and axonal damage contributes to a state of chronic neurodegeneration in MS, leading to progressive disability (Mahad et al., 2015).

Chronic neurodegeneration becomes particularly prominent in the progressive stages of MS. Longitudinal studies have shown that the rate of brain atrophy accelerates over time in MS patients (Eshaghi et al., 2018). This atrophy is not limited to white matter but significantly affects gray matter as well. In particular, atrophy of the cortex and deep gray matter structures has been strongly associated with cognitive impairment and physical disability (Steenwijk et al., 2016).

Recent research has begun to elucidate the mechanisms underlying chronic neurodegeneration:

4.3.1 Neuroinflammation and Glial Activation

Ongoing low-grade inflammation in chronic MS lesions is a significant factor perpetuating neurodegeneration. Activated microglia and astrocytes continue to release pro-inflammatory cytokines and reactive oxygen species, leading to persistent neuronal injury (Lassmann, 2018). Recent evidence suggests that this chronic glial activation induces epigenetic reprogramming in microglia, reinforcing a pro-inflammatory phenotype and potentially driving progressive neurodegeneration (Colonna & Butovsky, 2017).

4.3.2 Proteostasis Disruption

Disrupted protein homeostasis (proteostasis) has also been proposed to contribute to neurodegeneration in MS, through the accumulation of misfolded proteins, dysfunction of the ubiquitin-proteasome system, and abnormal accumulation of tau protein in axons.

4.3.3 Neurovascular Unit Dysfunction

Disruption of the neurovascular unit in multiple sclerosis (MS) has been investigated as a significant contributor to chronic neurodegeneration. One of the central pathological features is blood-brain barrier (BBB) dysfunction, which allows the entry of immune cells, cytokines, and neurotoxic molecules into the central nervous system (CNS), exacerbating inflammation and tissue damage. Moreover, BBB impairment alters cerebral perfusion and oxygenation, potentially accelerating neuronal loss and cognitive decline (Minagar & Alexander, 2003).

Recent neuroimaging studies have demonstrated that small vessel disease (SVD) is commonly observed in MS patients and is significantly associated with cognitive impairment, brain atrophy, and reduced cortical thickness, particularly in progressive forms of the disease (Andravizou et al., 2019). The implication is that SVD and neurovascular dysfunction may represent a parallel or synergistic pathology alongside traditional demyelinating mechanisms in MS, thereby presenting a potential target for adjunctive therapeutic strategies.

5. Remyelination and Repair Mechanisms

In addition to demyelination and axonal damage, the CNS's attempts at self-repair are important in MS. Remyelination, the process of forming new myelin sheaths around damaged axons, can contribute to partial recovery of neurological function in MS.

5.1. Oligodendrocyte Precursor Cells (OPCs)

The main players in remyelination are oligodendrocyte precursor cells (OPCs). These cells become activated in MS lesions, proliferate, and differentiate into mature oligodendrocytes, thereby producing new myelin (Franklin & Ffrench-Constant, 2017).

5.1.1 OPC Activation and Migration

In MS lesions, various factors activate OPCs and attract them to damaged areas. These include chemokines (e.g., CXCL12) and growth factors (e.g., PDGF and FGF-2). New research has shown that factors such as osteopontin produced by microglia also promote OPC migration and activation.

5.1.2 OPC Differentiation

The differentiation of OPCs into mature, myelin-producing oligodendrocytes is a critical step for remyelination. This process is regulated by a complex network of transcription factors. Recent studies have elucidated the role of key transcription factors such as MYRF and SOX10 in controlling OPC differentiation and myelin gene expression (Emery & Lu, 2015).

5.1.3 Myelin Formation

Mature oligodendrocytes form new myelin sheaths by contacting the axonal surface. This process involves an interaction between axonal signals and oligodendrocyte intracellular mechanisms. Recent advanced imaging techniques, such as live-cell imaging and super-resolution microscopy, have provided exceptional insights into the dynamics of myelin formation (Hughes & Appel, 2020).

5.2. Factors Influencing Remyelination Efficiency

Despite the presence of OPCs in MS lesions, remyelination often fails or is incomplete, particularly in chronic lesions. Several factors contribute to this remyelination failure:

5.2.1 Age-related Decline

Remyelination efficiency decreases with age, which is particularly relevant in MS as it often affects young adults and progresses over decades. Recent studies have identified age-related changes in OPCs, including reduced responsiveness to differentiation signals and altered epigenetic profiles (Neumann et al., 2019).

5.2.2 Inhibitory Factors in MS Lesions

The microenvironment of MS lesions often contains factors that inhibit OPC differentiation and remyelination.

These include chondroitin sulfate proteoglycans (CSPGs) and myelin debris. New research has identified specific signalling pathways, such as the Wnt pathway, that become dysregulated in MS lesions and inhibit remyelination (Guo et al., 2015).

5.2.3 Axonal Health

The state of demyelinated axons significantly influences remyelination success. Chronically demyelinated axons may lose their ability to support remyelination due to changes in axonal surface molecules and impaired metabolic support. Recent studies have identified specific axonal molecules, such as PSA-NCAM, that need to be downregulated for successful remyelination (Bacmeister et al., 2020).

5.3. Promoting Remyelination as a Therapeutic Strategy

Understanding the mechanisms of remyelination has led to the development of potential therapeutic strategies to enhance this process in MS:

5.3.1 Clemastine

This antihistamine has been shown to promote OPC differentiation and enhance remyelination in preclinical models and a small clinical trial (Green et al., 2017). Recent mechanistic studies have revealed that clemastine acts by modulating the M1 muscarinic receptor, providing insights for the development of more targeted therapies (Mei et al., 2016). Clinical interpretation should also consider the later disability-worsening signal in a small advanced-progressive-MS cohort; clemastine is not an established MS repair therapy (Kocot et al., 2025).

5.3.2 Anti-LINGO-1 Antibodies

LINGO-1 is a negative regulator of OPC differentiation. Antibodies targeting LINGO-1 have shown promise in promoting remyelination in animal models (Mi et al., 2013), but the anti-LINGO-1 antibody opicinumab did not meet its primary endpoints in phase II clinical trials. New research has elucidated the downstream signalling pathways of LINGO-1, identifying additional potential therapeutic targets (Jepson et al., 2012).

5.3.3 RXR Agonists

Retinoid X receptor (RXR) agonists have been shown to enhance OPC differentiation and remyelination in preclinical studies (Huang et al., 2011). Recent work has demonstrated that RXR agonists also have immunomodulatory effects, potentially providing dual benefits in MS (Chandraratna et al., 2016).

5.3.4 Cell-based Therapies

Recent advancements in cell-based therapies have shown promise for treating progressive forms of multiple sclerosis (MS). A notable development is the Phase I clinical trial conducted by Leone et al. (2023), which evaluated the safety and tolerability of intracerebroventricular transplantation of allogeneic human neural stem/progenitor cells (hNSCs) in patients with secondary progressive MS (SPMS). In this open-label, dose-escalation study, 15 participants received hNSC transplants and were monitored over a 12-month period. The results indicated that the procedure was safe and well-tolerated, with no treatment-related deaths or serious adverse events reported. Moreover, clinical and radiological assessments suggested disease stabilization in treated individuals.

These findings indicate the potential of hNSC-based therapies as a viable approach for managing progressive MS. However, further studies with larger cohorts and longer follow-up periods are necessary to fully ascertain the efficacy and long-term safety of this treatment modality.

6. Emerging Concepts and Future Directions

6.1. The Role of the Gut Microbiome

Recent research has highlighted the importance of the gut-brain axis in MS pathogenesis. Alterations in the gut microbiome composition have been observed in MS patients, and these changes may influence both immune responses and CNS repair processes (Cekanaviciute et al., 2017).

6.1.1 Microbiome-derived Metabolites

Specific metabolites produced by gut bacteria, such as short-chain fatty acids, have been shown to have immunomodulatory and neuroprotective effects. Recent studies have demonstrated that administration of these metabolites can ameliorate disease in animal models of MS (Haghikia et al., 2015).

6.1.2 Microbiome-based Therapies

Emerging therapeutic approaches targeting the gut microbiome, such as fecal microbiota transplantation (FMT) and probiotic supplementation, are under investigation for multiple sclerosis (MS). Small pilot studies have assessed the safety and tolerability of FMT in people with MS and have reported it to be generally well tolerated, but these were uncontrolled or early-phase studies without demonstrated clinical benefit. Microbiome-based therapies therefore remain an avenue of investigation rather than an established treatment.

6.2. Extracellular Vesicles in MS Pathogenesis and Repair

Extracellular vesicles (EVs) have developed into important mediators of intercellular communication in the CNS. In MS, EVs play diverse roles in both disease pathogenesis and repair processes:

6.2.1 Pathogenic Role of EVs

EVs derived from activated immune cells can carry pro-inflammatory molecules and autoantigens, potentially contributing to BBB disruption and spreading inflammation in the CNS (Dolcetti et al., 2020).

6.2.2 EVs in Repair and Remyelination

Mesenchymal stem cell (MSC)-derived exosomes have demonstrated significant immunomodulatory and neuroprotective effects in experimental models of multiple sclerosis (MS). These extracellular vesicles can modulate immune responses and promote neural repair mechanisms. Recent studies suggest that engineering MSC-derived exosomes to overexpress specific microRNAs (miRNAs) enhances their therapeutic efficacy. For instance, miR-146a-5p-enriched exosomes have been shown to reduce neuroinflammation and promote neuroprotection by modulating microglial activation and inflammatory signalling pathways (Tahmasebi et al., 2024). miRNA-engineered exosomes therefore merit study as a cell-free therapeutic strategy in MS.

6.3. Precision Medicine Approaches in MS

The heterogeneity of MS in terms of clinical presentation, disease course, and response to therapy has led to increased interest in precision medicine approaches:

6.3.1 Biomarker Development

Advanced proteomic and metabolomic techniques are being employed to identify biomarkers that can predict disease courses, treatment response, and prognosis. A recent large-scale study identified a panel of serum proteins that could predict conversion to secondary progressive MS with high accuracy (Siller et al., 2019).

6.3.2 Pharmacogenomics

Pharmacogenomics explores how genetic variations affect individual responses to medications, aiming to optimise therapeutic strategies. In the context of multiple sclerosis (MS), a study by Carrasco-Campos et al. (2021) investigated the association between certain gene polymorphisms and the efficacy of IFN-β therapy in RRMS patients. The study found that carriers of at least one copy of the C allele of the CTSS rs1136774 polymorphism exhibited a better response to IFN-β treatment. Additionally, individuals with the TT genotype of the TNFRSF10A rs20576 polymorphism had a higher probability of maintaining stable disability status over a 24-month treatment period, as measured by the Expanded Disability Status Scale (EDSS). On this evidence, genetic profiling of CTSS and TNFRSF10A polymorphisms may serve as predictive markers for IFN-β therapy responsiveness in RRMS patients.

6.3.3 Advanced Imaging Techniques

Advanced imaging modalities are being developed to enhance the monitoring of disease activity and treatment response in multiple sclerosis (MS). Among these, myelin water imaging (MWI) and positron emission tomography (PET) using myelin-binding tracers have shown promise in evaluating demyelination and remyelination processes in vivo. Myelin water imaging allows the estimation of myelin content based on T2 relaxation times, while PET with ligands such as [^{11}C]PIB or [^{11}C]MeDAS can provide molecular-level insights into myelin integrity. These approaches are increasingly utilised not only in research settings but also in longitudinal clinical studies aiming to quantify therapeutic efficacy in remyelination-promoting interventions (van der Weijden et al., 2023).

Our understanding of the mechanisms underlying demyelination and axonal damage in MS has greatly expanded in recent years. The interaction between immune-mediated processes, neurodegenerative changes, and repair mechanisms presents both challenges and opportunities for therapeutic intervention. As we continue to unravel the intricacies of MS pathophysiology, we move closer to developing more effective, targeted treatments that can halt disease progression, protect neurons, and promote repair in this devastating neurological disorder.

7. Neuroplasticity and Adaptive Mechanisms in MS

While much attention has been focused on the pathological processes in MS, recent research has begun to elucidate the adaptive and compensatory mechanisms that occur in response to CNS damage.

7.1. Cortical Reorganization

Functional MRI studies have revealed significant cortical reorganization in MS patients, particularly in the early stages of the disease:

7.1.1 Motor System Plasticity

In multiple sclerosis (MS), motor system plasticity is a critical component of functional recovery. Studies have shown that MS patients often exhibit increased activation in ipsilateral motor areas and recruit additional brain regions during motor tasks, suggesting adaptive neuroplastic changes. Tomassini et al. (2012) reviewed this evidence, including enhanced activation of the ipsilateral motor cortex during simple motor tasks, which may reflect compensatory mechanisms aimed at preserving motor function despite disease-related damage.

Furthermore, longitudinal studies indicate that the extent of motor cortex reorganization correlates with functional outcomes. In their review, Tomassini et al. (2012) described studies in which motor training in people with MS was accompanied by functional MRI changes in motor networks, suggesting that motor cortex plasticity could serve as a marker of functional recovery; this possibility requires prospective validation.

7.1.2 Cognitive Network Adaptation

Alterations in cognitive networks have been observed in multiple sclerosis (MS) patients, with increased activation of prefrontal areas during cognitive tasks (Schoonheim et al., 2015). Recent studies employing graph theory approaches have demonstrated that MS patients with preserved cognitive function exhibit more efficient network reorganization, characterised by enhanced global efficiency and reduced path length, suggesting adaptive neuroplasticity mechanisms (Rocca et al., 2016).

7.2. Synaptic Plasticity

At the cellular level, synaptic plasticity drives adapting to MS-related damage:

7.2.1 Long-term Potentiation (LTP) Alterations

Studies in animal models of MS have shown alterations in LTP, a key mechanism of synaptic plasticity. Stampanoni Bassi et al. (2018) reviewed whether pharmacological manipulation of LTP could favour the effects of motor rehabilitation in MS, an approach that remains hypothetical.

7.2.2 Dendritic Spine Remodeling

Changes in dendritic spine density and morphology have been observed in MS and may represent a compensatory mechanism. Two-photon imaging in living mice has shown that dendritic spines are dynamic structures (Grutzendler et al., 2002), and experimental models of MS have revealed spine loss and remodeling in inflamed grey matter; in vivo imaging of dendritic spines is not possible in patients.

7.3. Neurotrophic Factors

Neurotrophic factors are central to neuroprotection and plasticity in MS:

7.3.1 Brain-Derived Neurotrophic Factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is essential to neuroplasticity and neuroprotection, both of which are central in the context of multiple sclerosis (MS). Alterations in BDNF levels have been observed in MS patients, potentially impacting disease progression and symptomatology.

Recent studies have explored the impact of aerobic exercise on BDNF levels in individuals with MS. For instance, a study by Gravesteijn et al. (2023) investigated the effects of aerobic exercise training on neuroprotective biomarkers in MS patients. The findings indicated that aerobic exercise led to increased BDNF levels, suggesting enhanced neuroprotective mechanisms. This elevation in BDNF was associated with improved cognitive function and reduced fatigue, which makes aerobic exercise worth testing as an intervention in MS.

7.3.2 Nerve Growth Factor (NGF)

Nerve Growth Factor (NGF) is a neurotrophin known for its role in neuronal survival, differentiation, and repair. In the context of multiple sclerosis (MS), NGF has demonstrated neuroprotective effects, particularly through its influence on astrocytes and oligodendrocyte precursor cells (OPCs).

Aloe et al. (2012) reviewed nerve growth factor from its early discovery to its potential clinical use, including experimental work in which NGF administration reduced disease severity in animal models of neuroinflammation. Proposed mechanisms include modulation of immune responses and support for the survival and maturation of oligodendrocytes, the myelinating cells of the central nervous system.

Furthermore, research indicates that astrocytes, a type of glial cell, can produce NGF in response to CNS injury. This endogenous production of NGF by astrocytes contributes to the repair processes following demyelination. Whether astrocyte-derived NGF is sufficient to support neuronal survival and to influence OPC differentiation during remyelination has not been established.

These findings point to the therapeutic potential of NGF in MS, both through direct administration and by recruiting the body's endogenous repair mechanisms involving astrocytes.

8. The Role of Glial Cells in MS Pathogenesis and Repair

While traditionally viewed as supporting cells, glial cells are now recognised as key players in MS pathogenesis and repair:

8.1 Astrocytes

Astrocytes play diverse roles in MS, contributing to both pathology and repair:

8.1.1 Reactive Astrogliosis

Reactive astrocytes give rise to glial scars in multiple-sclerosis (MS) lesions, a process that can hamper remyelination. Yet single-cell and transcriptomic studies now show that reactive astrocytes are functionally heterogeneous: alongside the classical neurotoxic "A1" phenotype, other subpopulations actively foster repair. As reviewed by Liddelow et al. (2020), microglia-derived IL-1α, TNF-α and C1q drive astrocytes toward an A1 state that kills neurons and oligodendrocytes. Conversely, astrocytes spared this cytokine cocktail (or shifted toward an "A2-like" state) secrete trophic factors and support oligodendrocyte progenitor-cell (OPC) differentiation.

Building on this concept, Molina-Gonzalez et al. (2023) used in-vivo single-nucleus RNA-seq and functional manipulation in demyelinated mice to uncover a pro-regenerative transcriptional program in astrocytes. The program is marked by suppressed NRF2 signalling and enhanced cholesterol-biosynthesis/export genes; pharmacological NRF2 inhibition or stimulation of astrocytic cholesterol efflux rebiased astrocytes toward this state, rescued oligodendrocyte survival and restored remyelination in chronic lesions. These data provide a tractable molecular target for pushing astrocytes toward a repair-promoting phenotype in progressive MS.

8.1.2 Astrocyte-Mediated Glutamate Homeostasis

Dysregulation of glutamate homeostasis by astrocytes contributes to excitotoxicity in MS. New research has identified specific astrocytic glutamate transporters as potential therapeutic targets (Pitt et al., 2003).

8.2 Oligodendrocytes

Beyond their role in myelination, oligodendrocytes have come to be seen as important contributors in axonal support and metabolic coupling:

8.2.1 Metabolic Support of Axons

Oligodendrocytes provide metabolic support to axons through the transport of lactate. Whether enhancing this metabolic support can protect axons from degeneration in MS is an active question, and the relevant pathways are being defined in experimental systems.

8.2.2 Adaptive Myelination

New research has revealed that myelination is more dynamic than previously thought, with evidence for activity-dependent adaptive myelination. Sampaio-Baptista et al. (2020) showed in adult rats that experience alters white matter structure and myelin-related gene expression, supporting the concept of experience-dependent myelination; whether this can be harnessed therapeutically in MS remains untested.

9. Emerging Therapeutic Approaches

As our understanding of MS pathophysiology deepens, novel therapeutic approaches are being developed:

9.1. Nanoparticle-Based Therapies

Nanoparticles are being investigated for additional roles for targeted drug delivery in MS:

9.1.1 Remyelination-Promoting Nanoparticles

Recent advancements in nanotechnology have shown promise in promoting remyelination in multiple sclerosis (MS) models. A study by Osorio-Querejeta et al. (2020) demonstrated that extracellular vesicles enriched with miR-219a-5p induced oligodendrocyte precursor cell differentiation and improved experimental autoimmune encephalomyelitis (EAE) more efficiently than liposomes and polymeric nanoparticles, suggesting that the carrier itself influences therapeutic performance.

9.1.2 Immunomodulatory Nanoparticles

Nanoparticles designed to induce tolerance to myelin antigens have shown promise in preclinical studies. A phase I clinical trial of antigen-specific tolerogenic nanoparticles in MS patients is currently underway (Li et al., 2024).

9.2. Gene Therapy Approaches

Advancements in gene therapy techniques are opening new avenues for MS treatment:

9.2.1 CRISPR-Based Therapies

CRISPR/Cas9 genome editing has opened new avenues for precisely dissecting and targeting the molecular drivers of autoreactive T-cell behaviour in MS. In a landmark in vivo proof-of-concept, Kendirli et al. (2023) performed a genome-wide CRISPR screen in a rat experimental autoimmune encephalomyelitis (EAE) model to identify key genes regulating $CD4^+$ T-cell migration into the CNS. They discovered five "brake" and eighteen "facilitator" genes, among which α4-integrin and CXCR3 were validated: targeted deletion of either gene in MBP-reactive T cells markedly reduced their CNS infiltration and ameliorated clinical disease severity. This work demonstrates that CRISPR-mediated disruption of T-cell migratory pathways can effectively limit pathogenic CNS entry, highlighting novel therapeutic targets for MS.

9.2.2 Gene Delivery for Neuroprotection

Viral vector-mediated delivery of neuroprotective genes, such as brain-derived neurotrophic factor (BDNF), has demonstrated early findings in experimental models of multiple sclerosis (MS). BDNF plays a critical role in supporting neuronal survival, promoting synaptic plasticity, and enhancing remyelination. Although a first-in-human clinical trial of AAV-mediated BDNF delivery in progressive MS has not yet commenced, recent studies emphasize the potential of BDNF-based gene therapy in providing neuroprotection and modulating disease progression.

9.3. Exosome-Based Therapies

Exosomes, a type of extracellular vesicle, are being investigated as both therapeutic agents and drug delivery vehicles in MS:

9.3.1 Mesenchymal Stem Cell-Derived Exosomes

Extracellular vesicle effects depend on their source and preparation. Pusic et al. (2014) studied IFNγ-stimulated dendritic cell vesicles in preclinical remyelination experiments. This work does not establish efficacy of engineered MSC-derived vesicles in MS patients; see Section 5.7 for the study design.

9.3.2 Exosome-Mediated Drug Delivery

Exosomes are being explored as vehicles for delivering therapeutic molecules across the BBB. Li et al. (2022) reviewed how exosomes regulate the NLRP3 inflammasome in disease, a pathway of interest for limiting neuroinflammation.

10. The Impact of Environmental Factors on MS Pathogenesis

Growing evidence suggests that environmental factors contribute substantially to MS risk and progression:

10.1. Vitamin D

Low vitamin D levels have been associated with an increased risk of developing multiple sclerosis (MS) as well as heightened disease activity.

10.1.1 Epigenetic Modulation

Emerging evidence suggests that vitamin D may influence the risk of MS through epigenetic regulation of gene expression. In particular, vitamin D receptor (VDR) binding sites have been found to colocalize with MS-associated genetic variants. Handel et al. (2010) reviewed environmental factors and their timing in adult-onset MS, including the evidence linking vitamin D status to disease risk. This supports the notion that vitamin D can modulate gene expression at disease-susceptibility sites, providing a potential mechanistic explanation for how vitamin D deficiency contributes to MS pathogenesis.

10.1.2 Immunomodulatory Effects

Vitamin D has been shown to promote regulatory T cell function and modulate B cell responses in MS. In a randomised study, Smolders et al. (2020) found that vitamin D3 supplementation did not significantly change neurofilament light chain levels in MS, indicating that supplementation effects on measures of neuroaxonal damage remain unproven.

10.2. Epstein-Barr Virus (EBV)

The link between EBV infection and MS risk has been strengthened by recent research:

10.2.1 Molecular Mimicry

Recent research has provided compelling evidence linking Epstein–Barr virus (EBV) infection to multiple sclerosis (MS) through molecular mimicry mechanisms. A key study by Lanz et al. (2022) identified clonally expanded B cells in MS patients that produce antibodies targeting both the EBV nuclear antigen 1 (EBNA1) and the central nervous system (CNS) protein GlialCAM. This cross-reactivity suggests that EBV infection may trigger autoimmune responses against CNS components, contributing to MS pathogenesis. Structural analyses revealed that the antibodies' binding to GlialCAM is enhanced by post-translational modifications, such as serine phosphorylation.

Furthermore, immunization with EBNA1 exacerbated disease in a mouse model of MS, providing functional evidence for the role of molecular mimicry in disease progression. EBV-induced molecular mimicry thus appears significant in MS and highlight potential avenues for therapeutic intervention.

10.2.2 EBV-Targeted Therapies

Novel therapeutic approaches targeting EBV are being developed for MS. A phase I trial of autologous EBV-specific T-cell therapy in progressive MS reported acceptable safety and clinical improvement in some participants (Pender et al., 2018), but the subsequent randomised phase II trial of an allogeneic EBV-targeted T-cell product (ATA188, EMBOLD) did not meet its primary endpoint.

Our understanding of the pathophysiology of multiple sclerosis continues to expand, revealing new layers of complexity and potential therapeutic targets. The interplay between immune-mediated processes, neurodegenerative changes, and repair mechanisms presents both challenges and opportunities for intervention. As we unravel the intricate mechanisms underlying MS, we move closer to developing more effective, targeted treatments that can halt disease progression, protect neurons, and promote repair in this devastating neurological disorder. The integration of advanced technologies, from single-cell genomics to in vivo imaging, promises to accelerate our progress towards better management and potential cures for MS in the coming years.

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2.3. THE ROLE OF NEUROINFLAMMATION

1. Introduction

Multiple sclerosis (MS) is a complex, chronic autoimmune disorder characterised by inflammation, demyelination, and neurodegeneration within the central nervous system (CNS). This debilitating condition affects millions of individuals worldwide, with varying presentations and progressive patterns (Dobson & Giovannoni, 2019). At the core of MS pathogenesis lies neuroinflammation, a varied process that initiates and perpetuates immune-mediated damage to nerve fibres, ultimately leading to the hallmark symptoms and disability associated with the disease (Bitirgen et al., 2017). See Figure 2.3.1.

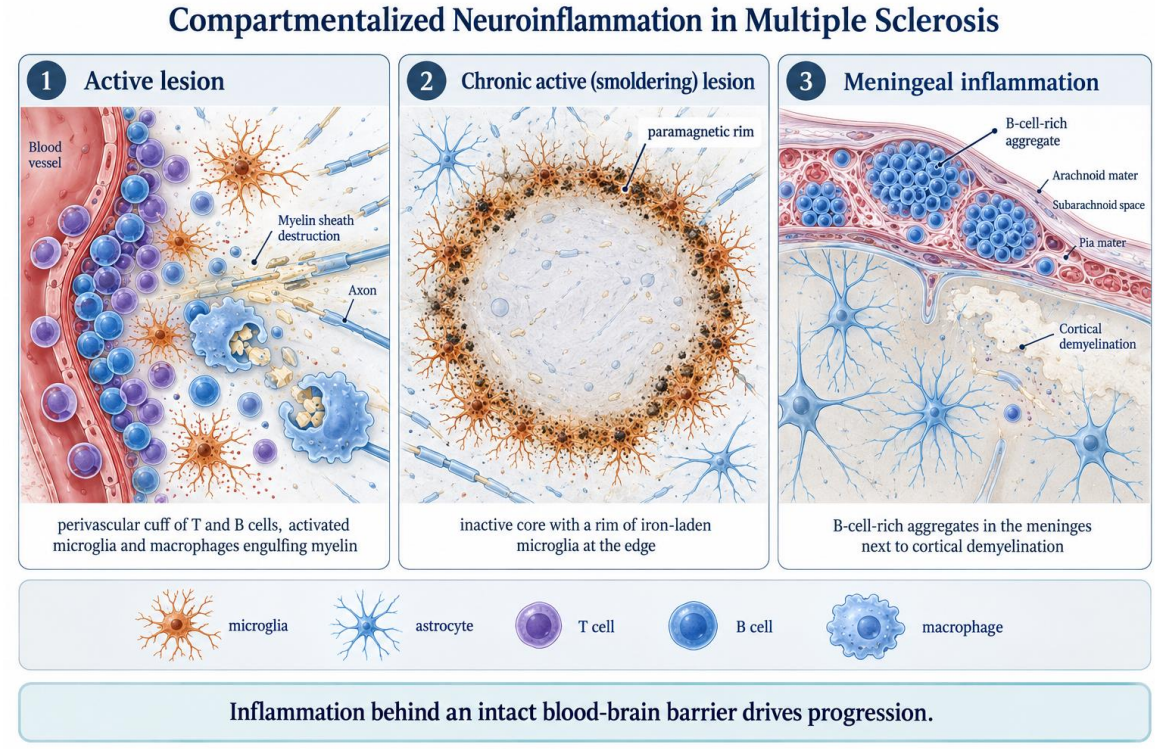


Figure 2.3.1. Compartmentalized neuroinflammation in multiple sclerosis: active lesions, chronic active (smoldering) lesions with a paramagnetic rim, and B-cell-rich meningeal aggregates adjacent to cortical demyelination.

Neuroinflammation in MS is not a simple, linear process but rather an interaction of various immune cells, molecular mediators, and CNS-resident cells. This inflammatory cascade involves both the innate and adaptive immune systems, leading to a sustained inflammatory environment that damages the myelin sheath, oligodendrocytes, and ultimately the axons themselves (Dendrou et al., 2015).

2. Pathophysiology of Neuroinflammation in MS

2.1. Initiation of the Inflammatory Response

The exact trigger for the autoimmune response in MS remains elusive, but several theories exist. One prominent hypothesis suggests that molecular mimicry between viral or bacterial antigens and myelin components may lead to the activation of autoreactive T cells (Mentis et al., 2017). Once activated, these T cells can cross the blood-brain barrier (BBB) and initiate an inflammatory cascade within the CNS.

2.2. Blood-Brain Barrier Disruption

The integrity of the BBB is important for maintaining CNS homeostasis. In MS, this barrier becomes compromised, allowing for the influx of immune cells and inflammatory mediators. Recent research has elucidated the molecular mechanisms underlying BBB dysfunction in MS, highlighting the role of matrix metalloproteinases (MMPs) and vascular cell adhesion molecules (VCAMs) in facilitating immune cell migration (Ortiz et al., 2014).

2.3. T Cell-Mediated Inflammation

T cells, particularly CD4+ T helper cells and CD8+ cytotoxic T cells, are key determinants of MS pathogenesis. These cells are activated in the periphery by antigen-presenting cells (APCs) presenting myelin-derived peptides,

leading to their clonal expansion and migration into the CNS. Within the CNS, reactivated T cells orchestrate a complex immune response, releasing pro-inflammatory cytokines such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), and interleukin-17 (IL-17) (Lassmann & Bradl, 2017).

The differentiation of naive CD4+ T cells into specific T helper subsets, including Th1, Th17, and regulatory T cells (Tregs), is closely involved in shaping the inflammatory response in MS. The balance between pro-inflammatory Th1 and Th17 cells and anti-inflammatory Tregs is often dysregulated in MS, favoring a pro-inflammatory environment (Fletcher et al., 2010).

2.4. B Cell Involvement in MS Neuroinflammation

While T cells have long been recognised as key players in MS pathogenesis, the role of B cells has gained increasing attention in recent years.

B cells contribute to MS neuroinflammation through several distinct mechanisms. They produce autoantibodies that can target myelin and other CNS components, leading to complement activation and antibody-dependent cell-mediated cytotoxicity (Krumbholz et al., 2012), and they act as efficient antigen-presenting cells, activating and reactivating autoreactive T cells (Li et al., 2018). They also secrete both pro-inflammatory cytokines such as IL-6 and TNF-α and anti-inflammatory cytokines such as IL-10, thereby modulating the overall inflammatory environment (Li et al., 2015). Finally, in some MS patients B cells contribute to the formation of ectopic lymphoid follicles within the meninges, structures that may serve as sites of sustained inflammation and antibody production (Serafini et al., 2004).

The presence of oligoclonal bands in the cerebrospinal fluid of MS patients, a hallmark diagnostic feature, reflects intrathecal antibody production in the disease process (Stangel et al., 2013).

2.5. Innate Immune Cell Activation

In addition to adaptive immunity, innate immune responses contribute to MS neuroinflammation. Microglia can participate in both tissue injury and repair, depending on their activation state and local environment (Voet et al., 2019). Activated microglia may release inflammatory mediators, present antigens, and phagocytose myelin debris.

Astrocytes, another type of CNS-resident cell, also participate in the inflammatory response. These cells can both promote and inhibit inflammation depending on the context. In MS, reactive astrocytes contribute to BBB dysfunction, cytokine production, and glial scar formation (Ponath et al., 2018).

Dendritic cells (DCs), while less abundant in the CNS, drive the initiation and perpetuation of the inflammatory response in MS. DCs act as potent APCs, presenting myelin antigens to T cells and producing cytokines that shape the adaptive immune response (Nuyts et al., 2013).

3. Molecular Mediators of Neuroinflammation in MS

3.1. Cytokines and Chemokines

The inflammatory milieu in MS is characterised by a complex network of cytokines and chemokines that orchestrate immune cell activation, migration, and effector functions.

Several cytokines are of particular importance in this network. IFN-γ, produced primarily by Th1 cells, activates macrophages and microglia, enhances MHC class II expression, and promotes BBB disruption (Arellano et al., 2015). TNF-α is a pleiotropic cytokine that contributes to oligodendrocyte death, demyelination, and axonal damage, yet it also has neuroprotective properties, which highlights the complex nature of cytokine signalling in MS (Probert, 2015). IL-17, produced by Th17 cells, promotes BBB disruption, recruits neutrophils, and enhances the pro-inflammatory functions of other immune cells (Kolbinger et al., 2016), while GM-CSF, produced by various T cell subsets, activates myeloid cells and promotes their inflammatory functions (Rasouli et al., 2015).

Chemokines are central to immune cell trafficking to the CNS. Key chemokines involved in MS pathogenesis include CXCL13, which attracts B cells, and CCL2 and CCL5, which recruit T cells and monocytes (Bielekova & Becker, 2010).

3.2. Adhesion Molecules

Adhesion molecules facilitate the migration of immune cells across the BBB. VCAM-1, expressed on endothelial cells, interacts with VLA-4 on lymphocytes and enables their transmigration across the barrier (Engelhardt & Ransohoff, 2012), while ICAM-1 binds to LFA-1 on lymphocytes and is upregulated on endothelial cells during inflammation (Sonar & Lal, 2018). The selectins, E-selectin and P-selectin, mediate the initial rolling of leukocytes along the endothelium, facilitating their subsequent firm adhesion and transmigration (Carrithers et al., 2002).

3.3. Matrix Metalloproteinases (MMPs)

MMPs are proteolytic enzymes that degrade extracellular matrix components and contribute to BBB disruption in MS. MMP-2 and MMP-9, in particular, have been implicated in MS pathogenesis, with elevated levels detected in the CSF and serum of MS patients (Yong et al., 2007).

3.4. Oxidative Stress Mediators

Oxidative stress is essential to the neurodegenerative processes associated with MS. The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by activated immune cells and dysfunctional mitochondria contributes to lipid peroxidation, protein oxidation, and DNA damage.

Among the key oxidative mediators, nitric oxide (NO), produced by activated microglia and macrophages, can lead to mitochondrial dysfunction and axonal degeneration (Smith & Lassmann, 2002). Superoxide, generated by activated immune cells, can combine with NO to form peroxynitrite, a highly reactive molecule that causes extensive cellular damage (Friese et al., 2014), and hydroxyl radicals can cause direct damage to cellular components, including lipids, proteins, and DNA (Haider et al., 2011).

3.5. Complement System

The complement system, a component of the innate immune response, has been implicated in MS pathogenesis. Complement activation can lead to the formation of the membrane attack complex, causing direct damage to oligodendrocytes and neurons. Additionally, complement proteins can enhance phagocytosis of myelin by macrophages and microglia (Ingram et al., 2009).

4. Novel Molecular Players in MS Neuroinflammation

Recent research has identified several novel molecular players that contribute to neuroinflammation in MS:

4.1. Repulsive Guidance Molecule A (RGMa)

RGMa has become a significant factor in MS, acting as a potent inhibitor of neuronal regeneration and a regulator of cell death (Demicheva et al., 2015). RGMa signalling has been shown to promote T cell activation and differentiation, as well as to inhibit axonal growth and remyelination. Targeting RGMa has shown promise in promoting neuroprotection and regeneration in experimental models of MS.

4.2. Inflammasome Activation

The inflammasome, a multiprotein complex responsible for the activation of inflammatory caspases and the production of pro-inflammatory cytokines, has been implicated in MS pathogenesis. The NLRP3 inflammasome, in particular, has been observed at elevated levels in both MS lesions and peripheral immune cells of patients. Activation of the NLRP3 inflammasome leads to the production of IL-1β and IL-18, potent pro-inflammatory cytokines that contribute to BBB disruption and myelin damage (Barclay & Shinohara, 2017).

4.3. MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. Several miRNAs have been implicated in MS pathogenesis, modulating various aspects of the inflammatory response. For example, miR-155 has been shown to promote pro-inflammatory T cell responses and contribute to BBB dysfunction in MS (McCoy, 2017).

4.4. Sphingosine-1-Phosphate (S1P) Signaling

S1P signalling contributes substantially to lymphocyte trafficking and has become a target for MS therapy. S1P receptors are expressed on various immune cells and CNS-resident cells, modulating their activation and migration. Disruption of S1P signalling through drugs like fingolimod has shown efficacy in reducing MS disease activity (Chun & Hartung, 2010).

5. Neuroinflammation and Neurodegeneration in MS

While inflammation is a primary driver of tissue damage in MS, the relationship between neuroinflammation and neurodegeneration is complex and bidirectional. Chronic inflammation can lead to irreversible axonal damage and neuronal loss, but neurodegenerative processes can also perpetuate and exacerbate the inflammatory response.

5.1. Mechanisms of Neurodegeneration in MS

Several mechanisms contribute to neurodegeneration in MS. Inflammatory cells and their products can directly damage axons and neurons through cytotoxic mechanisms (Friese et al., 2014), while inflammatory mediators and oxidative stress impair mitochondrial function, leading to energy deficits and axonal degeneration (Witte et al., 2014). Demyelination exposes axonal ion channels, producing ionic imbalances and eventual axonal degeneration (Craner et al., 2004), and elevated levels of glutamate in MS

lesions can cause excitotoxic damage to neurons and oligodendrocytes (Pitt et al., 2000). Inflammation and oxidative stress can also disrupt axonal transport mechanisms, contributing to neuronal dysfunction and death (Hares et al., 2014).

5.2. Neuroinflammation in Progressive MS

While acute inflammation is more prominent in relapsing-remitting MS, chronic neuroinflammation is a key determinant of progressive forms of the disease. In progressive MS, compartmentalised inflammation within the CNS, often involving meningeal inflammation and microglial activation, contributes to ongoing neurodegeneration (Mahad et al., 2015).

6. Imaging Neuroinflammation in MS

Advanced imaging techniques have substantially changed our ability to visualise and quantify neuroinflammation in MS patients. These techniques not only aid in diagnosis and monitoring of disease progression but also provide insights into the underlying pathological processes.

6.1. Magnetic Resonance Imaging (MRI)

MRI remains the gold standard for MS diagnosis and monitoring, and various techniques assess different aspects of neuroinflammation. Gadolinium-enhanced T1-weighted imaging allows the visualisation of active inflammatory lesions with BBB disruption (Filippi et al., 2019), while T2-weighted and FLAIR sequences detect both acute and chronic lesions and provide information on overall disease burden (Wattjes et al., 2015). Diffusion tensor imaging (DTI) can detect microstructural changes in normal-appearing white matter, offering insight into tissue damage beyond visible lesions (Sbardella et al., 2013), and magnetic resonance spectroscopy (MRS) allows the measurement of brain metabolites, including markers of neuroinflammation such as choline and myo-inositol.

6.2. Positron Emission Tomography (PET)

PET imaging with specific radioligands has been investigated as a powerful tool for visualizing neuroinflammation in MS. Translocator protein (TSPO) is upregulated in activated microglia and astrocytes, and TSPO-PET can therefore be used to quantify neuroinflammation in patients (Airas et al., 2015), while novel PET tracers targeting myelin can provide information on demyelination and remyelination processes (Bodini et al., 2016).

6.3. Optical Coherence Tomography (OCT)

OCT has developed into a powerful tool for assessing retinal changes in MS patients, offering insights into disease progression and the extent of axonal loss (Cellerino et al., 2019). Studies utilising OCT have demonstrated significant thinning of the retinal nerve fibre layer (RNFL) and the ganglion cell-inner plexiform layer (GCIPL) in MS patients, correlating with disease severity and duration (Garcia-Martin et al., 2017).

The use of OCT in MS research and clinical practice extends beyond mere structural assessment. Recent studies have shown that retinal layer thinning, as measured by OCT, can predict treatment failure in relapsing MS, which suggests a role for the technique in guiding treatment decisions (Bsteh et al., 2021).

7. Biomarkers of Neuroinflammation in MS

The identification and validation of biomarkers for neuroinflammation in MS is an active area of research. These biomarkers can aid in diagnosis, prognosis, and monitoring of treatment response.

7.1. Cerebrospinal Fluid (CSF) Biomarkers

CSF biomarkers provide direct insight into the inflammatory processes occurring within the CNS. The presence of oligoclonal bands (OCBs) is a hallmark of MS, reflecting intrathecal antibody production; OCBs are found in about 95% of patients and are included in the McDonald diagnostic criteria (Thompson et al., 2018), and recent studies suggest that OCB-negative patients may represent a distinct subgroup with different pathophysiological mechanisms and potentially different treatment responses (Dobson et al., 2013). Neurofilament light chain (NfL), a structural protein of neurons released into the CSF upon axonal damage, correlates with disease activity, disability progression, and brain atrophy (Khalil et al., 2018), and longitudinal studies have shown that CSF NfL levels can predict long-term disability outcomes (Barro et al., 2018). Chitinase 3-like protein 1 (CHI3L1), also known as YKL-40, is expressed by astrocytes and microglia and is elevated in the CSF of MS patients, with levels correlating with disease progression and with conversion from clinically isolated syndrome to clinically definite MS (Comabella et al., 2010). Glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed by astrocytes, reflects astrocyte activation and correlates with disease progression, particularly in progressive forms of the disease (Abdelhak et al., 2018). Myelin basic protein (MBP) and myelin

oligodendrocyte glycoprotein (MOG) are released into the CSF during active demyelination, so their levels can reflect ongoing myelin damage and may help differentiate MS from other neurological disorders. Finally, the levels of inflammatory mediators such as CXCL13, IL-6, and TNF-α provide insight into the inflammatory status of the CNS (Bielekova et al., 2012).

7.2. Blood-based Biomarkers

While CSF biomarkers provide direct insight into CNS pathology, blood-based biomarkers offer the advantage of easier accessibility and the potential for repeated measurements:

Serum NfL is the most developed of these. Recent advances in ultrasensitive assay technologies have enabled its reliable measurement, serum levels correlate strongly with CSF NfL and reflect ongoing neuroaxonal damage (Disanto et al., 2017), and the marker has shown promise for monitoring disease activity and treatment response. RNA profiling may identify gene expression signatures associated with disease activity; a 2009 study identified a 29-gene signature that could distinguish between relapsing and remitting phases of MS (Baranzini et al., 2009). Several circulating miRNAs have also been proposed as biomarkers, among them miR-145, which is upregulated in the blood of MS patients and correlates with disease activity (Keller et al., 2014). Markers of oxidative stress, such as advanced oxidation protein products (AOPPs) and isoprostanes, are elevated in serum and may reflect ongoing neuroinflammation and neurodegeneration (Adamczyk & Adamczyk-Sowa, 2016), and serum levels of matrix metalloproteinases, particularly MMP-9, have been associated with disease activity and BBB disruption (Benešová et al., 2009).

7.3. Imaging Biomarkers

Advances in neuroimaging have produced several imaging biomarkers of neuroinflammation. T2 lesion load, expressed as the number and volume of T2 hyperintense lesions, reflects the overall burden of disease (Filippi et al., 2019), while gadolinium-enhancing lesions represent areas of active inflammation with BBB disruption and serve as a sensitive marker of acute disease activity (Traboulsee et al., 2016). Measures of brain volume loss, particularly grey matter atrophy, correlate with disability progression and reflect the neurodegenerative component of the disease (De Stefano et al., 2016), and the detection of cortical lesions, especially with advanced techniques such as double inversion recovery (DIR), provides insight into cortical pathology (Calabrese et al., 2010). Magnetization transfer ratio (MTR) measurements give information on myelin content and integrity, offering a potential marker for demyelination and remyelination (Vargas et al., 2015), and TSPO-PET, as noted above, visualizes and quantifies microglial activation and thereby provides a direct measure of neuroinflammation (Airas et al., 2015).

8. Therapeutic Targeting of Neuroinflammation in MS

Understanding the complex mechanisms of neuroinflammation in MS has led to the development of various therapeutic strategies aimed at modulating the immune response and protecting the CNS from damage.

8.1 Current Immunomodulatory Therapies

Interferon-β was among the first approved treatments for MS and modulates the immune response through multiple mechanisms, including reduced T cell activation and migration across the BBB (Kieseier, 2011). Glatiramer acetate, a synthetic polypeptide, is thought to act as a decoy for myelin-reactive T cells and to promote the development of regulatory T cells (Aharoni, 2013). Natalizumab, a monoclonal antibody targeting the α4 integrin subunit, prevents lymphocyte migration across the BBB and thereby reduces CNS inflammation (Polman et al., 2006), while fingolimod, the first oral therapy approved for MS, modulates sphingosine-1-phosphate receptors and prevents lymphocyte egress from lymph nodes (Chun & Hartung, 2010). Dimethyl fumarate has both immunomodulatory and antioxidant properties, activating the Nrf2 pathway and reducing oxidative stress in the CNS (Gold et al., 2012), and teriflunomide, an inhibitor of dihydroorotate dehydrogenase, reduces the proliferation of activated T and B cells (O'Connor et al., 2011). Alemtuzumab, an anti-CD52 monoclonal antibody, causes profound depletion of circulating T and B cells followed by a repopulation phase that may reset the immune system (Coles et al., 2012). Ocrelizumab, the first B cell-depleting therapy approved for both relapsing and primary progressive MS, targets CD20+ B cells and thereby highlights the important role of B cells in MS pathogenesis (Hauser et al., 2017).

8.2 Emerging Therapeutic Approaches

Bruton's tyrosine kinase (BTK) inhibitors target both B cells and myeloid cells and may therefore address the inflammatory and neurodegenerative aspects of MS together (Montalban et al., 2019). Remyelinating therapies, including compounds such as clemastine fumarate and

bexarotene, aim to promote oligodendrocyte differentiation and myelin repair, addressing the degenerative component of the disease (Green et al., 2017), while neuroprotective strategies targeting mitochondrial dysfunction, such as biotin and idebenone, have been investigated for their potential to slow neurodegeneration (Witte et al., 2014), although subsequent trials of both agents in progressive MS were negative. Among cell-based approaches, autologous haematopoietic stem cell transplantation (AHSCT) primarily uses conditioning and immune reconstitution to suppress inflammatory disease activity and should not be equated with direct neural regeneration; MSCs and neural progenitor approaches are investigated for immunomodulation, trophic support, or repair, with different and generally less mature efficacy evidence (Scolding et al., 2017). Finally, emerging evidence for a role of the gut microbiome in MS pathogenesis has prompted investigation of therapeutic approaches targeting the gut-brain axis, such as fecal microbiota transplantation (Berer et al., 2017).

9. Future Directions in MS Neuroinflammation Research

As our understanding of neuroinflammation in MS continues to evolve, several key areas are likely to shape future research and therapeutic development:

9.1. Precision Medicine Approaches

The heterogeneity of MS in terms of clinical presentation, disease course, and treatment response argues for individually tailored treatment. Future research will likely focus on identifying biomarkers and genetic factors that can predict individual disease trajectories and treatment responses, enabling more tailored therapeutic approaches (Comabella & Montalban, 2014).

9.2. Targeting Chronic Neuroinflammation in Progressive MS

While current therapies are effective in managing relapsing forms of MS, treatment options for progressive MS remain limited. Future research will likely focus on developing strategies to target compartmentalised CNS inflammation and chronic microglial activation in progressive MS (Mahad et al., 2015).

9.3. Combination Therapies

Given the complex pathophysiology of MS, combining therapies that target different aspects of the disease (e.g., anti-inflammatory, neuroprotective, and remyelinating agents) may provide synergistic benefits. Investigating optimal combination strategies and their long-term effects will be an important area of future research (Ontaneda et al., 2017).

9.4. Advanced Imaging Techniques

Continued development of advanced imaging techniques, such as high-field MRI, molecular imaging, and novel PET ligands, will provide deeper insights into the dynamics of neuroinflammation and neurodegeneration in MS (Filippi et al., 2019).

9.5. Elucidating the Role of B Cells in MS Pathogenesis

The success of B cell-depleting therapies in MS has highlighted the need for a deeper understanding of B cell biology in MS. Future research will likely focus on characterising different B cell subsets, their functions in the CNS, and their contribution to both inflammatory and regulatory processes in MS (Li et al., 2018).

9.6. Investigating the Gut-Brain Axis in MS

The emerging role of the gut microbiome in MS pathogenesis opens up new avenues for research and potential therapeutic interventions. Future studies will likely explore the mechanisms by which gut microbiota influence neuroinflammation and the potential for microbiome-based therapies in MS (Berer et al., 2017).

9.7. Neuroprotection and Remyelination Strategies

Developing effective neuroprotective and remyelinating therapies remains a key goal in MS research. Future studies will likely focus on identifying novel targets for promoting neuroprotection and enhancing endogenous repair mechanisms in the CNS (Plemel et al., 2017).

10. The Role of Glial Cells in MS Neuroinflammation

While much attention has been focused on the role of infiltrating immune cells in MS pathogenesis, the contribution of CNS-resident glial cells to neuroinflammation is increasingly recognised as key.

10.1. Microglia

Microglia, the resident immune cells of the CNS, can contribute to both tissue injury and repair in MS, depending on activation state, lesion context, and disease stage.

Microglia, the primary immune cells of the CNS, play a complex and heterogeneous role in MS. They can adopt various activation states, ranging from pro-inflammatory

(M1-like) to anti-inflammatory and repair-promoting (M2-like) phenotypes, and the balance between these states influences the overall inflammatory environment in MS lesions (Voet et al., 2019). Activated microglia present antigens to T cells, potentially perpetuating the autoimmune response (Becher et al., 2006), and release pro-inflammatory mediators including TNF-α, IL-1β, and nitric oxide, although they can also produce anti-inflammatory factors such as IL-10 and TGF-β (Luo et al., 2017). Phagocytosis is a further essential function, since clearing myelin debris is essential for remyelination, yet sustained phagocytosis may also contribute to ongoing inflammation (Grajchen et al., 2018). Aberrant microglial pruning of synapses may additionally contribute to cognitive dysfunction in MS (Di Filippo et al., 2016).

Recent studies have highlighted the heterogeneity of microglial populations in MS lesions, with distinct subsets exhibiting different functional properties (Masuda et al., 2019).

10.2. Astrocytes

Astrocytes, the most abundant glial cells in the CNS, contribute to both harmful and protective processes in MS. They are central components of the BBB, and their dysfunction can contribute to barrier disruption (Sofroniew, 2015). Like microglia, they produce both pro-inflammatory factors such as IL-6 and CCL2 and anti-inflammatory factors such as IL-10 (Ponath et al., 2018), and under inflammatory conditions they can upregulate MHC class II molecules and present antigens to T cells (Fontana et al., 1984). Reactive astrocytes form glial scars around MS lesions, which can impede axonal regeneration but also limit the spread of inflammation (Sofroniew, 2015), and astrocyte dysfunction can lead to impaired glutamate uptake, potentially contributing to excitotoxicity (Pitt et al., 2000).

Recent research has identified distinct subpopulations of reactive astrocytes in MS lesions, with some exhibiting neurotoxic properties and others promoting tissue repair (Liddelow et al., 2017).

10.3. Oligodendrocytes and Oligodendrocyte Precursor Cells (OPCs)

Although they are primarily targets of the immune response in MS, oligodendrocytes and OPCs also play active roles in neuroinflammation. Oligodendrocytes can produce pro-inflammatory cytokines such as IL-1β and CCL2 in response to injury, and under inflammatory conditions they can express MHC class I molecules and present antigens to CD8+ T cells (Höftberger et al., 2004). OPCs, for their part, are important for remyelination, but their function can be impaired by the inflammatory environment of MS lesions (Franklin & Ffrench-Constant, 2008).

11. Oxidative Stress and Mitochondrial Dysfunction in MS Neuroinflammation

Oxidative stress and mitochondrial dysfunction are increasingly recognised as key contributors to neuroinflammation and neurodegeneration in MS.

11.1. Sources of Oxidative Stress in MS

Oxidative stress in MS arises from several sources. Infiltrating macrophages and activated microglia produce large amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Haider et al., 2011), damaged mitochondria constitute a further significant source of ROS within lesions (Witte et al., 2014), and increased iron deposition can catalyze the production of highly reactive hydroxyl radicals through the Fenton reaction (Hametner et al., 2013).

11.2. Consequences of Oxidative Stress

The consequences of this oxidative burden are extensive. Oxidative damage to myelin lipids contributes to demyelination and impairs remyelination (Haider et al., 2011), while oxidative modification of proteins can lead to enzyme inactivation and cellular dysfunction (Bizzozero et al., 2005). Oxidative DNA damage can trigger apoptosis and contribute to neurodegeneration (Haider et al., 2011), and oxidative injury to mitochondrial DNA and proteins produces energy deficits and further ROS production (Witte et al., 2014).

11.3. Mitochondrial Dysfunction in MS

Mitochondrial dysfunction affects MS pathology along several lines. Impaired mitochondrial function reduces ATP production and thereby contributes to axonal degeneration (Mahad et al., 2015), and it impairs calcium buffering, potentially leading to excitotoxicity (Su et al., 2009). Damaged mitochondria can also release pro-apoptotic factors that trigger cell death (Lassmann & van Horssen, 2011), and the accumulation of mtDNA mutations in MS lesions may contribute to progressive neurodegeneration (Campbell et al., 2011).

12. The Role of Adaptive Immunity in MS Neuroinflammation

While innate immune mechanisms and glial cell activation are important in MS pathogenesis, the adaptive immune response remains a central driver of neuroinflammation.

12.1. T Cell-Mediated Inflammation

Different subsets of CD4+ T cells contribute to MS pathogenesis: Th1 cells produce IFN-γ and mediate macrophage activation (Fletcher et al., 2010), Th17 cells produce IL-17 and are particularly important in BBB disruption (Kebir et al., 2007), and regulatory T cells suppress inflammation, although their function is often impaired in MS (Viglietta et al., 2004). CD8+ T cells can directly damage oligodendrocytes and neurons through cytotoxic mechanisms (Friese & Fugger, 2009), while γδ T cells, a subset capable of rapidly producing inflammatory cytokines, may contribute to the early stages of MS pathogenesis (Wucherpfennig et al., 1992).

12.2. B Cell Involvement

The role of B cells in MS has gained increasing attention, particularly following the success of B cell-depleting therapies:

B cells produce autoreactive antibodies that can contribute to demyelination and axonal damage (Krumbholz et al., 2012), and they are efficient antigen-presenting cells, activating and reactivating autoreactive T cells (Li et al., 2018). They secrete both pro-inflammatory cytokines such as TNF-α and IL-6 and anti-inflammatory cytokines such as IL-10, shaping the overall inflammatory environment (Li et al., 2015), and in some patients they contribute to the formation of ectopic lymphoid follicles in the meninges that may serve as sites of sustained inflammation (Serafini et al., 2004).

13. Environmental Factors Influencing Neuroinflammation in MS

Various environmental factors have been implicated in modulating the risk and course of MS, many of which may act through effects on neuroinflammation:

13.1. Vitamin D

Vitamin D influences MS neuroinflammation on several levels. It promotes regulatory T cell function and suppresses pro-inflammatory T cell responses (Correale et al., 2009), and it regulates the expression of multiple genes involved in immune function and myelin maintenance (Disanto et al., 2012). Epidemiologically, low vitamin D levels are associated with increased MS risk and greater disease activity (Munger et al., 2006).

13.2. Epstein-Barr Virus (EBV) Infection

EBV antigens may cross-react with myelin antigens through molecular mimicry, potentially triggering autoimmune responses (Lünemann et al., 2008), and EBV infection of B cells may promote their survival and activation, contributing to ongoing inflammation (Pender, 2011). The epidemiological link is striking: nearly all MS patients are seropositive for EBV, compared to about 90% of the general population (Ascherio & Munger, 2007).

13.3. Smoking

Cigarette smoke components can increase BBB permeability, potentially facilitating immune cell entry into the CNS (Hedström et al., 2009), and smoking increases oxidative stress, which may exacerbate neuroinflammation and tissue damage (Costenbader & Karlson, 2006). Nicotine itself has been shown to enhance the activation and proliferation of autoreactive T cells (Shi et al., 2009).

13.4. Gut Microbiome

Emerging evidence suggests that the gut microbiome may influence neuroinflammation in MS:

Gut microbiota can shape both innate and adaptive immune responses and may thereby influence autoimmunity (Berer et al., 2011). Microbial-derived metabolites such as the short-chain fatty acid butyrate have anti-inflammatory properties and may influence disease courses in MS (Duscha et al., 2020), and the gut microbiome may affect CNS inflammation through various routes, including vagus nerve signalling and the systemic circulation of bacterial products (Kirby & Ochoa-Repáraz, 2018).

14. Neuroplasticity and Repair Mechanisms in MS

While neuroinflammation is primarily associated with damage in MS, the nervous system also attempts to compensate and repair itself through various mechanisms.

14.1. Adaptive Neuroplasticity

Functional MRI studies have shown that MS patients often exhibit altered patterns of brain activation, which suggests compensatory cortical reorganization (Rocca et al., 2007). Changes in synaptic strength and connectivity may help compensate for neuronal loss and demyelination (Zeller & Classen, 2014), and although neurogenesis is limited in the adult brain, some evidence suggests that it may

be influenced by inflammatory damage in MS (Ekdahl et al., 2003).

14.2. Remyelination

Remyelination is a key repair mechanism in MS, although its efficiency often decreases as the disease progresses:

Demyelination triggers the activation and proliferation of oligodendrocyte precursor cells, which can differentiate into new oligodendrocytes (Franklin & Ffrench-Constant, 2008). The process is regulated by various molecules, including growth factors such as PDGF and FGF, chemokines such as CXCL12, and transcription factors such as Olig2 (Kremer et al., 2011). Its efficiency tends to decrease with age, which may contribute to the progressive nature of MS in later stages (Sim et al., 2002).

14.3. Neuroprotective Mechanisms

Several endogenous neuroprotective mechanisms are activated in response to inflammatory damage in MS:

Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are upregulated in MS lesions and can promote neuronal survival (Kerschensteiner et al., 1999). Heat shock proteins are induced in response to cellular stress and can protect against protein misfolding and aggregation (Cwiklinska et al., 2010), and the Nrf2 pathway, which regulates the expression of various antioxidant enzymes, is activated in MS lesions as a protective mechanism against oxidative stress (Licht-Mayer et al., 2015).

15. Chronic Neuroinflammation and Progressive MS

While acute inflammation is a hallmark of relapsing-remitting MS, chronic neuroinflammation is closely involved in progressive forms of the disease.

15.1. Compartmentalized Inflammation

In progressive MS, inflammation becomes partially trapped behind a repaired BBB, leading to compartmentalised inflammation within the CNS:

Aggregates of immune cells in the meninges, including B cell follicle-like structures, may contribute to subpial cortical demyelination and neurodegeneration (Magliozzi et al., 2007). Chronic activation of microglia in normal-appearing white and grey matter contributes to ongoing neurodegeneration (Lassmann et al., 2012), and the accumulation of oxidative damage, partly due to mitochondrial dysfunction, is a key feature of progressive MS (Fischer et al., 2012).

15.2. Neurodegeneration in Progressive MS

Several mechanisms contribute to ongoing neurodegeneration in progressive MS:

Chronic demyelination leads to increased energy demands in axons, resulting in a state of "virtual hypoxia" and eventual axonal degeneration (Trapp & Stys, 2009). Increased iron deposition in the CNS can promote oxidative stress and neurodegeneration (Hametner et al., 2013), while altered glutamate homeostasis can lead to excitotoxic damage to neurons and oligodendrocytes (Werner et al., 2001). Chronic demyelination also leads to the redistribution of sodium channels along axons, contributing to energy deficits and axonal degeneration (Craner et al., 2004).

16. Emerging Therapeutic Approaches Targeting Neuroinflammation

Based on our evolving understanding of neuroinflammation in MS, several novel therapeutic approaches are being developed:

16.1. Targeting Chronic Microglial Activation

Compounds that inhibit the colony-stimulating factor 1 receptor (CSF1R) can modulate microglial activation and potentially slow neurodegeneration (Olmos-Alonso et al., 2016), and targeting metabolic pathways in microglia, such as glycolysis, may help shift their phenotype from pro-inflammatory to neuroprotective.

16.2. Enhancing Neuroprotection and Repair

Compounds that activate the Nrf2 pathway, such as dimethyl fumarate, can enhance antioxidant responses and potentially protect against neurodegeneration (Linker et al., 2011). Agents that promote OPC differentiation and remyelination, such as clemastine fumarate and bexarotene, are being investigated for their potential to enhance repair (Green et al., 2017), and mesenchymal stem cell transplantation is being explored for its potential immunomodulatory and neuroprotective effects in MS (Scolding et al., 2017).

16.3. Targeting B Cells in Progressive MS

Direct administration of B cell-depleting antibodies into the CSF is being investigated as a means of targeting compartmentalised inflammation in progressive MS (Komori et al., 2016), and Bruton's tyrosine kinase (BTK) inhibitors, which target both B cells and myeloid cells, are being evaluated for their potential in this setting (Torke et al., 2020).

16.4. Combination Therapies

Given the complex pathophysiology of MS, combining therapies that target different aspects of the disease may provide synergistic benefits:

Combining immunomodulatory drugs with neuroprotective compounds may help address both the inflammatory and the neurodegenerative components of MS (Ontaneda et al., 2017), and combining therapies that suppress inflammation with those that enhance remyelination may improve overall outcomes (Plemel et al., 2017).

This extensive exploration of neuroinflammation in MS highlights the complexity of the disease process and the many factors that contribute to its pathogenesis. Understanding these mechanisms is essential for developing more effective and targeted therapies for MS patients, particularly for progressive forms of the disease where current treatment options are limited.

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2.4. BLOOD-BRAIN BARRIER DISRUPTION IN MULTIPLE SCLEROSIS

1. Introduction

Multiple sclerosis (MS) stands as one of the most enigmatic and challenging neurological disorders of our time. At its core lies an interaction of immune dysfunction, neurodegeneration, and vascular abnormalities. Central to this intricate pathophysiology is the disruption of the blood-brain barrier (BBB), a critical interface that normally maintains the delicate homeostasis of the central nervous system (CNS) (Sweeney et al., 2019). Figure 2.4.1 compares the barrier in health and in MS and sets out the consequences for drug delivery.

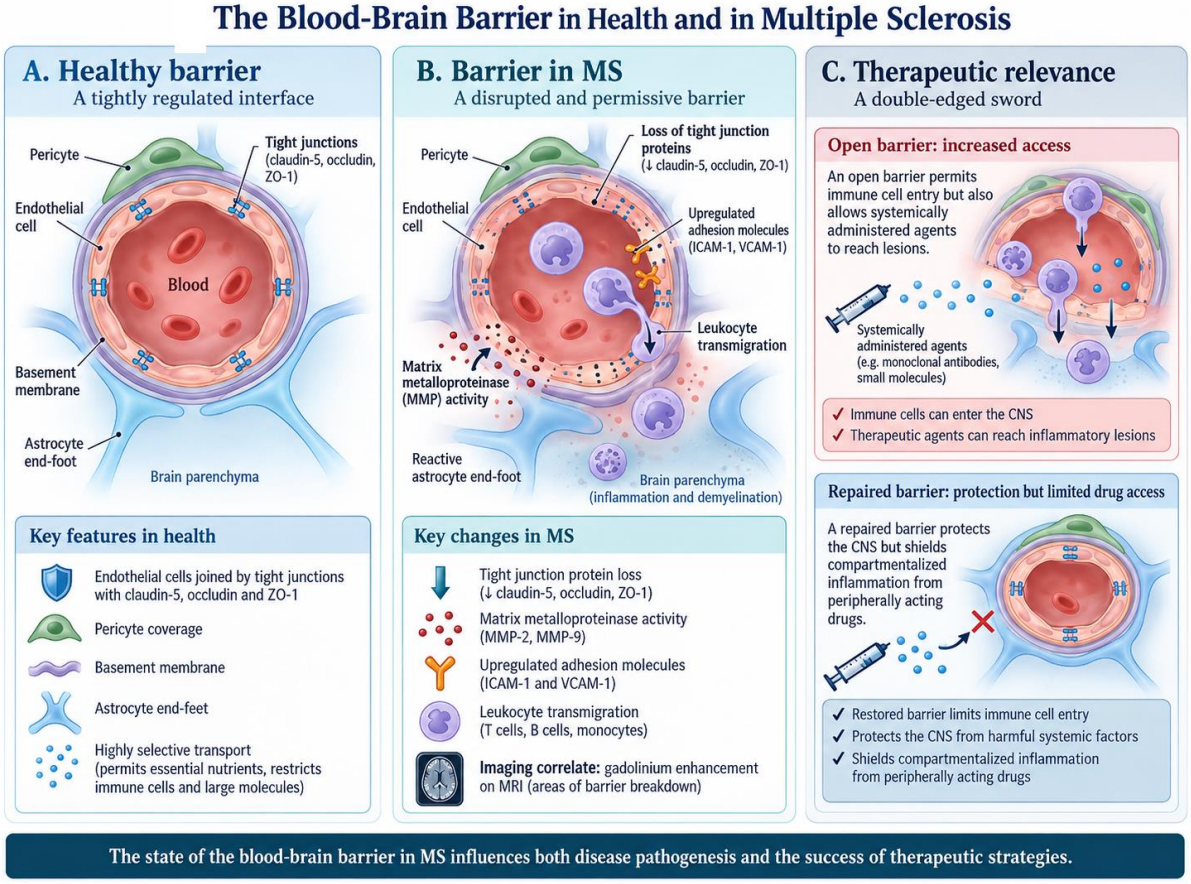


Figure 2.4.1. The blood-brain barrier in health and in multiple sclerosis, and the consequences of barrier state for drug delivery.

The BBB, far from being a simple physical barrier, is a dynamic and highly specialized structure. It comprises a network of endothelial cells, pericytes, and astrocyte end-feet, collectively forming what is known as the neurovascular unit (Daneman & Prat, 2015). In healthy individuals, this barrier serves as a vigilant gatekeeper, meticulously regulating the passage of substances between the bloodstream and the CNS. It allows essential nutrients to enter while simultaneously preventing potentially harmful agents from accessing this sensitive environment (Abbott et al., 2010).

However, in the context of MS, this protective shield becomes compromised. The breakdown of the BBB is not merely a consequence of the disease but matters for its initiation and progression. This disruption sets the stage for a cascade of events that ultimately lead to the hallmark features of MS: inflammation, demyelination, and neurodegeneration (Ortiz et al., 2014).

Recent advancements in neuroimaging and molecular biology have shed new light on the intricacies of BBB dysfunction in MS. For instance, high-resolution magnetic resonance imaging (MRI) techniques have allowed researchers to visualise BBB leakage in real-time, providing unusual insights into the early stages of lesion formation (Cramer et al., 2014). Moreover, single-cell RNA sequencing studies have revealed the molecular signatures of BBB components during disease progression, offering potential new targets for therapeutic intervention (Vanlandewijck et al., 2018).

From targeted immunomodulators to advanced cell-based therapies, the field offers a range of possibilities for enhancing BBB integrity and, by extension, mitigating the devastating effects of this disease.

BBB disruption links immune-cell entry, endothelial injury, and CNS inflammation. The mechanisms described below also determine how drugs and investigational cell or vesicle products reach their intended targets.

2. Mechanisms of BBB Disruption in MS

The integrity of the BBB is compromised in MS through a complex process involving various cellular and molecular players. Understanding these mechanisms is important for developing targeted therapies and identifying potential biomarkers of disease progression.

2.1. Inflammatory Mediators and Cytokines

At the heart of BBB disruption in MS lies a complex web of inflammatory processes. Pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon-gamma (IFN-γ), play important roles in altering BBB permeability (Minagar & Alexander, 2003). These cytokines exert their effects through multiple mechanisms:

Three mechanisms are central here. Pro-inflammatory cytokines downregulate tight junction proteins: TNF-α and IL-1β decrease the expression of claudin-5 and occludin, key components of endothelial tight junctions (Kebir et al., 2007), and this downregulation increases paracellular permeability, allowing inflammatory cells and potentially harmful substances to pass into the CNS. The same cytokines induce the production and activation of matrix metalloproteinases, particularly MMP-9, enzymes that degrade extracellular matrix components and tight junction proteins and thereby further compromise BBB integrity (Aung et al., 2015); recent studies have also implicated MMP-2 and MMP-3, suggesting a more interaction of proteolytic activity in barrier disruption (Rempe et al., 2016). Finally, cytokines promote the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, facilitating leukocyte adhesion and transmigration across the BBB (Calabresi et al., 2001), and recent research has highlighted the role of activated leukocyte cell adhesion molecule (ALCAM) in this process, providing a potential new target for therapeutic intervention (Cayrol et al., 2008).

Th17 cells and their associated cytokines have been implicated in BBB disruption. IL-17 and IL-22 can alter tight-junction integrity and promote CNS inflammation, and IL-17 may interact with other inflammatory mediators to increase barrier permeability (Kebir et al., 2007; Huppert et al., 2010).

Interestingly, recent studies have also implicated gut microbiome in modulating BBB integrity in MS. The concept of the "gut-brain axis" has gained traction, with evidence suggesting that alterations in gut microbial composition can influence BBB permeability through the production of short-chain fatty acids and other metabolites (Buscarinu et al., 2017). This emerging field of research opens up new avenues for potential therapeutic interventions targeting the gut microbiome to enhance BBB integrity in MS patients.

2.2. Immune Cell Infiltration

The compromised BBB in MS allows for increased infiltration of immune cells into the CNS, a process that involves an interaction between circulating leukocytes and the endothelial cells of the BBB. This infiltration process can be broken down into several key steps:

The migration of leukocytes across the barrier proceeds in three coordinated steps. Initial contact between leukocytes and the endothelium is mediated by selectins on endothelial cells interacting with their ligands on leukocytes, an interaction that initiates the adhesion cascade and allows leukocytes to roll along the vessel wall (Engelhardt & Ransohoff, 2012). Firm adhesion follows, as integrins on leukocytes bind to adhesion molecules such as ICAM-1 and VCAM-1 on endothelial cells, arresting the cells at the vessel wall and preparing them for transmigration (Larochelle et al., 2011). In the final step, leukocytes cross the endothelial barrier through either paracellular or transcellular routes, a process guided by chemokine gradients and involving the coordinated action of various adhesion molecules and junctional proteins.

Recent research has identified the specific subsets of immune cells involved in BBB disruption in MS. While Th1 and Th17 cells have long been recognised as key players, emerging evidence suggests a more nuanced role for other cell types:

CD8+ cytotoxic T cells contribute to BBB disruption through the release of perforin and granzymes, which can directly damage endothelial cells (Johnson et al., 2012), and recent studies have highlighted their role in forming memory populations within the CNS, potentially contributing to disease chronicity (Smolders et al., 2018). B cells, traditionally viewed primarily as antibody-producing cells,

are now understood to play a more complex role: they can contribute to BBB disruption through the production of pro-inflammatory cytokines and the presentation of antigens to T cells (Li et al., 2018), and the success of B cell-depleting therapies in MS has further showed their importance in disease progression. Mounting evidence also implicates innate immune cells, including monocytes, dendritic cells, and natural killer cells; recent studies have shown, for example, that patrolling monocytes can facilitate the early stages of T cell infiltration across the BBB (Audoy-Rémus et al., 2008).

The intricate dance of immune cell infiltration across the BBB in MS presents both challenges and opportunities for therapeutic intervention. Understanding the temporal and spatial dynamics of this process could lead to more targeted approaches for preventing or mitigating BBB disruption.

2.3. Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress drives BBB disruption in MS, contributing to a vicious cycle of inflammation and tissue damage. The excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can lead to:

Oxidative stress acts on the barrier along three routes. Lipid peroxidation of endothelial cell membranes alters membrane fluidity and function, potentially compromising the barrier properties of the BBB (Witte et al., 2014); ROS can directly modify tight junction proteins, leading to their degradation or altered function (Lochhead et al., 2010); and oxidative stress can trigger redox-sensitive signalling cascades, including the NF-κB pathway, which promotes inflammation and further BBB disruption (Morgan & Liu, 2011).

Recent studies have shown increased levels of oxidative stress markers in the serum and cerebrospinal fluid (CSF) of MS patients, correlating with disease activity and BBB permeability (Ljubisavljevic et al., 2014). Furthermore, advanced oxidation protein products (AOPPs) have come to be seen as potential biomarkers of BBB disruption and disease progression in MS (Fiorini et al., 2013).

Mitochondrial dysfunction in endothelial cells has also been implicated in BBB disruption. Impaired energy metabolism can compromise the maintenance of tight junctions and active transport mechanisms critical for BBB function (Witte et al., 2014). Recent research has uncovered several mechanisms linking mitochondrial dysfunction to BBB disruption in MS:

Mitochondrial dysfunction can lead to dysregulation of intracellular calcium levels, affecting endothelial cell contractility and junctional integrity, and impaired mitochondrial function reduces ATP availability, potentially compromising the energy-dependent processes important for maintaining BBB integrity (Witte et al., 2014). Dysfunctional mitochondria can themselves become a significant source of ROS, exacerbating oxidative stress and barrier damage (Mao et al., 2013).

The interplay between oxidative stress and mitochondrial dysfunction creates a self-perpetuating cycle of BBB disruption in MS. Targeting these processes through antioxidant therapies or mitochondrial-protective agents is a possible route for therapeutic intervention.

2.4. Astrocyte and Pericyte Dysfunction

While much attention has been focused on endothelial cells, recent research has highlighted the key roles of astrocytes and pericytes in maintaining BBB integrity. In MS, these cells undergo functional changes that contribute to barrier dysfunction:

In response to inflammatory stimuli, astrocytes become reactive and produce inflammatory mediators that further compromise BBB integrity; activated astrocytes have been shown to downregulate the expression of tight junction proteins in endothelial cells and to alter the distribution of water channels, contributing to edema formation (Argaw et al., 2012). Pericytes, which stabilize blood vessels and regulate BBB permeability, detach from the vessel wall in MS, leading to increased permeability and vascular instability (Armulik et al., 2010); recent studies have also implicated them in the regulation of immune cell trafficking across the barrier, suggesting a more complex role in disease pathogenesis (Rustenhoven et al., 2017). Altered astrocyte-endothelial interactions complete this picture, since changes in astrocyte end-feet coverage and aquaporin-4 distribution can disrupt water homeostasis and contribute to edema formation in MS lesions (Wolburg-Buchholz et al., 2009), and recent research has uncovered a role for astrocytic extracellular vesicles in modulating BBB function, opening new avenues for therapeutic intervention (Dickens et al., 2017).

The Sonic hedgehog (Shh) pathway has become a critical mediator of astrocyte-induced BBB maintenance. Alvarez et al. (2011) demonstrated that astrocyte-derived Shh is essential for maintaining BBB integrity under inflammatory conditions. Disruption of this pathway in MS may

contribute to barrier dysfunction, presenting a potential target for therapeutic strategies.

Moreover, recent studies have highlighted the role of the Wnt/β-catenin signalling pathway in BBB maintenance and repair. Lengfeld et al. (2017) showed that activation of this pathway in endothelial cells could reduce immune cell infiltration and BBB disruption in an experimental autoimmune encephalomyelitis (EAE) model of MS. This finding suggests that targeting the Wnt pathway could be a novel approach to restoring BBB integrity in MS patients.

Strategies aimed at preserving or restoring the function of astrocytes and pericytes could prove invaluable in maintaining BBB integrity and mitigating disease progression in MS.

3. Clinical Implications of BBB Disruption in MS

The disruption of the BBB in MS has far-reaching clinical implications, influencing disease progression, diagnosis, and treatment strategies. Understanding these implications is central for developing more effective management approaches for MS patients.

3.1. Disease Progression and Lesion Formation

BBB disruption is intimately linked to the formation and evolution of MS lesions. Gadolinium-enhancing lesions on magnetic resonance imaging (MRI) are indicative of active BBB breakdown and correlate with disease activity (Gaitán et al., 2011). The entry of immune cells and inflammatory mediators through the compromised BBB leads to a cascade of events:

Infiltrating immune cells attack myelin sheaths, producing the characteristic plaques observed in MS (Lassmann et al., 2007), and recent studies have revealed the complexity of these demyelination processes, including the role of cytotoxic T cells in direct axonal injury and the contribution of antibody-mediated mechanisms (Machado-Santos et al., 2018). The inflammatory milieu and oxidative stress contribute to axonal injury and neurodegeneration (Trapp & Nave, 2008), and advanced imaging techniques such as diffusion tensor imaging have provided new insights into the extent and progression of axonal damage (Filippi et al., 2018). Reactive astrocytes form glial scars that can impede remyelination and axonal regeneration (Nair et al., 2008), although recent research has uncovered both beneficial and detrimental roles for astrocytes in MS lesions, highlighting the complexity of glial responses in the disease (Liddelow et al., 2017).

The relationship between BBB disruption and lesion formation is not unidirectional. Emerging evidence suggests that subtle BBB alterations may precede visible lesion formation, potentially providing an early marker of disease activity (Cramer et al., 2014).

Recent studies have also highlighted the role of BBB disruption in cortical pathology. Calabrese et al. (2015) reviewed the origins of grey matter damage in MS, including the potential contribution of barrier dysfunction and meningeal inflammation to cortical lesions, atrophy, and cognitive impairment. This work argues for considering both white and grey matter pathology in understanding MS progression.

3.2. Biomarkers of BBB Disruption

The disruption of the BBB in MS has led to the identification of several potential biomarkers that may aid in diagnosis, prognosis, and monitoring of treatment efficacy. These biomarkers not only provide insights into disease activity but also offer opportunities for personalised treatment approaches.

Elevated levels of MMP-9 in serum and CSF have been associated with BBB breakdown and disease activity (Yong et al., 2007), and recent studies have expanded our understanding of MMP dynamics in MS, revealing distinct patterns of expression in different disease subtypes (Benešová et al., 2009). Fragments of occludin and claudin-5 in CSF may serve as markers of barrier integrity (Blyth et al., 2009), and advanced proteomic approaches have identified additional tight junction components that could serve as sensitive indicators of BBB dysfunction (Haqqani et al., 2013). The CSF/serum albumin ratio is an indicator of overall BBB permeability and has been used to assess barrier function in MS (Obermeier et al., 2013), with longitudinal studies demonstrating its prognostic value in predicting disease progression (Uher et al., 2015). Serum and CSF levels of neurofilament light chain correlate with BBB disruption and axonal damage (Disanto et al., 2017), and technological advances such as single-molecule array (Simoa) technology have dramatically improved the sensitivity of NfL detection, allowing its measurement in blood samples and potentially advancing MS monitoring (Khalil et al., 2018). Glial fibrillary acidic protein, an astrocyte-derived protein, has attracted attention as a promising marker of BBB disruption and disease activity, since elevated levels in CSF and serum correlate with gadolinium-enhancing lesions on MRI and predict long-term disability progression (Abdelhak et al., 2018). Finally, specific miRNA signatures

have been associated with BBB disruption; miR-155 in particular is central to barrier breakdown and could serve as both a biomarker and a therapeutic target (Lopez-Ramirez et al., 2014).

The integration of these biomarkers with advanced imaging techniques has opened new avenues for assessing BBB integrity in MS. Dynamic contrast-enhanced MRI (DCE-MRI) allows for quantitative measurement of BBB permeability, providing insights into subtle changes in barrier function that may precede visible lesion formation (Cramer et al., 2014). Furthermore, novel MRI techniques such as diffusion-weighted imaging and magnetization transfer ratio mapping offer complementary information on tissue microstructure and myelin content, enhancing our ability to monitor disease progression and treatment response (Filippi et al., 2019).

Emerging technologies, such as positron emission tomography (PET) with novel radiotracers targeting specific aspects of BBB function, promise to further refine our ability to assess BBB integrity in vivo. For instance, the development of radiotracers targeting P-glycoprotein, a key efflux transporter at the BBB, could provide valuable insights into drug penetration and BBB function in MS patients (van Assema et al., 2012).

The combination of these biomarkers and imaging techniques offers the potential for a broader and personalised approach to MS diagnosis and management. By providing a more nuanced understanding of BBB dysfunction in individual patients, these tools may guide treatment decisions and allow for earlier intervention in those at highest risk of disease progression.

3.3. Impact on Treatment Strategies

Understanding the mechanisms of BBB disruption in MS has significant implications for therapeutic approaches, influencing drug development, delivery strategies, and treatment monitoring:

The compromised BBB presents both challenges and opportunities for drug delivery. Increased permeability may enhance the penetration of certain therapeutic agents into the CNS, but it can also lead to undesirable side effects and reduced efficacy through non-specific distribution (Kiptoo et al., 2011); recent advances in nanotechnology have produced novel delivery systems designed to exploit BBB disruption in MS while minimising off-target effects (Saraiva et al., 2016). Developing treatments that specifically address barrier dysfunction has become a focus of research: modulators of the sphingosine-1-phosphate receptor, such as fingolimod, reduce BBB permeability and lymphocyte infiltration into the CNS (Chun & Hartung, 2010), and therapies targeting specific aspects of the barrier, such as endothelial cell adhesion molecules or tight junction proteins, have more recently entered clinical trials. Because barrier disruption is complex, combination therapies targeting multiple aspects of dysfunction may prove more effective than single-agent approaches; combining anti-inflammatory agents with BBB stabilizers or antioxidants, for instance, could provide synergistic benefits in preserving integrity and reducing disease progression. The heterogeneity of BBB disruption between patients also argues for individually tailored strategies, since integrating biomarker data with advanced imaging may allow clinicians to tailor treatment to an individual's specific pattern of dysfunction (Correale et al., 2017). Assessment of barrier integrity through imaging and biomarker analysis further provides valuable information on treatment response: the resolution of gadolinium-enhancing lesions on MRI is often used as an indicator of efficacy in clinical trials (Barkhof et al., 2009), and changes in serum NfL levels have more recently been shown to correlate with treatment response, offering a less invasive means of monitoring (Novakova et al., 2017). Ongoing research continues to uncover new targets for preserving or restoring barrier integrity, among them the Wnt/β-catenin signalling pathway, whose agonists could be developed as BBB-stabilizing therapies (Lengfeld et al., 2017), and specific microRNAs involved in barrier regulation such as miR-155 (Lopez-Ramirez et al., 2014). Cell-based approaches complete this picture: mesenchymal stem cells have demonstrated the ability to promote BBB repair through the secretion of anti-inflammatory and neurotrophic factors (Xue et al., 2018), and recent advances include engineered MSCs that overexpress specific factors to enhance their barrier-protective effects (Yu et al., 2019).

The integration of advanced imaging techniques, biomarker analysis, and personalised medicine approaches promises to advance MS management, potentially leading to improved outcomes and quality of life for patients living with this challenging disease.

4. Therapeutic Approaches Targeting BBB Disruption

The central role of BBB disruption in MS pathogenesis has led to the development of various therapeutic strategies aimed at preserving or restoring barrier integrity. These approaches range from established immunomodulatory

treatments to recent cell-based therapies and targeted interventions.

4.1. Anti-inflammatory Therapies

Many current MS treatments aim to modulate the immune response and reduce inflammation, indirectly affecting BBB integrity:

Interferon-β, a first-line therapy, stabilizes the BBB by reducing the expression of adhesion molecules and MMPs (Kraus & Oschmann, 2006), and recent studies have elucidated additional mechanisms, including the upregulation of claudin-1 expression in endothelial cells (Hashioka et al., 2010). Glatiramer acetate may protect barrier integrity by promoting the production of anti-inflammatory cytokines and neurotrophic factors (Aharoni et al., 2008) and by enhancing the regulatory T cell response (Haas et al., 2009). Dimethyl fumarate activates the Nrf2 antioxidant pathway, potentially reducing oxidative stress-induced barrier damage (Linker et al., 2011), and has also been shown to modulate endothelial cell function directly, enhancing BBB integrity through multiple mechanisms. Natalizumab targets α4-integrin, preventing leukocyte adhesion to endothelial cells and reducing permeability (Polman et al., 2006); long-term studies have confirmed its efficacy in reducing barrier disruption and disease activity, although concerns about progressive multifocal leukoencephalopathy necessitate careful patient selection and monitoring (Butzkueven et al., 2014). Finally, sphingosine-1-phosphate receptor modulators such as fingolimod and siponimod reduce BBB permeability and limit lymphocyte infiltration into the CNS (Chun & Hartung, 2010), and recent research indicates that these agents exert direct protective effects on endothelial cells and astrocytes, contributing to barrier preservation beyond their immunomodulatory properties (Spampinato et al., 2015).

Recent studies have also explored the potential of more targeted anti-inflammatory strategies. For instance, selective inhibition of the p38 mitogen-activated protein kinase (MAPK) pathway has shown promise in mitigating BBB disruption and CNS inflammation in experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS. Pharmacologic inhibition of p38 MAPK ameliorated EAE in mice, suggesting that this pathway has potential for pharmacologic intervention in MS (Krementsov et al., 2013). Similarly, targeting specific inflammatory mediators such as interleukin-17 (IL-17) and granulocyte-macrophage colony-stimulating factor (GM-CSF) offers a possible avenue for future therapeutic development (Stoolman et al., 2014).

4.2. Cell-based Therapies

Stem cell-based approaches have shown promise in restoring BBB integrity in preclinical models of MS:

Mesenchymal stem cells (MSCs) are thought to promote BBB repair through the secretion of anti-inflammatory and neurotrophic factors; controlled trials in ischemic stroke provide some supporting clinical data (Xue et al., 2018), and engineered MSCs overexpressing factors such as IL-10 or brain-derived neurotrophic factor are being explored to enhance barrier-protective effects. Transplanted neural precursor cells promote neuroprotection through an immunomodulatory mechanism in EAE (Pluchino et al., 2005), and later work has linked such effects to metabolic signalling between neural stem cells and inflammatory phagocytes (Peruzzotti-Jametti et al., 2018). Endothelial progenitor cells have been studied for vascular repair mainly in stroke, where research has focused on optimising their safety and efficacy (Kaneko et al., 2012); their role in barrier restoration in MS remains to be established. Induced pluripotent stem cells represent a further approach to personalised cell therapy, since iPSC-derived neural or endothelial cells can be generated for individual patients, although their potential for BBB repair in MS is still experimental.

4.3. Targeting Specific BBB Components

Emerging therapies aim to directly target components of the BBB to enhance its integrity:

Molecules that enhance the expression or function of claudin-5, a key tight junction protein, are being investigated for their potential to stabilize the barrier (Greene et al., 2019), and recent studies have identified small molecule enhancers of claudin-5 expression that warrant further investigation in preclinical models (Campbell et al., 2012). Selective matrix metalloproteinase inhibitors, particularly those targeting MMP-9, have shown promise in reducing barrier breakdown (Rempe et al., 2016), with recent work focusing on more specific inhibitors with improved pharmacokinetic profiles to minimise off-target effects (Vandenbroucke & Libert, 2014). Advanced drug delivery systems are being developed to transport therapeutic agents across the BBB while minimising disruption to its integrity (Saraiva et al., 2016); recent innovations include the use of exosomes as natural nanocarriers (Alvarez-Erviti et al., 2011) and the development of BBB-penetrating peptides

for enhanced drug targeting (Oller-Salvia et al., 2016). Modulators of the Wnt/β-catenin signalling pathway have also attracted attention, since agonists of this pathway reduce immune cell infiltration and barrier disruption in EAE models (Lengfeld et al., 2017). Finally, given the important role of pericytes in maintaining barrier integrity, strategies aimed at preserving or restoring pericyte function are a possible route, and recent studies have explored platelet-derived growth factor receptor-β agonists to promote pericyte survival and BBB stability (Giannoni et al., 2018).

4.4. Antioxidant and Mitochondrial-targeted Therapies

Given the role of oxidative stress in BBB disruption, antioxidant therapies have gained attention:

N-acetylcysteine has reduced oxidative damage in other models of neurological injury (Kamboj et al., 2010), but whether it limits BBB permeability in MS remains to be shown. Mitochondria-targeted antioxidants such as MitoQ, which target mitochondrial ROS production, delayed disease progression and alleviated pathology in an EAE mouse model (Mao et al., 2013); MitoQ also interacts with the Nrf2 pathway, which may influence its cellular effects (Rao et al., 2010). Activators of nuclear factor erythroid 2-related factor 2 (Nrf2) enhance the expression of antioxidant enzymes, and loss of Nrf2 exacerbates EAE (Johnson et al., 2010), with newer activators offering improved BBB penetration and reduced off-target effects (Quinti et al., 2016). Coenzyme Q10 and idebenone may protect mitochondrial function and reduce oxidative stress-induced barrier damage, although evidence in MS models is limited. Combining mitochondria-targeted antioxidants with anti-inflammatory agents is a rational strategy, given the shared contribution of mitochondrial dysfunction and oxidative stress across neurodegenerative diseases (Alqahtani et al., 2023), but it has not yet been tested systematically in EAE.

The integration of antioxidant strategies with other BBB-protective approaches may allow broader treatment of MS-related BBB dysfunction.

5. Future Directions and Challenges in BBB Disruption Research in MS

While significant progress has been made in understanding BBB disruption in MS, several challenges and opportunities for future research remain. Addressing these areas will be important for developing more effective treatments targeting BBB dysfunction in MS.

5.1. Advanced Imaging Techniques for BBB Assessment

Advances in ultra-high-field MRI have significantly improved the ability to detect the subtle BBB dysfunctions that occur in MS, allowing more precise assessment of barrier integrity not only within visible lesions but also in regions of normal-appearing white matter, and thereby offering deeper insight into disease progression and subclinical inflammation (Varatharaj & Galea, 2017). Alongside this, dynamic contrast-enhanced MRI protocols are being refined to quantify permeability changes over time and in response to treatment (Cramer et al., 2014), while PET and SPECT techniques with novel radiotracers targeting specific BBB proteins or transporters are advancing the molecular imaging of barrier components (van Assema et al., 2012).

Challenges include standardising imaging protocols across centres and correlating imaging findings with clinical outcomes.

5.2. BBB-specific Biomarkers in MS

Work in this area centres on identifying and validating blood-based markers that reflect barrier integrity, such as tight junction proteins or endothelial-derived vesicles (Blyth et al., 2009), and on developing more sensitive and specific CSF markers of barrier dysfunction, potentially including microRNAs or metabolomic profiles. A further priority is the integration of imaging, fluid, and clinical biomarkers into detailed BBB disruption profiles for individual patients (Correale et al., 2017).

The main challenge lies in translating these biomarkers from research settings to clinical practice, ensuring reproducibility and cost-effectiveness.

5.3. Targeted Therapies for BBB Repair in MS

Three directions stand out. Cell-based approaches include stem cell treatments specifically aimed at restoring barrier integrity, such as engineered endothelial progenitor cells (Xue et al., 2018). Nanoparticle-based drug delivery aims to produce BBB-penetrating carriers that can deliver therapeutic agents directly to sites of barrier disruption (Saraiva et al., 2016). A third line investigates therapies that modulate tight junctions, transporters, or basement membrane components in order to enhance barrier function in MS (Greene et al., 2019).

Challenges include ensuring the specificity of these treatments to avoid unintended effects on healthy BBB regions and demonstrating long-term efficacy and safety.

5.4. BBB Dysfunction in Progressive MS

Chronic BBB alterations are increasingly recognised as a key pathological feature of progressive MS, and persistent barrier dysfunction is thought to contribute significantly to ongoing neurodegeneration by facilitating low-grade immune cell infiltration and chronic inflammation (Minagar & Alexander, 2003). Compartmentalized inflammation behind an apparently intact barrier has also been implicated, since ectopic lymphoid follicles and localised inflammatory responses within the CNS can persist independently of peripheral immune activity, which presents a major therapeutic challenge (Machado-Santos et al., 2018). Against this background, BBB-focused neuroprotection has been investigated as an investigational strategy, aiming to prevent continuous neuronal damage and limit the progression of disability in progressive forms of the disease (Feinstein et al., 2015).

The main challenge remains the development of treatments that can effectively target BBB dysfunction within the complex and compartmentalised pathophysiology of progressive MS.

5.5. BBB and Remyelination in MS

Research here addresses how barrier dysfunction affects oligodendrocyte precursor cell migration and differentiation in MS lesions (Lubetzki et al., 2020), whether restoring BBB integrity can create a more favourable environment for remyelination (Yao et al., 2014), and how the transport of remyelination-promoting factors across the barrier might be enhanced (Chountoulesi & Demetzos, 2020; van Schaik et al., 2022; Rittchen et al., 2015).

Challenges include untangling the complex interactions between BBB repair, inflammation resolution, and remyelination processes in MS.

5.6. Personalized Approaches to BBB Dysfunction in MS

Priorities include identifying genetic variants that predispose to barrier dysfunction in MS and assessing their potential as therapeutic targets, developing methods to characterize individual patterns of BBB disruption in order to guide treatment (Varatharaj & Galea, 2017), and creating AI-driven models that can predict individual responses to barrier-targeted therapies from multi-modal data.

The main challenge is integrating diverse data types (genetic, imaging, clinical) to create actionable, personalised treatment plans targeting BBB dysfunction in MS.

5.7. Gut-Brain Axis and BBB Integrity in MS

This line of work investigates how gut microbiota alterations in MS affect barrier function and integrity (Buscarinu et al., 2017), seeks to identify specific gut-derived metabolites that modulate BBB permeability and could serve as therapeutic targets (Vascellari et al., 2020), and explores nutritional approaches that might support barrier integrity through modulation of the gut microbiome (Riccio & Rossano, 2018).

Challenges include establishing clear causal relationships between gut microbiome changes, BBB dysfunction, and MS pathogenesis, as well as developing targeted interventions.

By focusing on these BBB-specific areas of research, we can advance our understanding of MS pathogenesis and develop more targeted therapies to address BBB dysfunction in this complex neurological disorder. Bringing these approaches together should improve diagnosis, treatment, and quality of life for individuals living with MS.

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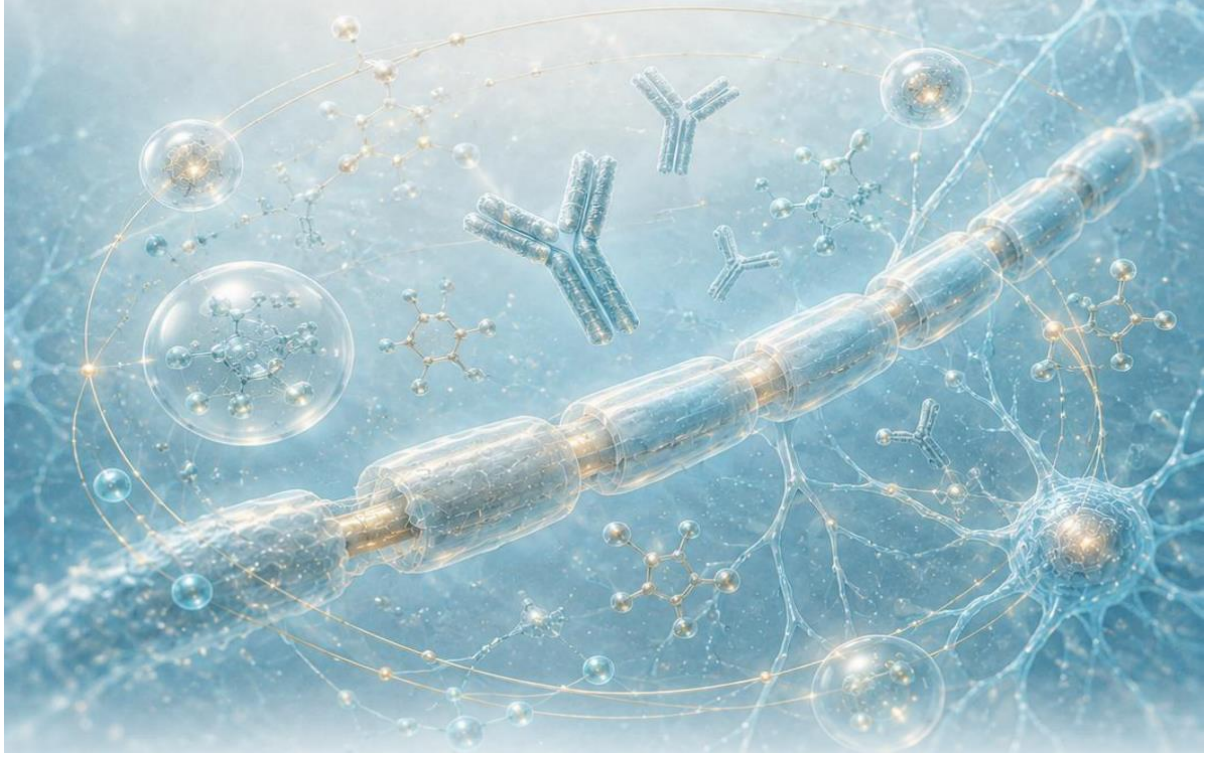
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CHAPTER 3

CURRENT TREATMENT LANDSCAPE



3.1. DISEASE-MODIFYING THERAPIES (DMTs)

1. Introduction

DMTs differ in mechanism, efficacy, safety, and monitoring requirements. Figure 3.1.1 groups the agents by mechanism; the following discussion examines how these differences influence treatment selection.

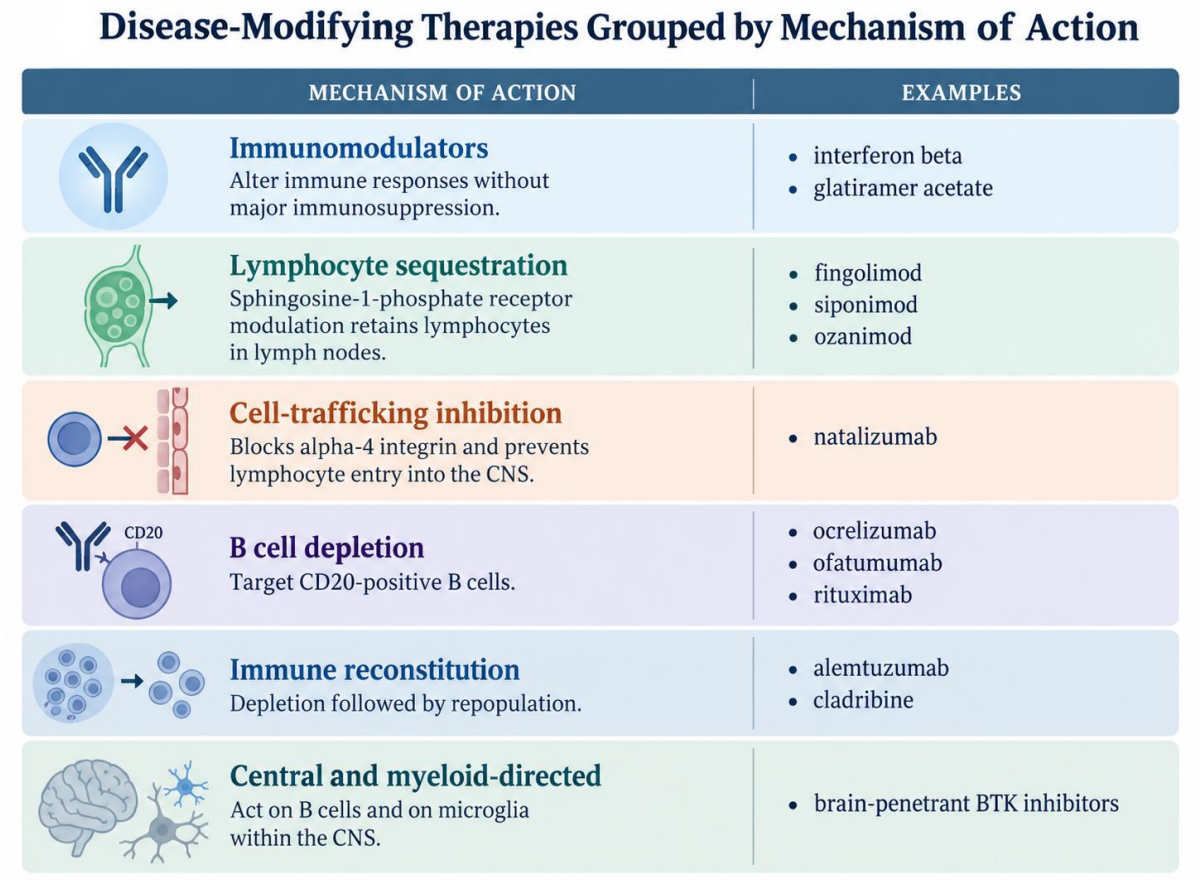


Figure 3.1.1. Disease-modifying therapies grouped by mechanism of action. Agents are listed as examples of each class and not in order of efficacy.

2. Historical Perspective and Evolution of DMTs

The development of DMTs for MS began in the early 1990s with the introduction of interferon beta-1b, marking the beginning of disease-modifying treatment in MS (Wingerchuk & Carter, 2014). This milestone was quickly followed by the approval of other interferons and glatiramer acetate, collectively known as platform therapies. These early DMTs, while modest in their efficacy compared to modern standards, represented a significant leap forward in the ability to modify the course of MS (Torkildsen et al., 2016). See Figure 3.1.2.

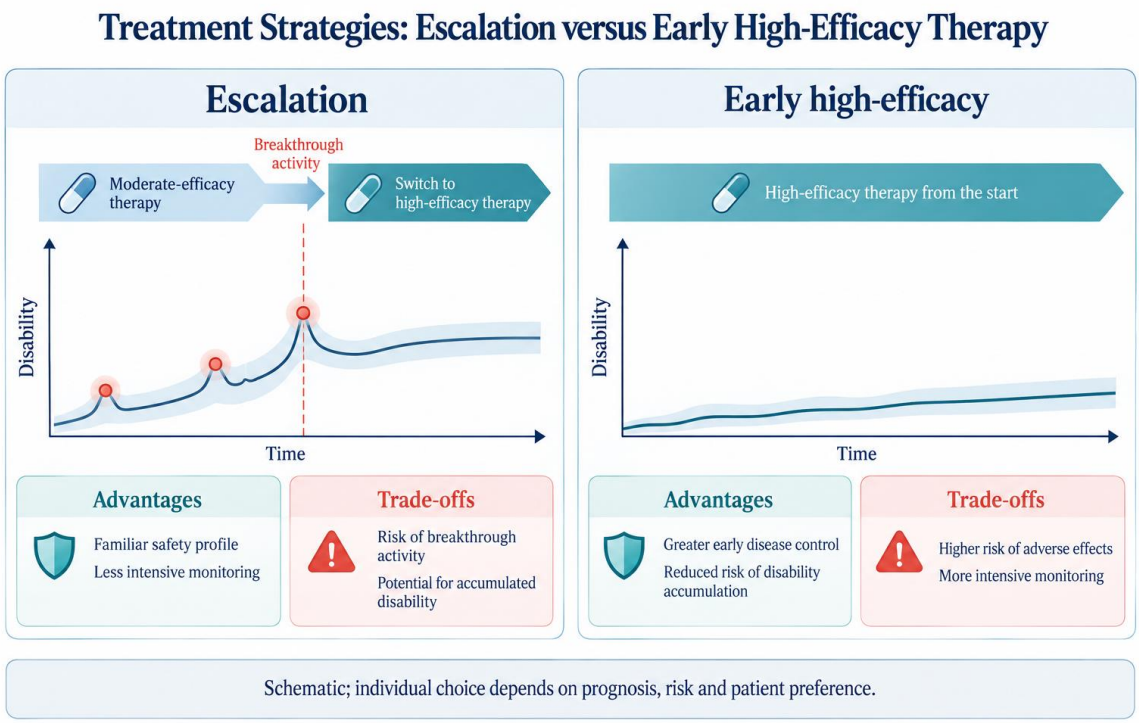


Figure 3.1.2. Escalation versus early high-efficacy treatment strategies. The curves are schematic; the choice depends on prognosis, risk and patient preference.

As the pathophysiology of MS became clearer, the range of available DMTs expanded rapidly. The early 2000s saw the introduction of natalizumab, the first monoclonal antibody therapy for MS, which showed substantial efficacy in controlled studies but came with the risk of progressive multifocal leukoencephalopathy (PML), a rare but serious adverse event (Polman et al., 2006). This development highlighted the delicate balance between efficacy and safety that would become a recurring theme in MS treatment.

The subsequent years witnessed a proliferation of new DMTs, including oral therapies like fingolimod, teriflunomide, and dimethyl fumarate, as well as high-efficacy monoclonal antibodies such as alemtuzumab, ocrelizumab, and ofatumumab (Zhang et al., 2021). Each new therapy brought unique mechanisms of action, efficacy profiles, and safety considerations, expanding the toolkit available to clinicians and patients for managing MS.

3. Current Landscape of DMTs

Today, the arsenal of approved DMTs for MS includes at least 18 therapies, spanning various mechanisms of action and routes of administration (Zhang et al., 2021). This diversity allows for more personalised treatment approaches, considering factors such as disease activity, patient preferences, comorbidities, and lifestyle considerations.

4. Mechanism of Action and Efficacy Profiles

DMTs in MS operate through various mechanisms to modulate the immune system and reduce the aberrant immune response characteristic of the disease. These mechanisms can be broadly categorized into several groups:

4.1. Immunomodulators:

Including interferons and glatiramer acetate, these therapies alter the immune response without causing significant immunosuppression (Torkildsen et al., 2016).

4.2. Immunosuppressants:

Such as fingolimod and siponimod, which sequester lymphocytes in lymph nodes (Chun & Hartung, 2010).

4.3. Cell-trafficking inhibitors:

Natalizumab, which prevents lymphocyte migration into the central nervous system (Polman et al., 2006).

4.4. B-cell depleting agents:

Ocrelizumab and ofatumumab, which target CD20+ B cells (Hauser et al., 2017).

4.5. Immune cell modulators:

Dimethyl fumarate and cladribine, which affect multiple aspects of immune function (Gold et al., 2012; Giovannoni et al., 2010).

The efficacy of these DMTs varies considerably, with newer therapies generally demonstrating superior effects on relapse rates, disability progression, and MRI outcomes compared to earlier platform therapies. For instance, natalizumab has shown superior effects on patient-reported outcomes compared to other DMTs, demonstrating the potential for these therapies to significantly improve patients' quality of life (Chen et al., 2022).

High-efficacy DMTs, particularly monoclonal antibodies, have demonstrated substantial efficacy in controlling disease activity. Ocrelizumab, for example, has shown the ability to achieve no evidence of disease activity (NEDA) in a significant proportion of patients with relapsing-remitting MS (RRMS) (Hauser et al., 2017). Similarly, alemtuzumab has demonstrated long-lasting effects on disease control, even after the completion of treatment courses (Coles et al., 2012).

The concept of treating MS with high-efficacy therapies early in the disease course, often referred to as "hit hard and early," has gained traction in recent years. This approach aims to prevent irreversible neurological damage and potentially alter the long-term trajectory of the disease (Comi et al., 2017). Evidence suggests that early intervention with high-efficacy DMTs may offer superior long-term outcomes compared to escalation approaches, particularly in patients with highly active disease (He et al., 2020).

5. Agent-by-Agent Overview of Approved DMTs

The following overview brings together, in one place, the individual agents in current use, grouped by generation. Each entry summarises the mechanism of action, the central trial evidence, the principal adverse effects and the practical limitations of the agent. The safety and tolerability issues raised here are examined in greater depth in Section 3.3.

5.1. First-Generation Injectable DMTs

5.1.1. Interferon Beta

Interferon beta was one of the first approved DMTs for MS treatment. It acts by modulating the immune system through multiple mechanisms, including inhibition of T cell activation and proliferation, reduction of blood-brain barrier permeability, and increasing the production of anti-inflammatory cytokines.

It is available in two forms, interferon beta-1a (Avonex, Rebif) and interferon beta-1b (Betaseron, Extavia). Its mechanism of action is varied: it inhibits T cell proliferation and shifts the cytokine profile from pro-inflammatory Th1 to anti-inflammatory Th2, reduces blood-brain barrier permeability and thereby limits lymphocyte migration into the CNS, and down-regulates MHC Class II expression, reducing antigen presentation. Potential neuroprotective effects through the induction of nerve growth factors have also been proposed. In terms of efficacy, interferon beta produces approximately a 30% reduction in relapse rates, slows new lesion formation on MRI by about 50-70%, and yields a modest delay in disability progression, with a 20-30% reduction in two-year confirmed disability progression.

Long-term data from the 21-year follow-up of the pivotal interferon beta-1b trial showed that early treatment was associated with improved survival (Goodin et al., 2012).

Side Effects:

1. Flu-like symptoms (fever, chills, myalgia) - affect up to 60% of patients
2. Injection site reactions - redness, pain, and rarely necrosis
3. Elevated liver enzymes - typically transient and asymptomatic
4. Depression - may exacerbate pre-existing depression
5. Thyroid dysfunction - both hyper- and hypothyroidism reported

The limitations of interferon beta are several. Its efficacy is only partial, and many patients continue to have disease activity despite treatment. The injectable route of administration may affect patient compliance, and the development of neutralizing antibodies can reduce efficacy in up to 35% of patients. Interferon beta was formerly labelled as contraindicated during pregnancy; since 2019, European labelling has allowed its use during pregnancy when clinically needed, on the basis of registry data showing no increase in adverse pregnancy outcomes (Hellwig et al., 2020). Treatment decisions around conception and pregnancy nevertheless require careful, individualized family planning.

5.1.2. Glatiramer Acetate

Glatiramer acetate is a synthetic polypeptide composed of four amino acids found in myelin basic protein. It acts by

altering T cell reactivity and increasing the number of regulatory T cells (Derwenskus, 2011).

Its mechanism of action involves competitive inhibition of myelin antigen binding to MHC molecules, the induction of antigen-specific suppressor T cells, and a shift in the T cell population from pro-inflammatory Th1 to anti-inflammatory Th2; potential neuroprotective effects through increased production of brain-derived neurotrophic factors (BDNF) have also been described. In terms of efficacy, glatiramer acetate produces approximately a 30% reduction in relapse rates and a reduction in MRI activity, including new T2 lesions and gadolinium-enhancing lesions, and it may slow brain atrophy, although data on this point are mixed.

Long-term data from the 15-year follow-up of the US Glatiramer Acetate trial showed sustained efficacy and safety (Ford et al., 2010).

Side Effects:

1. Injection site reactions - most common side effect, affecting up to 90% of patients

2. Immediate post-injection systemic reaction - flushing, chest tightness, palpitations, anxiety (occurs in about 10% of patients)

3. Lipoatrophy - with long-term use at injection sites

Its limitations include modest efficacy, similar to that of interferon beta, and the need for frequent subcutaneous injection (20 mg daily or 40 mg three times weekly), which may affect patient compliance. The lack of biomarkers to predict response is a further difficulty, since it makes ideal candidates for treatment hard to identify.

5.2. Second-Generation DMTs

5.2.1. Natalizumab

Natalizumab is a humanized monoclonal antibody against α4-integrin. It reduces inflammation in MS by preventing lymphocytes from crossing the blood-brain barrier (Rudick et al., 2006).

Its mechanism of action begins with binding to α4β1-integrin on lymphocytes, which prevents their adhesion to vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells; this inhibits lymphocyte migration across the blood-brain barrier, and additional effects on cell signalling and activation have also been proposed. Natalizumab is highly effective, producing up to a 68% reduction in the annualised relapse rate, an 83% reduction in new or enlarging T2 lesions on MRI, and a 42% reduction in the risk of disability progression at two years.

The AFFIRM trial demonstrated the high efficacy of natalizumab in relapsing-remitting MS (Polman et al., 2006).

Side Effects:

1. Infusion reactions - headache, dizziness, nausea (common but usually mild)

2. Liver enzyme elevations - usually asymptomatic and reversible

3. Increased risk of progressive multifocal leukoencephalopathy (PML) - rare but potentially fatal

Its limitations are significant. The risk of PML is the foremost concern, particularly in JC virus-positive patients with prolonged treatment duration, and rebound disease activity upon discontinuation can lead to severe relapses. The monthly intravenous infusion requires regular hospital visits, and the high cost may limit access in some healthcare systems.

5.2.2. Fingolimod

Fingolimod was the first oral DMT approved for MS. It is a sphingosine-1-phosphate receptor modulator that acts by preventing lymphocytes from exiting lymph nodes (Galán Sánchez-Seco et al., 2017).

Its mechanism of action is based on functional antagonism of S1P receptors, which causes internalization of these receptors on lymphocytes and results in the sequestration of lymphocytes in lymph nodes, reducing their availability to enter the CNS; direct effects on CNS cells, including neuroprotective and repair-promoting actions, have also been proposed. Fingolimod produces a 54% reduction in the annualised relapse rate compared to placebo, a 74% reduction in new or enlarging T2 lesions on MRI, and a 30% reduction in disability progression at two years.

The FREEDOMS and TRANSFORMS trials demonstrated the efficacy of fingolimod in relapsing-remitting MS (Kappos et al., 2010; Cohen et al., 2010).

Side Effects:

1. Bradycardia and atrioventricular block - particularly with the first dose

2. Macular edema - occurs in about 0.5% of patients

3. Elevated liver enzymes - usually asymptomatic but require monitoring

4. Lymphopenia - increases risk of infections

5. Increased risk of herpes virus infections

Several limitations shape its use. First-dose observation is required because of cardiac effects, and regular ophthalmological and dermatological screening is needed during

treatment. The risk of infections is increased, particularly varicella zoster virus reactivation, and rebound disease activity may follow discontinuation. The drug is also contraindicated in pregnancy owing to teratogenic effects observed in animal studies.

5.3. Third-Generation DMTs

5.3.1. Dimethyl Fumarate

Dimethyl fumarate is an oral DMT that activates the Nrf2 pathway, exerting anti-inflammatory and antioxidant effects (Bomprezzi, 2015).

Its mechanism of action involves activation of the Nrf2 transcriptional pathway, leading to upregulation of antioxidant genes, inhibition of the NF-κB pathway, which reduces pro-inflammatory cytokine production, and modulation of immune cell differentiation in favor of anti-inflammatory phenotypes. Dimethyl fumarate produces a 53% reduction in the annualised relapse rate, an 85% reduction in new or enlarging T2 lesions on MRI, and a 38% reduction in disability progression at two years.

The DEFINE and CONFIRM trials demonstrated the efficacy of dimethyl fumarate in relapsing-remitting MS (Gold et al., 2012; Fox et al., 2012).

Side Effects:

1. Flushing - affects up to 40% of patients, usually decreases over time
2. Gastrointestinal symptoms - nausea, diarrhea, abdominal pain
3. Lymphopenia - increases risk of infections, including PML

Its limitations centre on tolerability and monitoring: gastrointestinal side effects may affect patient compliance, especially in the first few weeks of treatment, and the risk of severe lymphopenia requires regular monitoring of lymphocyte counts. Rare cases of PML have been reported, mostly in the setting of severe, prolonged lymphopenia.

5.3.2. Teriflunomide

Teriflunomide is an oral DMT that inhibits dihydroorotate dehydrogenase, an enzyme necessary for de novo pyrimidine synthesis. This leads to a reduction in proliferation of activated T and B cells (Oh & O'Connor, 2013).

Its mechanism of action rests on the inhibition of rapidly dividing lymphocytes through pyrimidine synthesis blockade, alongside alteration of integrin-mediated cell adhesion and potential antiviral effects. Teriflunomide produces a 31-36% reduction in the annualised relapse rate, a 67-80% reduction in gadolinium-enhancing lesions on MRI, and a 26-31% reduction in disability progression at two years.

The TEMSO and TOWER trials demonstrated the efficacy of teriflunomide in relapsing-remitting MS (O'Connor et al., 2011; Confavreux et al., 2014).

Side Effects:

1. Hair thinning - usually reversible
2. Elevated liver enzymes - require regular monitoring
3. Gastrointestinal symptoms - nausea, diarrhea
4. Peripheral neuropathy - rare but potentially serious

Its most important limitation is teratogenicity, which requires strict contraception and a prolonged elimination procedure if pregnancy is planned. Its efficacy is moderate compared to some newer DMTs, and its effects on CYP enzymes create the potential for drug interactions.

5.3.3. Ocrelizumab

Ocrelizumab is a humanized monoclonal antibody targeting CD20+ B cells. It is the first DMT approved for both relapsing and primary progressive forms of MS (Hauser et al., 2017; Montalban et al., 2017).

Its mechanism of action is the depletion of CD20+ B cells through antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, which reduces antigen presentation and pro-inflammatory cytokine production by B cells and may also affect pathogenic B cell-T cell interactions. In relapsing MS, ocrelizumab produces a 46-47% reduction in the annualised relapse rate compared with interferon beta-1a, a 94-95% reduction in gadolinium-enhancing lesions on MRI, and approximately a 40% lower hazard of disability progression confirmed for 12 weeks in the pooled 96-week OPERA trials. In primary progressive MS, it was associated with a 24% lower hazard of disability progression confirmed for 12 weeks compared with placebo during ORATORIO follow-up, and with less worsening on the timed 25-foot walk at week 120; this functional endpoint should be distinguished from confirmed EDSS progression.

The OPERA I and II trials demonstrated efficacy in relapsing MS, while the ORATORIO trial showed efficacy in primary progressive MS.

Side Effects:

1. Infusion-related reactions - most common side effect, usually mild to moderate
2. Increased risk of infections - particularly upper respiratory tract infections

3. Slight increase in malignancies observed in clinical trials

Its limitations reflect its relative novelty: long-term safety data are still limited, there is a potential increased risk of certain infections and malignancies, and the effects of long-term B cell depletion are not fully understood. The high cost of the drug may also limit access in some healthcare systems.

5.4. Bruton's Tyrosine Kinase Inhibitors

Bruton's tyrosine kinase (BTK) inhibitors are the most substantial addition to MS therapeutics since the anti-CD20 antibodies, and they differ from every agent discussed above in one important respect: several are brain-penetrant and act not only on peripheral B cells but also on microglia and other myeloid cells within the central nervous system. This mechanism is directly relevant to the argument of this book, because chronic compartmentalised inflammation behind an intact blood–brain barrier is precisely the process that peripheral immunomodulation leaves untouched, and it is also the process that cell- and vesicle-based approaches aim to address.

Evobrutinib was the first agent of this class to reach phase III in relapsing MS, but its two phase III trials failed to demonstrate superiority over teriflunomide on annualised relapse rate, and development in MS was not pursued further (Montalban et al., 2024).

Tolebrutinib, a brain-penetrant covalent BTK inhibitor, produced the first positive randomised phase III result in non-relapsing secondary progressive MS. In HERCULES, 1,131 participants with non-relapsing SPMS were randomised 2:1 to tolebrutinib or placebo; 6-month confirmed disability progression was reduced relative to placebo (hazard ratio 0.69; 95% CI 0.55-0.88), with confirmed disability improvement in 10% versus 5% of participants (Fox et al., 2025). In relapsing MS, however, GEMINI 1 and 2 did not show superiority over teriflunomide for annualised relapse rate, so the disability findings from the pooled analysis should be interpreted as secondary to the failed primary efficacy comparison (Oh et al., 2025). PERSEUS in primary progressive MS did not meet its primary endpoint. Liver injury remains the principal safety concern and requires laboratory monitoring. The regulatory picture also changed during 2026: after the U.S. complete response letter in December 2025, the European Union granted marketing authorization for tolebrutinib (Cenrifki) on 19 June 2026 for adults with secondary progressive MS without relapses in the preceding 2 years (European Medicines Agency, 2026).

Fenebrutinib, a reversible, non-covalent and highly selective BTK inhibitor, completed a three-trial phase III program. In the two relapsing-MS studies, FENhance 1 and FENhance 2, which together randomised 1,497 participants against teriflunomide over at least 96 weeks, annualised relapse rate was reduced by 51.1% and 58.5%, respectively, with significant reductions in gadolinium-enhancing and new or enlarging T2 lesions; disability-progression endpoints showed favourable trends without reaching clear separation. In FENtrepid, in primary progressive MS, fenebrutinib met its primary endpoint of non-inferiority to ocrelizumab on 12-week composite confirmed disability progression. Liver enzyme elevations were comparable to teriflunomide, but a numerical excess of deaths in the fenebrutinib arms of the relapsing-MS studies has been the subject of continuing analysis, and full peer-reviewed publication of these datasets should be awaited before firm conclusions are drawn (Roche, 2026b, 2026c). As of 6 October 2026, the FDA had accepted the new drug application under priority review; acceptance for review is not marketing authorisation (Roche, 2026a).

For the purposes of this book, the significance of the BTK inhibitor class is twofold. It demonstrates that progression independent of relapse activity is, at least in part, a treatable target, which raises the evidential bar that cell- and exosome-based therapies must eventually clear; and it establishes central, myeloid-directed mechanisms as a legitimate therapeutic axis, which is the same axis on which mesenchymal stromal cell and extracellular vesicle products are hypothesised to act.

6. Challenges in DMT Selection and Management

While the expanding array of DMTs has broadened treatment options, it has also introduced complexity into the decision-making process for clinicians and patients. Several key challenges have emerged in the selection and management of DMTs:

6.1. Individualized Treatment Selection

The heterogeneity of MS presentation and progression complicates treatment decisions, necessitating a personalised approach to therapy selection (Sormani et al., 2016). The choice of DMT is influenced by disease activity and severity, by patient age and comorbidities, and by pregnancy planning, as well as by patient preferences and lifestyle considerations, the safety profile and monitoring requirements of the agent, and its route of administration.

Balancing these factors requires a nuanced understanding of each DMT's risk-benefit profile and careful consideration of individual patient characteristics. The development of decision support tools and treatment algorithms has aimed to assist clinicians in navigating these complex choices (Ontaneda et al., 2019).

6.2. Treatment Adherence

Adherence to DMTs remains a critical challenge in MS management, with studies indicating suboptimal adherence rates among patients (Banks et al., 2020). Non-adherence is driven by treatment fatigue, by side effects and tolerability issues, and by a perceived lack of efficacy, as well as by complex administration schedules and by cost and access barriers.

Addressing these barriers through patient education, support programs, and shared decision-making is key for optimising treatment outcomes (Köpke et al., 2017). The development of long-acting formulations and novel delivery systems offers potential solutions to some adherence challenges but also introduces new considerations in treatment selection and management (Kolb-Mäurer et al., 2019).

Patient perceptions of treatment convenience significantly influence adherence to therapy. Oral DMTs are associated with higher satisfaction due to ease of use, while infusion therapies are rated higher for overall satisfaction and perceived effectiveness (Turčáni et al., 2020).

6.3. Safety Monitoring and Risk Management

The immunomodulatory nature of DMTs necessitates careful safety monitoring and risk management strategies. The principal safety concerns are opportunistic infections, such as PML with natalizumab; autoimmune complications, such as thyroid disorders with alemtuzumab; cardiovascular risks, such as bradycardia on fingolimod initiation; and malignancy risks, particularly with long-term immunosuppression.

Implementing robust safety monitoring protocols, including regular laboratory testing, imaging, and clinical assessments, is essential for mitigating these risks. The development of risk stratification tools, such as the JC virus antibody index for PML risk with natalizumab, has improved the ability to tailor treatment decisions to individual risk profiles (Ho et al., 2017).

6.4. Economic Considerations and Access

The high costs associated with DMTs present significant challenges for healthcare systems and patients alike. The economic burden of MS, largely driven by DMT costs, has raised concerns about the sustainability of current treatment paradigms (Hartung, 2021). Strategies proposed to address these challenges include the development of biosimilars and follow-on products, value-based pricing models, cost-effectiveness analysis to inform treatment decisions, and patient assistance programs to improve access.

Balancing the need for effective treatment with economic considerations remains a complex challenge, requiring collaboration between healthcare providers, payers, policymakers, and the pharmaceutical industry (Simoens, 2022).

7. Special Populations and Considerations

Certain patient populations require special consideration in the management of MS with DMTs:

7.1. Pediatric MS

The management of pediatric MS presents unique challenges, given the potential long-term impacts of both the disease and its treatments on developing systems. Key considerations are the limited number of therapies approved for pediatric use, concerns about long-term safety and about effects on growth and development, the impact of the disease on cognitive function and academic performance, and the psychosocial aspects of managing a chronic disease in youth.

Recent trends indicate a shift towards earlier initiation of DMTs in pediatric MS, with therapies like fingolimod gaining approval for use in this population (Yearwood & Wilbur, 2023). However, long-term safety data remain limited, necessitating careful risk-benefit assessment and close monitoring in pediatric patients (Ghezzi et al., 2010).

7.2. Pregnancy and Family Planning

Managing MS in women of childbearing age requires careful consideration of the potential impacts of DMTs on fertility, pregnancy outcomes, and fetal development. The main issues are the teratogenic potential of certain DMTs, the need for contraception during treatment with some therapies, the potential for disease reactivation upon treatment discontinuation, and the balance between maternal health and fetal safety.

Recent research has focused on developing evidence-based guidelines for DMT use in pregnant women with MS, aiming to optimise outcomes for both mother and child (Coyle et al., 2019). The establishment of pregnancy registries and long-term follow-up studies has provided valuable insights into the safety of various DMTs during pregnancy and lactation (Hellwig et al., 2020).

7.3. Aging Population with MS

As the MS population ages, new challenges emerge in the management of DMTs in older adults. Risk-benefit profiles change with advancing age and accumulating comorbidities, the question of DMT discontinuation arises in stable patients, age-related comorbidities must be managed alongside MS, and cognitive and physical rehabilitation needs become more prominent.

Studies have investigated the appropriateness of DMT discontinuation in older patients with MS, considering factors such as disease stability, side effect burden, and overall health status (Zhu & Xia, 2024). The development of tailored treatment strategies for older adults with MS remains an active area of research (Sanai et al., 2016).

8. Emerging Trends and Future Directions

The field of MS treatment continues to expand, with several trends shaping the future of DMT use:

8.1. Personalized Medicine and Biomarkers

Advances in biomarker research offer the potential for more precise treatment selection and monitoring. Serum neurofilament light chain (sNfL) has developed into a marker of disease activity and treatment response (Kuhle et al., 2019), genetic markers such as HLA types may indicate treatment response and risk (Fragoso, 2018), and advanced imaging techniques are being developed for assessing disease progression and treatment effects.

These biomarkers may enable more targeted use of DMTs, potentially improving outcomes and reducing unnecessary treatment exposure.

8.2. Novel Therapeutic Approaches

Ongoing research is exploring several new avenues for MS treatment: remyelination therapies to promote repair of damaged myelin (Lubetzki et al., 2020), neuroprotective agents to prevent neurodegeneration, cell-based therapies including stem cell transplantation (Burt et al., 2019), and combination therapies targeting multiple aspects of MS pathology.

These innovative approaches hold the potential to complement existing DMTs, addressing aspects of MS not fully targeted by current treatments.

8.3. Advanced Drug Delivery Systems

Recent advancements in drug delivery technologies aim to enhance treatment adherence and reduce the therapeutic burden for patients with MS. Long-acting formulations of disease-modifying therapies are being developed to decrease dosing frequency, novel administration routes such as subcutaneous formulations of therapies traditionally given intravenously are improving patient comfort and convenience, and nanotechnology-based delivery systems are being used to enhance CNS penetration and ensure more effective targeting of therapeutic agents.

These innovations are designed to improve the convenience and efficacy of DMT administration, potentially leading to better adherence and increased patient satisfaction (Ezike et al., 2023).

8.4. Real-World Evidence and Comparative Effectiveness Research

The importance of real-world evidence in guiding MS treatment decisions has gained increasing recognition. Large-scale observational studies, registries, and advanced analytical techniques are providing valuable insights into the long-term effectiveness and safety of DMTs in clinical practice (Kalincik et al., 2017). This real-world data complements information from randomised controlled trials, offering a more thorough view of DMT performance across diverse patient populations and treatment settings.

Comparative effectiveness research, leveraging advanced statistical methods and big data analytics, is enhancing our understanding of how different DMTs perform relative to one another in real-world settings. These studies inform clinical decision-making, health policy, and guideline development, contributing to more evidence-based and patient-centered MS care (Tramacere et al., 2015).

8.5. Precision Dosing and Treatment Optimization

Efforts to optimise DMT use through precision dosing and individualized treatment regimens are gaining momentum. Proposed approaches include therapeutic drug monitoring to guide dosing decisions, pulsed or intermittent dosing strategies to minimise long-term immunosuppression, and combination therapies tailored to individual disease characteristics.

These strategies aim to maximize efficacy while minimising side effects and long-term risks associated with DMT use (Sellebjerg et al., 2020).

9. Conclusion

The range of disease-modifying therapies (DMTs) for multiple sclerosis (MS) has undergone a substantial transformation over the past three decades. The extensive body of literature reviewed here reflects the broad scope of research

in the field, covering various aspects including the efficacy and safety of DMTs, their economic impacts, use in special populations, and future directions. The complexity and continuously evolving nature of MS treatment are pointed to by this diverse array of studies.

The role of DMTs in MS management has evolved far beyond a simple treatment approach. Advancements such as personalised medicine, the use of biomarkers, novel therapeutic targets, and the integration of real-world data continue to transform the care of MS patients. These developments offer marked options for controlling disease activity and improving patient outcomes. Better knowledge of pathophysiology, together with a wider choice of treatments, brings genuinely individualized management within closer reach.

However, significant problems remain in the field of MS treatment. These include optimising treatment selection, addressing adherence issues, managing special populations, and ensuring equitable access to these life-changing therapies. The future of MS treatment lies in addressing these challenges through the integration of advanced therapeutics, personalised medicine approaches, and integrated care models that address all aspects of patient well-being.

Looking ahead, research in this field is likely to focus on developing more effective and safer treatments, optimising treatment strategies, and gaining a better understanding of the underlying mechanisms of MS. By leveraging emerging technologies, real-world evidence, and patient-centered research, the field of MS management continues to advance, offering hope for improved outcomes and quality of life for individuals living with this challenging condition.

Continued improvement in MS care will depend on coordinated work among researchers, clinicians, patients, and policymakers. The practical priority is to translate trial evidence into treatment choices that improve clinical outcomes and quality of life. Refinement of treatment strategies should be guided by comparative effectiveness, long-term safety data, patient priorities, and real-world outcomes rather than by novelty alone.

This detailed review of the literature shows how varied and unsettled DMT use has become in MS treatment. Clinicians, researchers, and policymakers should continue to use this wealth of information to develop evidence-based, patient-centered, and sustainable treatment strategies. By doing so, we can strive to improve the quality of life for MS patients, slow the progression of the disease, and ultimately lead to better outcomes for those affected by this complex neurological disorder.

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3.2. SYMPTOMATIC TREATMENTS

1. Introduction

Symptom management complements disease modification and addresses physical, cognitive, and psychological difficulties that may persist despite control of inflammatory activity (Shah, 2015; Thompson et al., 2018). Drug treatment, rehabilitation, and support need to be matched to the individual patient (Chiaravalloti & DeLuca, 2008; Frohman et al., 2011). Figure 3.2.1 summarises these components.

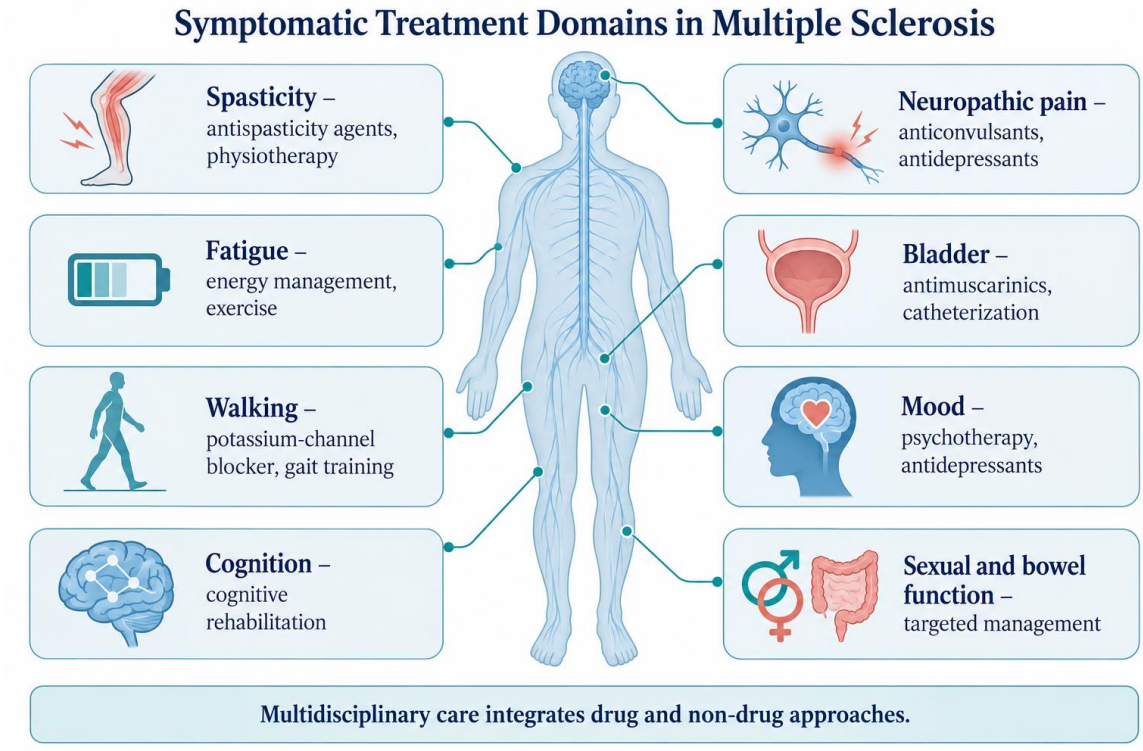


Figure 3.2.1. Symptomatic treatment domains in multiple sclerosis, combining pharmacological and non-pharmacological approaches within multidisciplinary care.

1.1. The Evolving Landscape of Symptomatic Management in MS

Symptomatic care aims to improve daily function and quality of life alongside DMT treatment (Abboud et al., 2017; Thompson et al., 2018). Assessment should identify the symptoms most relevant to each patient and the factors that aggravate them (Filippi et al., 2019; Krupp et al., 2010; Nasios et al., 2020).

1.2. Shift Towards Holistic and Patient-Centered Care Models

Patient-centred care considers physical symptoms, cognition, emotional well-being, and social circumstances together (Ben-Zacharia et al., 2023; Lakin et al., 2021; Zhang et al., 2020). Secondary effects, including reduced participation and psychological distress, also require attention (Carletto et al., 2016; Sintzel, 2018).

2. Pharmacological Interventions: A Cornerstone of Symptom Management

Pharmacological interventions remain a cornerstone in the management of MS symptoms, offering targeted relief for a wide range of manifestations (Frohman et al., 2011; Shah, 2015). The arsenal of medications available to MS patients has expanded significantly, providing more options for tailored treatment approaches (Grossman et al., 2010; Koch et al., 2011).

2.1. Cannabinoid-based Therapies

The use of cannabinoid-based therapies in MS has garnered considerable attention in recent years (Chiurchiù et al., 2018; Nouh et al., 2024). These interventions have shown promise in alleviating symptoms such as spasticity, pain, and tremors (Koppel et al., 2014; Novotna et al., 2011). The endocannabinoid system, which plays a role in regulating neurotransmission and neuroinflammation, has

come to be seen as a potential therapeutic target in MS (Tankou et al., 2018).

2.1.1. Sativex and Its Applications

Sativex (nabiximols), an oromucosal spray containing delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), has been approved in several countries for the treatment of MS-related spasticity (Novotna et al., 2011; Chiurchiù et al., 2018). Clinical trials have demonstrated its efficacy in reducing spasticity and improving sleep quality in MS patients (Nouh et al., 2024). However, the use of cannabinoids in MS management remains controversial in some regions due to regulatory constraints and concerns about long-term safety (Koppel et al., 2014).

2.2. Corticosteroids and Immunomodulators

Corticosteroids remain central to the management of acute MS exacerbations (Berkovich, 2013; Erwin et al., 2011). Pulse-dose methylprednisolone therapy is widely used to reduce inflammation and shorten the duration of relapses (Berkovich, 2013; de Sèze et al., 2007). While effective in the short term, the long-term use of corticosteroids must be carefully monitored due to potential side effects, including osteoporosis, diabetes, and increased susceptibility to infections (Vasylovskyy et al., 2024).

2.2.1. Interferon Therapy and Its Dual Role

Interferon therapy, primarily considered a disease-modifying treatment, also contributes to symptomatic management by reducing the frequency and severity of relapses (Alajbegovic et al., 2012; Iaffaldano et al., 2012). However, the potential for exacerbation of certain symptoms, such as trigeminal neuralgia, calls for careful monitoring and individualized treatment plans (Bischof & Sprenger, 2014; Brass et al., 2014).

2.2.2. Emerging Research on Disease-Modifying Therapies

Emerging research suggests that some disease-modifying therapies may have direct symptomatic benefits beyond their impact on disease progression (Chan et al., 2017; Otero-Romero et al., 2016). For instance, natalizumab, a monoclonal antibody used in relapsing-remitting MS, has been associated with improvements in fatigue and cognitive function (Iaffaldano et al., 2012).

3. Symptom-Specific Pharmacological Interventions

3.1. Spasticity Management

Spasticity, a common and often debilitating symptom of MS, requires a heterogeneous approach to management (Otero-Romero et al., 2016; Erwin et al., 2011). α2-adrenergic agonists like tizanidine hydrochloride have demonstrated effectiveness in reducing muscle tone and improving flexibility (Dalgas et al., 2009). Baclofen, a GABA-B receptor agonist, is another widely used antispasmodic medication in MS (Erwin et al., 2011; de Sèze et al., 2007). For severe cases of spasticity, intrathecal baclofen therapy via implanted pumps can provide more targeted relief with fewer systemic side effects (Erwin et al., 2011; Esquenazi et al., 2010).

3.1.1. Combination Therapies for Spasticity

The management of spasticity often necessitates a combination of pharmacological interventions and physical therapy, which is why a multidisciplinary approach matters (Otero-Romero et al., 2016; Papeix et al., 2015). Botulinum toxin injections have also emerged as an effective treatment for focal spasticity in MS, offering localised relief with minimal systemic effects (Esquenazi et al., 2010).

3.2. Fatigue Management

Fatigue, one of the most prevalent and disabling symptoms of MS, poses significant challenges in management (Krupp et al., 2010; Braley & Chervin, 2010). While the pathophysiology of MS-related fatigue is not fully understood, it is believed to involve both central and peripheral mechanisms (Lange et al., 2009; van den Akker et al., 2016).

3.2.1. Pharmacological Approaches to Fatigue

Amantadine and modafinil are sometimes used for MS fatigue, but the randomised TRIUMPHANT-MS crossover trial did not demonstrate superiority over placebo and reported more adverse events with active medication (Nourbakhsh et al., 2021). Earlier small-study signals should be interpreted in light of that controlled comparison.

3.2.2. Emerging Treatments for Fatigue

Histamine H3 receptor antagonists, which enhance histaminergic neurotransmission and promote wakefulness, have been proposed as candidates for managing MS-related fatigue, but controlled clinical evidence in MS is currently lacking (Braley & Chervin, 2010).

3.3. Cognitive Dysfunction

Cognitive impairment affects a considerable proportion of MS patients and can have profound impacts on quality of life and functional capacity (Chiaravalloti & DeLuca, 2008; Rosti-Otajärvi & Hämäläinen, 2014). While no medications are specifically approved for treating cognitive dysfunction in MS, several agents have been studied for their potential cognitive-enhancing effects (Amato et al., 2013; Iaffaldano et al., 2012).

3.3.1. Acetylcholinesterase Inhibitors and Cognitive Function

An early donepezil study reported memory-related benefit (Krupp et al., 2004). This does not establish routine efficacy for cognitive impairment in MS. The memantine trial by Peyro Saint Paul et al. (2016) concerns a different drug class and should be assessed separately.

3.3.2. NMDA Receptor Antagonists in Cognitive Impairment

Memantine, an NMDA receptor antagonist, has been tested for cognitive impairment in MS. The randomised placebo-controlled study by Peyro Saint Paul et al. (2016) did not demonstrate a significant benefit on the primary cognitive outcome. Its findings do not support presenting memantine as an established treatment for MS-related cognitive impairment.

3.4. Pain Management

Pain in MS can take various forms, including neuropathic pain, musculoskeletal pain, and headaches (Truini et al., 2013; Solaro & Uccelli, 2011). The management of MS-related pain often requires a multimodal approach, combining pharmacological and non-pharmacological interventions.

3.4.1. Neuropathic Pain Treatment

For neuropathic pain, anticonvulsants such as gabapentin and pregabalin are commonly prescribed (Truini et al., 2013; Koppel et al., 2014). These medications modulate calcium channels and neurotransmitter release, providing relief for conditions like trigeminal neuralgia and dysesthesias (Bischof & Sprenger, 2014).

3.4.2. Antidepressants in Pain Management

Tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors (SNRIs) have also shown efficacy in managing neuropathic pain in MS (Koch et al., 2011; Hind et al., 2014). These agents work by modulating pain pathways in the central nervous system.

3.4.3. Advanced Interventions for Refractory Pain

In cases of severe, refractory pain, interventional techniques such as spinal cord stimulation or intrathecal drug delivery systems may be considered (Solaro & Uccelli, 2011). These approaches can provide targeted pain relief while minimising systemic side effects (Truini et al., 2013).

3.5. Bladder and Bowel Dysfunction

Urinary and bowel symptoms are common in MS and can significantly impact quality of life (de Sèze et al., 2007; Cruz et al., 2011). Anticholinergic medications, such as oxybutynin and tolterodine, are often used to manage overactive bladder symptoms in MS patients (de Sèze et al., 2007).

3.5.1. Management of Urinary Retention

For patients with urinary retention, alpha-blockers or intermittent catheterization may be necessary (Cruz et al., 2011). In severe cases, botulinum toxin injections into the detrusor muscle have shown efficacy in reducing urinary incontinence and improving quality of life (Esquenazi et al., 2010; Otero-Romero et al., 2016).

3.5.2. Bowel Dysfunction Treatment

Management of bowel dysfunction in MS often involves a combination of dietary modifications, lifestyle changes, and pharmacological interventions (Otero-Romero et al., 2016). Laxatives, stool softeners, and prokinetic agents may be used to address constipation, while antidiarrheal medications can help manage bowel incontinence (Cruz et al., 2011).

4. Non-Pharmacological Approaches: Complementing Medical Management

While pharmacological interventions form a central component of MS symptom management, non-pharmacological approaches play an equally important role in comprehensive care (Marziniak et al., 2018; Corry et al., 2019). These interventions not only address specific symptoms but also contribute to overall well-being and functional improvement (Latimer-Cheung et al., 2013).

4.1. Exercise and Physical Rehabilitation

Exercise and physical activity have been associated with improvements in various MS-related symptoms, including fatigue, spasticity, and mobility limitations (Dalgas et al.,

2009; Motl et al., 2009). The benefits of exercise extend beyond physical symptoms, with emerging evidence suggesting positive effects on cognitive function and mood in MS patients (Latimer-Cheung et al., 2013; Snook & Motl, 2008).

4.1.1. Aerobic Exercise Benefits

Aerobic exercise has been shown to improve cardiovascular fitness, reduce fatigue, and enhance overall quality of life in MS patients (Motl et al., 2009; Dalgas et al., 2009). Resistance training can help maintain muscle strength and bone density, which are particularly important given the increased risk of osteoporosis in MS patients (Dalgas et al., 2009).

4.1.2. Specialized Exercise Programs

Specialized exercise programs, such as aquatic therapy and Pilates, have also demonstrated benefits in MS symptom management (Castro-Sánchez et al., 2012; Kalron et al., 2016). Aquatic therapy, in particular, can be beneficial for patients with heat sensitivity or balance issues, as the buoyancy of water provides support and reduces the risk of falls (Kalron et al., 2016).

4.2. Cognitive Rehabilitation

Cognitive rehabilitation represents a critical aspect of non-pharmacological management in MS (Chiaravalloti & DeLuca, 2008; Rosti-Otajärvi & Hämäläinen, 2014). With cognitive impairment affecting up to 70% of MS patients, targeted interventions aimed at improving cognitive function are increasingly recognised as essential components of comprehensive care (Amato et al., 2013; Goverover et al., 2008).

4.2.1. Cognitive Training Strategies

Cognitive rehabilitation programs typically focus on strategies to improve attention, memory, executive function, and information processing speed (Chiaravalloti et al., 2013; Peyro Saint Paul et al., 2016). These interventions may include computer-based cognitive training, strategy training, and compensatory techniques to manage cognitive deficits in daily life (Rosti-Otajärvi & Hämäläinen, 2014; Nasios et al., 2020).

4.2.2. Virtual and Augmented Reality in Cognitive Rehabilitation

Recent advances in cognitive rehabilitation for MS have explored the potential of virtual reality (VR) and augmented reality (AR) technologies (Maggio et al., 2019; Peruzzi et al., 2016). These immersive environments offer novel approaches to cognitive training, providing engaging and customizable exercises that can be tailored to individual patient needs (Marziniak et al., 2018).

4.3. Mind-Body Therapies

Mind-body therapies, such as yoga, meditation, and mindfulness-based stress reduction (MBSR), have gained attention for their potential in managing MS symptoms (Grossman et al., 2010; Rogers & MacDonald, 2015). These practices offer a holistic approach to symptom management, addressing both physical and psychological aspects of the disease (Carletto et al., 2016; Burschka et al., 2014).

4.3.1. Yoga and Its Benefits

Yoga has been shown to improve balance, flexibility, and strength in MS patients, while also reducing fatigue and improving overall quality of life (Cramer et al., 2014; Grossman et al., 2010). Mindfulness-based interventions have demonstrated efficacy in reducing stress, improving sleep quality, and enhancing emotional well-being in individuals with MS (Grossman et al., 2010).

4.3.2. Tai Chi in MS Management

Tai chi, an ancient Chinese practice combining gentle physical exercise with mindfulness, has also shown promise in MS symptom management (Burschka et al., 2014). Studies have reported improvements in balance, coordination, and quality of life among MS patients practicing tai chi regularly (Kalron et al., 2016).

4.4. Acupuncture and Traditional Chinese Medicine

While the evidence base is still developing, some MS patients report benefits from acupuncture and traditional Chinese medicine (TCM) in managing symptoms such as pain, spasticity, and fatigue (Quispe-Cabanillas et al., 2012; Carletto et al., 2016). Acupuncture, in particular, has been studied for its potential to modulate pain pathways and reduce inflammation (Quispe-Cabanillas et al., 2012).

5. The Role of Technology in Symptom Management

Advancements in technology have opened new avenues for symptom management in MS (Bove et al., 2019; Marziniak et al., 2018). These innovations not only enhance the delivery of care but also empower patients to take a more active role in managing their condition (Midaglia et al., 2019; Hsieh et al., 2021).

5.1. Mobile Health (mHealth) Applications

Mobile health (mHealth) applications offer innovative ways to monitor symptoms, track treatment efficacy, and enhance patient engagement in self-management (Corry et al., 2019; D'hooghe et al., 2018). These digital tools not only facilitate more personalised care but also provide valuable data for research and treatment optimisation (Marziniak et al., 2018; Midaglia et al., 2019).

5.1.1. MS-Specific Apps and Their Functions

MS-specific apps can help patients track symptoms, medication adherence, and activity levels. Some applications incorporate gamification elements to encourage adherence to exercise programs and rehabilitation activities (Bove et al., 2019; Hsieh et al., 2021).

5.2. Telemedicine and Remote Monitoring

The COVID-19 pandemic has accelerated the adoption of telemedicine in MS care (Bove et al., 2019; Marziniak et al., 2018). Remote consultations and monitoring systems allow healthcare providers to assess symptoms, adjust treatments, and provide support without the need for in-person visits (Corry et al., 2019; Midaglia et al., 2019). This approach is particularly beneficial for patients with mobility limitations or those living in rural areas with limited access to specialized MS care (Hsieh et al., 2021).

5.2.1. Wearable Devices and Continuous Monitoring

Wearable devices and sensors can provide continuous, real-time data on various aspects of MS symptoms and disease progression. For example, accelerometers and gait analysis systems can offer objective measures of mobility and balance, while wearable electroencephalography (EEG) devices may provide insights into cognitive function and fatigue (Hsieh et al., 2021).

5.3. Virtual and Augmented Reality in Rehabilitation

Virtual reality (VR) and augmented reality (AR) technologies are emerging as candidate tools in MS rehabilitation (Maggio et al., 2019; Peruzzi et al., 2016). These immersive technologies offer novel approaches to address balance and gait issues, as well as cognitive training, in a controlled and engaging environment (Marziniak et al., 2018).

5.3.1. VR-Based Balance Training

VR-based balance training programs have shown potential in improving postural control and reducing fall risk in MS patients (Kalron et al., 2016). AR applications can provide real-time feedback and guidance during physical therapy exercises, enhancing the effectiveness of home-based rehabilitation programs (Peruzzi et al., 2016; Hsieh et al., 2021).

6. Addressing Invisible Symptoms: The Hidden Burden of MS

The management of invisible symptoms, such as fatigue, cognitive dysfunction, and mood disorders, presents unique challenges in MS care (Lakin et al., 2021; Feinstein et al., 2014). These symptoms, while not immediately apparent, can significantly impact quality of life and functional capacity (Chiaravalloti & DeLuca, 2008; van den Akker et al., 2016).

6.1. Fatigue Management Strategies

Fatigue, one of the most common and debilitating symptoms of MS, requires a complex approach combining pharmacological interventions, energy conservation strategies, and lifestyle modifications (Blikman et al., 2013; D'hooghe et al., 2018).

6.1.1. Energy Conservation Techniques

Energy conservation techniques, such as pacing activities and prioritizing tasks, can help patients manage their limited energy resources more effectively (Blikman et al., 2013). Occupational therapists play a key role in teaching these strategies and helping patients adapt their environments to minimise unnecessary energy expenditure (van den Akker et al., 2016).

6.1.2. Cognitive Behavioral Therapy for Fatigue

Cognitive behavioural therapy (CBT) has shown promise in helping MS patients manage fatigue by addressing negative thought patterns and behaviours that may exacerbate fatigue symptoms (van den Akker et al., 2016; D'hooghe et al., 2018).

6.2. Cognitive Rehabilitation and Support

Cognitive impairment, affecting up to 70% of MS patients, necessitates thorough assessment and targeted interventions (Chiaravalloti & DeLuca, 2008; Rosti-Otajärvi & Hämäläinen, 2014). Cognitive rehabilitation programs, coupled with pharmacological treatments, aim to improve cognitive function and enhance daily living activities (Amato et al., 2013; Goverover et al., 2008).

6.2.1. Cognitive Remediation Therapy

Cognitive remediation therapy, which focuses on improving specific cognitive domains through targeted exercises, has shown efficacy in enhancing attention, information processing speed, and executive function in MS patients (Chiaravalloti et al., 2013; Peyro Saint Paul et al., 2016).

6.2.2. Compensatory Strategies

Compensatory strategies, such as the use of memory aids, organizational tools, and environmental modifications, can help patients manage cognitive difficulties in their daily lives (Rosti-Otajärvi & Hämäläinen, 2014; Nasios et al., 2020). Neuropsychologists and occupational therapists often work together to develop and implement these strategies (Goverover et al., 2008).

6.3. Addressing Mood Disorders

Depression and anxiety are common comorbidities in MS, with prevalence rates significantly higher than in the general population (Feinstein et al., 2014; Koch et al., 2011). These mood disorders not only impact quality of life but can also exacerbate other MS symptoms and affect treatment adherence (Hind et al., 2014).

6.3.1. Psychotherapy Approaches

Psychotherapy, particularly cognitive behavioural therapy (CBT), has demonstrated efficacy in treating depression and anxiety in MS patients (Hind et al., 2014; Koch et al., 2011). CBT can help individuals develop coping strategies, challenge negative thought patterns, and improve overall emotional well-being (van den Akker et al., 2016; Grossman et al., 2010).

6.3.2. Pharmacological Management of Mood Disorders

In addition to psychotherapy, pharmacological interventions are closely involved in managing mood disorders in MS. Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are commonly prescribed for depression and anxiety in MS patients (Koch et al., 2011; Hind et al., 2014). However, care must be taken to monitor for potential interactions with other MS medications and to address any side effects that may exacerbate existing MS symptoms (Feinstein et al., 2014).

6.3.3. Group Therapy and Support Groups

Group therapy and support groups can provide valuable emotional support and coping strategies for MS patients dealing with mood disorders (Marrie et al., 2015). These settings allow individuals to share experiences, learn from others, and reduce feelings of isolation often associated with chronic illness (Hind et al., 2014).

6.3.4. Mindfulness-Based Interventions

Mindfulness-based interventions have shown early findings in reducing symptoms of depression and anxiety in MS patients (Grossman et al., 2010; Rogers & MacDonald, 2015). Techniques such as mindfulness-based stress reduction (MBSR) can help individuals develop greater awareness of their thoughts and emotions, leading to improved emotional regulation and stress management (Carletto et al., 2016; Burschka et al., 2014).

7. The Importance of Holistic Care in MS Management

The complex nature of MS symptoms necessitates a holistic approach to care that addresses not only the physical manifestations of the disease but also its psychological and social impacts (Golla et al., 2012; Lakin et al., 2021). This multidisciplinary approach recognises the interconnectedness of various MS symptoms and the need for integrated management strategies (Miller et al., 2013; Papeix et al., 2015).

7.1. Multidisciplinary Care Teams

The concept of multidisciplinary care in MS has gained significant traction in recent years. These teams typically include neurologists, MS nurses, physiotherapists, occupational therapists, speech and language therapists, psychologists, and social workers (Papeix et al., 2015). The collaboration between these professionals ensures that all aspects of a patient's care are addressed in a coordinated manner (Miller et al., 2013).

7.1.1. Regular Team Meetings

Regular multidisciplinary team meetings allow for the sharing of expertise and the development of integrated care plans tailored to each patient's unique needs (Papeix et al., 2015; Miller et al., 2013). This approach has been shown to improve patient outcomes, enhance quality of life, and reduce healthcare utilization in MS patients (Papeix et al., 2015).

7.2. Patient-Centered Care and Shared Decision Making

The shift towards patient-centered care in MS management emphasizes the importance of involving patients in treatment decisions and care planning (Köpke et al., 2018).

Shared decision-making models encourage open communication between healthcare providers and patients, ensuring that treatment plans align with individual preferences, values, and goals (Miller et al., 2013).

7.2.1. Patient Education

Patient education matters for empowering individuals with MS to take an active role in their care (Köpke et al., 2018). Comprehensive education programs covering topics such as symptom management, treatment options, and lifestyle modifications can enhance patient self-efficacy and improve adherence to treatment plans (Miller et al., 2013).

7.3. Addressing Social and Occupational Impacts

MS can have significant impacts on an individual's social relationships and occupational functioning (Dorstyn et al., 2019; Lo et al., 2020). Holistic care approaches recognize the importance of addressing these aspects to improve overall quality of life (Edmonds et al., 2010; Marrie et al., 2015).

7.3.1. Vocational Rehabilitation

Vocational rehabilitation services can help MS patients maintain employment or explore alternative career options that accommodate their changing abilities (Dorstyn et al., 2019). These services may include workplace adaptations, retraining programs, and counseling on disability rights and employment laws (Lo et al., 2020).

7.3.2. Social Support Interventions

Social support interventions, such as peer mentoring programs and social skills training, can help individuals with MS maintain and develop social connections (Marrie et al., 2015). These interventions are particularly important given the high rates of social isolation and loneliness reported among MS patients (Grossman et al., 2010; Hind et al., 2014).

8. The Impact of Comorbidities on MS Symptom Management

Comorbidities significantly influence the severity and management of MS symptoms (Marrie et al., 2015; Lo et al., 2020). Conditions such as depression, anxiety, and sleep disorders not only exacerbate MS symptoms but also impact treatment efficacy and overall prognosis (Feinstein et al., 2014; Brass et al., 2014). A holistic approach to MS care must, therefore, include the identification and management of comorbid conditions to optimise outcomes (Marrie et al., 2015).

8.1. Cardiovascular Comorbidities

Cardiovascular risk factors, such as hypertension, hyperlipidemia, and diabetes, are more prevalent in MS patients and can contribute to disease progression and symptom severity (Marrie et al., 2015; Lo et al., 2020). Management of these conditions through lifestyle modifications and appropriate medical interventions is important for optimising MS outcomes (Dalgas et al., 2009).

8.1.1. Potential Benefits of Statins

Recent research has explored the potential benefits of statins in MS management, not only for their lipid-lowering effects but also for their potential neuroprotective properties (Chan et al., 2017). Although phase II data were encouraging (Chan et al., 2017), the subsequent phase III MS-STAT2 trial did not show that simvastatin slows disability progression in secondary progressive MS.

8.2. Sleep Disorders

Sleep disturbances are common in MS and can exacerbate fatigue, cognitive dysfunction, and mood disorders (Brass et al., 2014; Lange et al., 2009). Comprehensive sleep assessments and targeted interventions are essential components of holistic MS care (Brass et al., 2014; Koch et al., 2011).

8.2.1. Cognitive Behavioral Therapy for Insomnia

Cognitive behavioural therapy for insomnia (CBT-I) has shown efficacy in improving sleep quality in MS patients (Brass et al., 2014; van den Akker et al., 2016). This non-pharmacological approach addresses the behavioural and cognitive factors contributing to sleep disturbances and can be particularly beneficial for patients with comorbid anxiety or depression (Hind et al., 2014; Koch et al., 2011).

9. Emerging Therapies and Future Directions

New interventions for MS symptoms should be assessed against existing care using outcomes such as function, participation, and quality of life.

9.1. Neuromodulation Techniques

Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are non-invasive brain stimulation techniques that have shown promise in managing various MS symptoms (Palm et al., 2014). These approaches aim to modulate neural activity in specific brain

regions, potentially improving symptoms such as fatigue, cognitive dysfunction, and motor impairments.

9.1.1. Deep Brain Stimulation for Tremor

Thalamic stimulation, using deep brain stimulation (DBS) techniques, is being explored for the management of severe tremors in MS patients who have not responded to conventional treatments (Oliveria et al., 2017). While invasive, this approach has shown early findings in small studies, significantly improving tremors and quality of life in selected patients (Oliveria et al., 2017).

9.2. Bioengineering and Assistive Technologies

Advances in bioengineering are leading to the development of innovative assistive technologies for MS patients (Wolpaw et al., 2018; Marziniak et al., 2018). Exoskeletons and robotic gait training devices are being refined to improve mobility and gait in individuals with significant walking impairments (Schwartz et al., 2012; Kalron et al., 2016).

9.2.1. Brain-Computer Interfaces

Brain-computer interfaces (BCIs) represent another area of active work in MS management. These systems, which allow direct communication between the brain and external devices, have the potential to assist patients with severe motor impairments in controlling assistive devices or communicating more effectively (Wolpaw et al., 2018; Marziniak et al., 2018).

9.3. Gut Microbiome Modulation

Growing evidence suggests a link between the gut microbiome and MS pathogenesis and progression (Berer et al., 2017; Preziosi et al., 2013). This has led to interest in therapeutic approaches targeting the gut-brain axis. Probiotics, prebiotics, and fecal microbiota transplantation are being investigated for their potential to modulate the immune system and influence MS disease activity (Tankou et al., 2018; Berer et al., 2017).

10. Conclusion

Symptom treatment requires coordination across disciplines and should combine drug treatment, rehabilitation, and practical support according to the patient's priorities (Dalgas et al., 2009; Miller et al., 2013; Spain, 2022).

Improved understanding of MS pathophysiology may identify additional therapeutic targets and treatment modalities (Plemel et al., 2017; Iaffaldano et al., 2012). Clinical care nonetheless remains broader than disease modification alone and requires individualized symptom control, rehabilitation, and support for participation and quality of life (Thompson et al., 2018; Shah, 2015).

Care plans should be reviewed as symptoms, disability, and personal priorities change. Preserving independence and participation requires attention to physical, cognitive, and emotional needs throughout the disease course (Feinstein et al., 2014; Lakin et al., 2021; Miller et al., 2013; Papeix et al., 2015).

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3.3. LIMITATIONS AND SIDE EFFECTS OF CURRENT TREATMENTS

Treatment limitations include incomplete control of progression, adverse effects, administration burden, and uneven access to care. Figure 3.3.1 separates established treatment effects from outcomes that current therapies have not reliably achieved.

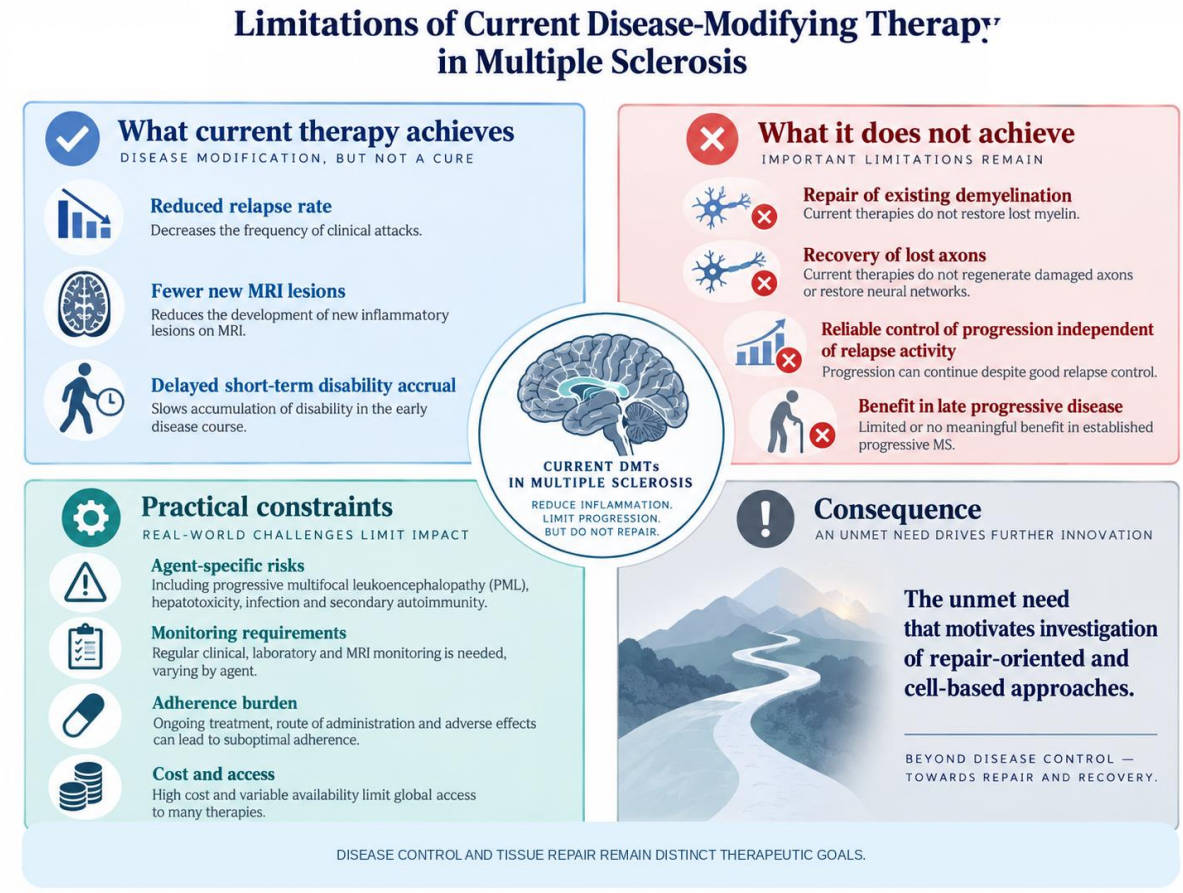


Figure 3.3.1. What current disease-modifying therapy achieves and does not achieve in multiple sclerosis, and the unmet need that motivates investigation of repair-oriented approaches.

1. Efficacy Limitations of Disease-Modifying Therapies

Disease-modifying therapies form the cornerstone of MS treatment, with the primary goals of reducing relapse rates, slowing disease progression, and preventing the accumulation of disability. However, these treatments face significant limitations in their efficacy, particularly in the long term and in more progressive forms of the disease.

1.1. First-Line Treatments: Interferon Beta and Glatiramer Acetate

Interferon beta and glatiramer acetate have long been staples in the treatment of relapsing-remitting MS (RRMS). However, their efficacy is only partial, and many patients continue to experience disease activity despite treatment.

A meta-analysis conducted by Filippini et al. (2013) provided a full evaluation of the efficacy of these first-line treatments. The study found that while these drugs reduced relapse rates by approximately 30% compared to placebo, their impact on disability progression was less clear. Over a two-year period, the effect on disability progression was not statistically significant, raising questions about the long-term benefits of these widely prescribed medications.

Shirani et al. (2012) examined interferon beta exposure and time to sustain EDSS 6 in an observational cohort of 2,656 patients. They found no statistically significant association with a lower hazard of reaching that endpoint. Confounding, treatment selection and differences between historical and contemporary controls limit causal interpretation. The finding should not be generalized to all DMTs or used to negate their demonstrated effects on relapses and MRI activity.

The limitations of these first-line therapies are further made clear by the phenomenon of breakthrough disease

activity. Rudick et al. (2006) showed in the SENTINEL trial that patients with continued disease activity on interferon beta-1a had fewer relapses and less MRI activity when natalizumab was added, illustrating that a considerable proportion of patients have breakthrough activity on first-line therapy. This breakthrough disease activity not only impacts patient outcomes but also necessitates frequent treatment re-evaluation and potential escalation to more potent therapies.

1.2. Second-Line Treatments: Natalizumab and Fingolimod

Second-line therapies such as natalizumab and fingolimod have demonstrated higher efficacy in controlling disease activity compared to first-line treatments. However, they too have limitations in their effectiveness, particularly in the long term and in progressive forms of MS.

In AFFIRM, natalizumab reduced relapse activity and the risk of sustained disability progression relative to placebo. The two-year cumulative probability of progression was 17% with natalizumab and 29% with placebo, with a hazard ratio of 0.58 (Polman et al., 2006). Residual disease activity remains clinically important, but the comparator and the correct absolute risks are necessary for a balanced account of treatment benefit.

In FREEDOMS, fingolimod reduced annualised relapse rates and the risk of confirmed disability progression relative to placebo over two years (Kappos et al., 2010). Some patients nevertheless experienced relapses or progression. These distinct outcomes should be reported separately, with the dose and comparator specified; residual activity does not imply absence of treatment benefit.

Moreover, the efficacy of these treatments in progressive forms of MS is limited. While some DMTs have shown modest benefits in primary progressive MS (PPMS) and secondary progressive MS (SPMS), their effect is less pronounced than in RRMS. Ontaneda et al. (2017) conducted a detailed review of treatment options for progressive MS and found that while some DMTs could slow disability progression to a certain extent, their overall impact on disease progression in these forms of MS was limited. This highlights a significant unmet need in MS treatment, as a substantial proportion of patients eventually transition to a progressive disease course.

1.3. Newer Oral Therapies: Dimethyl Fumarate and Teriflunomide

The introduction of oral therapies such as dimethyl fumarate and teriflunomide has expanded treatment options for MS patients. However, these medications also face efficacy limitations.

In DEFINE, the proportions with confirmed disability progression over two years were approximately 16% with twice-daily dimethyl fumarate, 18% with the three-times-daily regimen and 27% with placebo (Gold et al., 2012). These are progression outcomes, distinct from the proportion experiencing a relapse. The trial demonstrated treatment benefit while also showing that disability accumulation was not eliminated.

Teriflunomide, another oral therapy for RRMS, has demonstrated similar efficacy limitations. O'Connor et al. (2011) found that teriflunomide reduced the annualised relapse rate by 31-36% compared to placebo over two years. However, 20-22% of patients still experienced disability progression, again highlighting the incomplete nature of disease control with current therapies.

1.4. Limitations in Addressing Specific MS Symptoms

While DMTs focus on reducing overall disease activity, they often fall short in addressing specific MS symptoms that significantly impact quality of life. This limitation is particularly evident in the management of symptoms such as cognitive fatigue, neuropathic pain, and spasticity.

Cognitive fatigue, a debilitating symptom experienced by up to 80% of MS patients, is often inadequately addressed by current treatments. Cercignani et al. (2021) conducted a notable study using advanced neuroimaging techniques to investigate the neural correlates of cognitive fatigue in MS. They found that cognitive fatigue was associated with alterations in the functional connectivity of monoamine circuits, particularly involving the locus coeruleus and the ventral tegmental area. This study not only clarified the underlying mechanisms of cognitive fatigue but also highlighted the limited efficacy of current DMTs in addressing this symptom, as these treatments do not directly target the neural circuits involved in fatigue.

Pain in MS includes neuropathic, musculoskeletal and other syndromes. Prevalence estimates vary with the pain definition and study population (Foley et al., 2013). Assessment and treatment should distinguish pain mechanism, severity, comorbidity and functional impact.

Spasticity, characterised by muscle stiffness and involuntary muscle spasms, is another symptom that is often inadequately managed with current MS treatments. Otero-Romero et al. (2016) conducted a systematic review of spasticity management in MS and found that while various pharmacological and non-pharmacological interventions exist, their efficacy is often limited, and they can be associated with significant side effects. More targeted approaches are therefore needed to managing specific MS symptoms alongside disease-modifying therapies.

2. Side Effects and Safety Concerns

The side effect profiles of MS treatments pose significant challenges for patient management and treatment adherence. These side effects range from mild and manageable to severe and potentially life-threatening, necessitating careful risk-benefit analysis and ongoing monitoring.

2.1. Injection-Related Side Effects

Injectable therapies such as interferon beta and glatiramer acetate are associated with a range of injection-related side effects that can significantly impact patient quality of life and treatment adherence.

Wingerchuk and Weinshenker (2016) provided a general overview of the side effects associated with injectable MS therapies. They reported that up to 90% of patients experience injection-site reactions with glatiramer acetate, including localised pain, erythema, and induration. These reactions, while mild, can be distressing for patients and may lead to treatment discontinuation in some cases.

Interferon beta therapies are associated with flu-like symptoms in up to 75% of patients, particularly during the initial months of treatment. These symptoms typically include fever, chills, myalgia, and fatigue, often occurring within hours of injection and lasting for 24-36 hours. While these symptoms tend to diminish over time, they can significantly impact patient quality of life and work productivity, particularly in the early stages of treatment.

Repeated injections of interferon beta or glatiramer acetate can cause local tissue changes, including lipoatrophy. In a cross-sectional study of 229 people with MS who had received their first injectable DMT for at least two years, Balak et al. (2013) found lipoatrophy in 45 participants, approximately 20% of the full cohort. The glatiramer acetate subgroup comprised 44 patients, of whom 15 had lipoatrophy, approximately 34%. Such lesions warrant examination and discussion when injection tolerability and treatment adherence are reviewed.

2.2. Liver Toxicity and Cardiovascular Risks

Several MS treatments carry risks of liver toxicity and cardiovascular complications, necessitating regular monitoring and, in some cases, treatment discontinuation.

Biolato et al. (2021) analyzes pre-registration and post-marketing data concerning the hepatotoxicity of disease-modifying therapies (DMTs) used in relapsing-remitting multiple sclerosis. The study highlights that while serious liver adverse events are rare, no DMT is entirely free from potential hepatic toxic effects. Cases of acute liver failure have been reported for several DMTs, including beta-interferon, fingolimod, natalizumab, alemtuzumab, and ocrelizumab, through mechanisms such as idiosyncratic reactions, autoimmune hepatitis, or viral reactivation. The authors emphasize the importance of informing patients about possible hepatic side effects and recommend regular monitoring of liver function during treatment. They also discuss considerations for DMT use in patients with viral hepatitis or liver cirrhosis.

Teriflunomide, in particular, carries a boxed warning for hepatotoxicity. The drug's prescribing information mandates baseline liver function tests and regular monitoring throughout treatment. In some cases, elevated liver enzymes necessitate dose reduction or treatment discontinuation, impacting the overall treatment strategy for these patients.

Cardiovascular risks are another significant concern with certain MS treatments, particularly fingolimod. Kappos et al. (2010) reported that fingolimod initiation was associated with a transient decrease in heart rate and, in some cases, atrioventricular conduction block. This necessitates cardiac monitoring during treatment initiation, typically requiring at least 6 hours of continuous ECG monitoring after the first dose. In some patients with pre-existing cardiac conditions, overnight monitoring may be required, adding to the complexity of treatment initiation and potentially limiting its use in certain patient populations.

2.3. Opportunistic Infections

One of the most serious concerns with some MS treatments is the risk of opportunistic infections, particularly progressive multifocal leukoencephalopathy (PML).

Natalizumab, while highly effective in controlling MS disease activity, is associated with a risk of PML, a potentially fatal brain infection caused by the JC virus. Bloomgren et al. (2012) conducted a close analysis of PML risk in natalizumab-treated patients. They found that the risk of PML varied significantly based on three main factors: the

presence of anti-JC virus antibodies, prior use of immunosuppressants, and duration of natalizumab treatment. In high-risk patients (those positive for JC virus antibodies, with prior immunosuppressant use, and more than two years of natalizumab treatment), the risk of PML was estimated to be as high as 11.1 per 1,000 patients.

This significant risk necessitates careful patient selection, regular monitoring, and complex risk stratification strategies. Patients on natalizumab typically undergo regular JC virus antibody testing and MRI scans to monitor early signs of PML. In some cases, extended interval dosing of natalizumab has been employed as a strategy to mitigate PML risk while maintaining treatment efficacy, although the optimal approach remains a subject of ongoing research.

Fingolimod and dimethyl fumarate have also been associated with rare cases of PML, albeit at lower rates than natalizumab. Calabrese et al. (2017) discussed dimethyl fumarate as an exit strategy for patients at risk of severe adverse events on natalizumab, a situation in which the balance of PML risk between agents guides treatment decisions. Regular clinical and MRI monitoring remains important for patients on these medications, particularly those with risk factors for PML.

Other opportunistic infections have also been reported with various MS treatments. For instance, Varicella Zoster virus reactivation has been observed with fingolimod treatment, necessitating vaccination strategies in some patients prior to treatment initiation. Similarly, listeriosis has been reported in patients treated with alemtuzumab, leading to recommendations for dietary restrictions during and immediately after treatment courses.

2.4. Hematological Abnormalities

Several MS treatments can cause haematological abnormalities, necessitating regular blood monitoring and, in some cases, treatment discontinuation.

Teriflunomide has been associated with lymphopenia and neutropenia. Prescribing information for teriflunomide recommends obtaining a complete blood count within 6 months before initiation of treatment and monitoring for signs of bone marrow suppression during treatment. In clinical trials, approximately 15% of patients experienced lymphopenia, although severe cases were rare.

Alemtuzumab, a highly effective treatment for active RRMS, is associated with a range of haematological abnormalities. Coles et al. (2012) reported on the safety profile of alemtuzumab in the CARE-MS II trial. They found that 48% of patients developed lymphopenia, which was expected given the mechanism of action of the drug. More concerning were the autoimmune effects, including immune thrombocytopenia (ITP) in 1% of patients and autoimmune thyroid disorders in 16% of patients over two years. These autoimmune effects necessitate a complex monitoring program for patients on alemtuzumab, including monthly blood and urine tests for 48 months after the last dose.

Fingolimod treatment is associated with lymphopenia, which is part of its mechanism of action in treating MS. However, severe lymphopenia can increase the risk of infections. Regular monitoring of lymphocyte counts is required, and treatment discontinuation may be necessary if lymphocyte counts fall too low.

These haematological abnormalities not only pose health risks to patients but also add to the complexity of treatment management, requiring regular blood tests and ongoing vigilance for potential complications.

3. Treatment Adherence Challenges

The limitations and side effects of MS treatments significantly impact patient adherence, an important factor in long-term disease management. Poor adherence can lead to suboptimal treatment outcomes, increased healthcare utilization, and higher costs.

3.1. Injection Fatigue and Administration Burden

For patients on injectable therapies, the frequent need for self-administration can lead to injection fatigue, significantly impacting long-term adherence.

Crawford et al. (2014) conducted an in-depth review of treatment fatigue in MS patients on long-term therapy. They identified several factors contributing to injection fatigue, including injection anxiety, pain, and the psychological burden of regular injections. The authors noted that over time, patients may develop a negative association with the injection process, leading to missed doses or treatment discontinuation.

A study by Higuera et al. (2016) provided quantitative data on adherence rates to DMTs in MS. They found that adherence rates varied widely, ranging from 41% to 88%, depending on the specific medication and mode of administration. Injectable therapies generally had lower adherence rates compared to oral medications, highlighting the impact of administration route on treatment adherence.

The administration burden extends beyond the act of injection itself. Patients must manage storage requirements, travel considerations, and the disposal of used injection materials. These factors can significantly impact quality of life and may influence treatment choices, particularly with the availability of oral alternatives.

3.2. Cognitive Impairment and Treatment Management

Cognitive impairment, a common symptom in MS, can further complicate treatment adherence by affecting patients' ability to manage complex treatment regimens.

Chiaravalloti and DeLuca (2008) conducted a review of cognitive impairment in MS. They reported that up to 65% of MS patients experience some degree of cognitive dysfunction, with deficits commonly observed in information processing speed, attention, and executive function. These cognitive changes can significantly impact a patient's ability to adhere to complex treatment regimens, remember medication schedules, and manage side effects effectively.

Moreover, cognitive fatigue, as discussed earlier, can exacerbate these challenges. Patients experiencing severe cognitive fatigue may struggle to maintain the mental energy required to consistently adhere to their treatment regimen, especially if it involves frequent administration or complex monitoring protocols.

3.3. Financial Burden and Access Issues

The high cost of MS treatments presents another significant barrier to adherence, often leading to treatment discontinuation or suboptimal adherence.

Hartung et al. (2015) conducted a detailed analysis of the cost trends of disease-modifying therapies (DMTs) for MS in the United States. Their findings were striking: the average cost of DMTs increased from $8,000 to $11,000 per year in the 1990s to over $60,000 per year by 2015. This dramatic increase in cost outpaced inflation and other measures of drug cost changes, raising concerns about the sustainability of MS treatment.

The financial burden of MS treatments extends beyond the direct cost of medications. Patients often face high out-of-pocket expenses, including copayments, deductibles, and costs associated with monitoring and managing side effects. Adelman et al. (2013) systematically reviewed the cost burden of MS in the United States and found that disease-modifying therapies account for a large share of total costs. Cost-sharing may therefore affect access to and continuation of treatment.

Moreover, access to MS treatments varies significantly across different healthcare systems and geographical regions. Zeineddine and Yamout (2020) described the treatment of MS in special populations such as refugees, where access to diagnosis, monitoring, and disease-modifying therapy is frequently interrupted; similar constraints of cost and availability limit access to newer DMTs in many low- and middle-income countries. This global inequality in treatment access not only affects individual patient outcomes but also impacts the overall global burden of MS.

4. Limitations in Addressing Disease Progression

Perhaps the most significant limitation of current MS treatments is their limited efficacy in preventing long-term disability progression, particularly in progressive forms of MS.

4.1. Progressive Forms of MS

While significant progress has been made in treating relapsing-remitting MS (RRMS), the management of progressive forms of MS remains a major challenge. Primary progressive MS (PPMS) and secondary progressive MS (SPMS) are characterised by steady accumulation of disability with or without superimposed relapses.

ORATORIO randomised 732 patients with PPMS to ocrelizumab or placebo and followed them for at least 120 weeks. Disability progression confirmed for 12 weeks occurred in 32.9% and 39.3%, respectively, with a hazard ratio of 0.76 (Montalban et al., 2017). This modest but demonstrated benefit should be distinguished from evidence in RRMS; comparisons of effect sizes across different trials and populations do not establish comparative superiority.

The limited efficacy in progressive MS is thought to be due to the different underlying pathological mechanisms at play. While RRMS is predominantly driven by inflammatory processes, progressive MS is characterised by more diffuse inflammation, chronic microglial activation, and neurodegeneration. Current DMTs, which primarily target peripheral immune responses, may not effectively address these central nervous system-driven processes.

4.2. Long-term Disability Accumulation

Even in RRMS, the long-term efficacy of current treatments in preventing disability accumulation remains a subject of debate. Cree et al. (2016) conducted a long-term follow-up study of MS patients in the treatment era. They found that although long-term outcomes were better than in historical natural-history cohorts, a proportion of

patients still accumulated significant disability over time: at a median of 16.8 years after disease onset, 10.7% of patients had reached a level of disability requiring a cane for walking (EDSS ≥ 6), and 18.1% had converted to secondary progressive MS.

This long-term accumulation of disability despite treatment highlights the limitations of current therapies in fully halting the disease process. It argues for more effective neuroprotective and neuroregenerative strategies in MS treatment.

5. Challenges in Treatment Selection and Sequencing

The expanding array of treatment options for MS, while providing more choices for patients and clinicians, also introduces challenges in treatment selection and sequencing.

5.1. Heterogeneity of Treatment Response

MS is a heterogeneous disease, and patient responses to treatments can vary widely. Predicting which patients will respond best to which treatments remains a significant challenge. Sormani and Bruzzi (2015) reviewed the concept of treatment response in MS and highlighted the difficulties in defining and measuring treatment response. They noted that while group-level data from clinical trials provide information on average treatment effects, individual patient responses can deviate significantly from these averages.

This heterogeneity in treatment response necessitates a personalised approach to MS management, but current tools for predicting individual treatment responses are limited. Biomarkers that could reliably predict treatment response are an area of active research but are not yet available for routine clinical use.

5.2. Treatment Sequencing

The optimal sequencing of disease-modifying therapies (DMTs) in multiple sclerosis (MS) management remains a complex issue, particularly concerning long-term outcomes. While escalation strategies, initiating treatment with safer but potentially less effective therapies and progressing to more potent options as needed, are commonly employed, recent evidence suggests that early intensive treatment (EIT) may offer superior long-term benefits. Evidence from observational comparisons suggests that early intensive treatment may reduce the risk of disability progression relative to escalation, but randomised comparisons are still ongoing. Treatment discontinuation carries its own risks: in a systematic review and meta-analysis of real-world studies, Prosperini et al. (2023) examined outcomes after stopping disease-modifying treatments in MS. However, the choice between these strategies must consider individual patient factors, including disease activity, prognosis, and potential risks associated with high-efficacy treatments, such as increased susceptibility to infections or other adverse events. Moreover, discontinuation of certain therapies, notably fingolimod and natalizumab, has been linked to rebound disease activity, adding another layer of complexity to treatment sequencing decisions.

6. Pregnancy and Family Planning Considerations

MS often affects women of childbearing age, and the impact of treatments on pregnancy outcomes and family planning is an important consideration.

6.1. Teratogenic Risks

Several MS treatments carry teratogenic risks and are contraindicated during pregnancy. Teriflunomide, for instance, carries a boxed warning for embryofetal toxicity based on animal studies. Women of childbearing potential must use effective contraception during treatment and undergo an accelerated elimination procedure if pregnancy is planned.

Lu et al. (2012) conducted a systematic review of the safety of disease-modifying drugs for MS in pregnancy. They found that while some treatments (particularly interferons and glatiramer acetate) appear to be relatively safe during pregnancy, data on newer therapies are limited. The need to discontinue certain treatments before conception and during pregnancy can lead to disease reactivation, posing risks to both the mother and the developing fetus.

6.2. Impact on Fertility and Family Planning

Some MS treatments may impact fertility and complicate family planning. For instance, cyclophosphamide, sometimes used in aggressive MS, can cause ovarian failure and infertility. While not commonly used as a first-line treatment, its potential use in some cases makes the need clear for careful consideration of long-term implications when selecting treatments for younger patients.

The need to discontinue treatments for extended periods before conception (e.g., four months after each alemtuzumab course, or up to two years for teriflunomide unless an accelerated elimination procedure is used) can significantly impact family planning timelines. This often requires a delicate balance between managing disease activity and allowing for safe pregnancy planning.

7. Conclusion

Current treatment for multiple sclerosis, while significantly improved from previous decades, still faces substantial challenges. The limitations in efficacy, particularly in preventing long-term disability and managing progressive forms of MS, remain a significant concern. Moreover, the side effects associated with these treatments, ranging from mild injection-site reactions to severe opportunistic infections, pose considerable risks and management challenges.

The impact of these limitations and side effects extends beyond clinical outcomes, affecting patient quality of life, treatment adherence, and overall disease management. The complex balance between treatment efficacy and safety necessitates careful consideration in treatment selection and ongoing patient monitoring. Furthermore, challenges in sequencing treatment, addressing specific MS symptoms, and managing MS in the context of family planning add layers of complexity to patient care.

Unmet needs are most apparent in progressive disease and in symptoms that persist despite DMT treatment. Better safety, tolerability, and support for adherence remain necessary alongside the search for treatments that affect progression.

The development of biomarkers to predict treatment response and disease progression could significantly improve treatment selection and personalisation. Moreover, novel therapeutic approaches targeting neuroprotection and remyelination may address the underlying neurodegenerative processes in MS.

Ultimately, the goal of MS treatment should be not just to control disease activity, but to improve overall quality of life while minimising treatment burden and risks. Achieving this goal will require continued research, innovation in drug development, and a multidisciplinary approach to patient care that addresses both the biological and psychosocial aspects of living with MS.

MS management should remain patient-centered, with treatment decisions incorporating individual preferences, risk tolerance, comorbidity, reproductive plans, disability trajectory, and quality-of-life goals. This approach does not remove uncertainty, but it makes the trade-offs between efficacy, safety, convenience, and monitoring requirements explicit and clinically usable.

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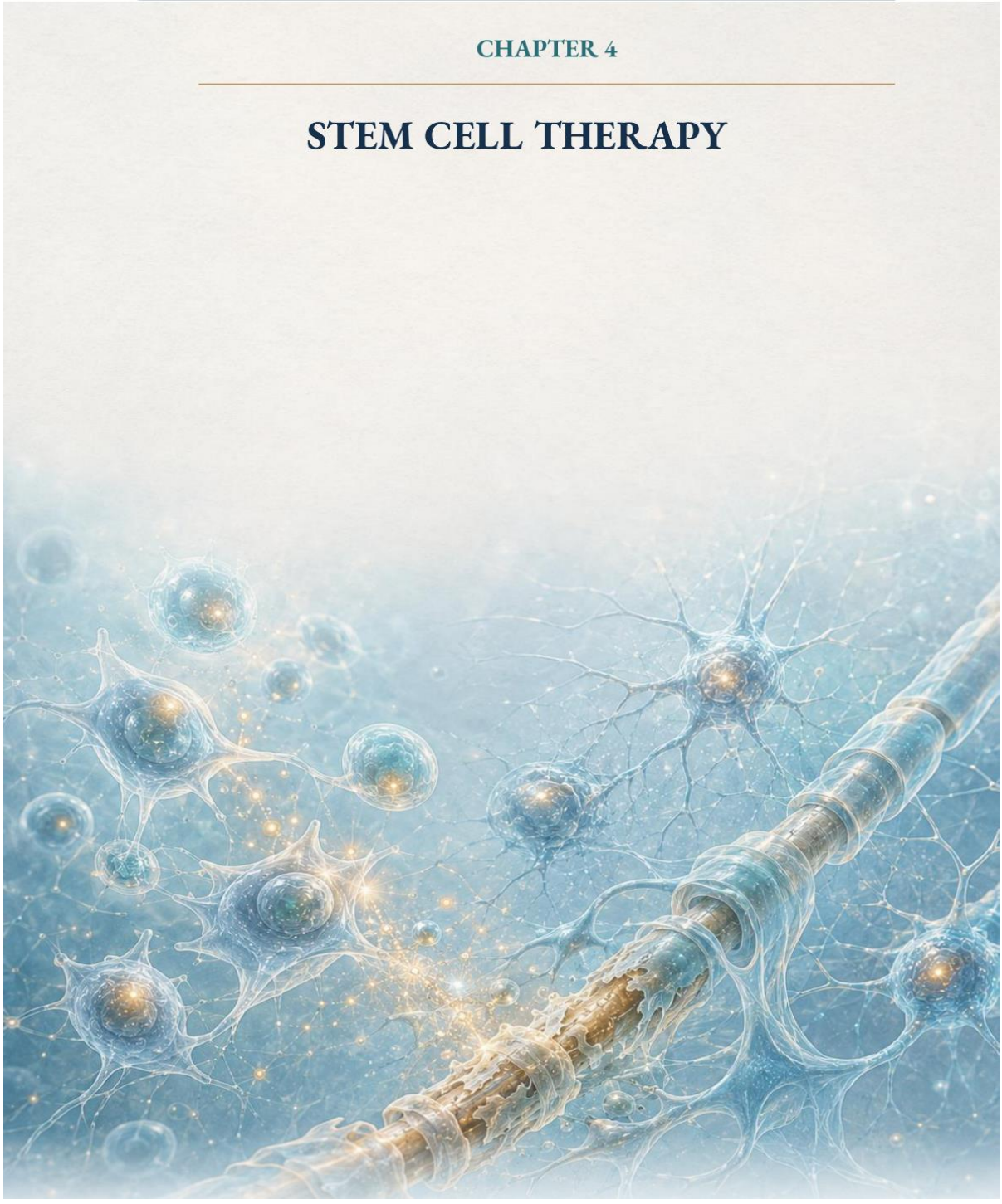
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CHAPTER 4

STEM CELL THERAPY



4.1. DEFINITION AND TYPES OF STEM CELLS (E.G., EMBRYONIC, MESENCHYMAL, HEMATOPOIETIC)

This chapter reviews the defining properties and principal classifications of stem cells, followed by established and investigational applications relevant to regenerative medicine. Stem-cell types are considered by origin, potency, and tissue source, with attention to clinical translation, artificial-intelligence-assisted research, manufacturing, safety, and regulatory constraints. The aim is to provide the biological background needed to evaluate later discussions of stem-cell approaches in MS. The classification used throughout this chapter is shown in Figure 4.1.1.

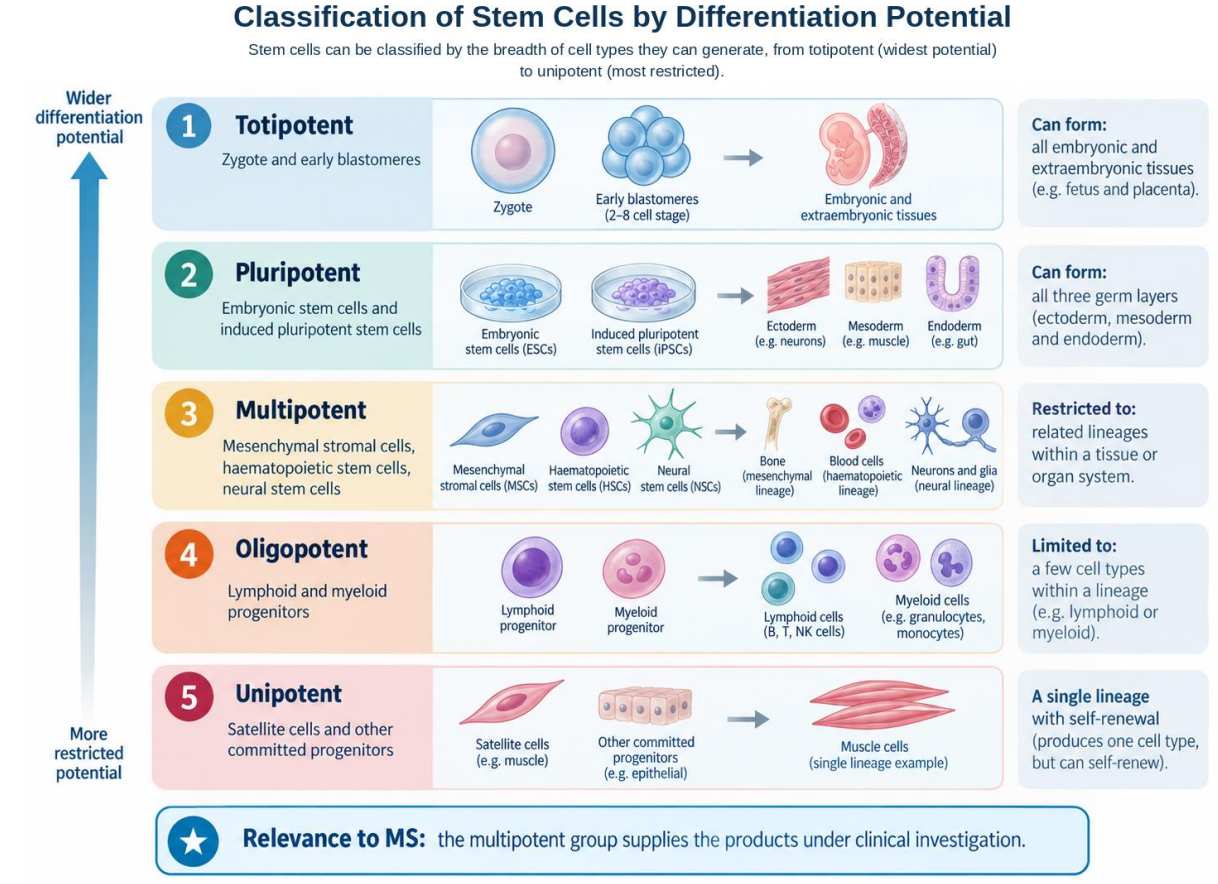


Figure 4.1.1. Classification of stem cells by differentiation potential. The multipotent group supplies the products currently under clinical investigation in multiple sclerosis.

1. Introduction

Self-renewal and differentiation make stem cells useful for studying development, modelling disease, and testing therapeutic hypotheses. Clinical applications depend on controlling these properties and defining the resulting cell product.

The concept of stem cell has its roots in the late 19th century, with the work of German biologist Ernst Haeckel, who coined the term "Stammzelle" (stem cell) to describe the fertilized egg that gives rise to all other cells (Ramalho-Santos & Willenbring, 2007). However, it wasn't until the mid-20th century that concrete scientific evidence for the existence of stem cells began to emerge.

A key moment in stem cell research came in 1961 when Canadian scientists James Till and Ernest McCulloch demonstrated the existence of self-renewing cells in mouse bone marrow (Till & McCulloch, 1961). Their notable work laid the foundation for our understanding of haematopoietic stem cells and provided a basis for bone marrow transplantation as a treatment for blood disorders and cancers.

The field took another significant leap forward in 1981 with the isolation of embryonic stem cells from mouse blastocysts by Martin Evans and Matthew Kaufman in the UK, and independently by Gail Martin in the USA (Evans & Kaufman, 1981; Martin, 1981). This achievement opened up new possibilities for studying early

development and set the stage for the later derivation of human embryonic stem cells.

The derivation of human embryonic stem cells in 1998 by James Thomson and colleagues at the University of Wisconsin-Madison marked a watershed moment in stem cell research (Thomson et al., 1998). These cells, capable of giving rise to any cell type in the human body, promised new avenues for studying human development, disease modelling, and regenerative medicine. However, their derivation also sparked ethical debates that continue to shape the field today.

A substantial change of approach occurred in 2006 when Shinya Yamanaka and his team at Kyoto University demonstrated that adult mouse fibroblasts could be reprogrammed into a pluripotent state by introducing just four genes (Takahashi & Yamanaka, 2006). This discovery of induced pluripotent stem cells (iPSCs), later repeated with human cells, substantially changed the field, providing a source of pluripotent stem cells that bypassed many of the ethical concerns associated with embryonic stem cells.

Stem cells are an important subject in regenerative medicine because of their capacity for self-renewal, differentiation, disease modelling, and experimental tissue repair. They can regenerate damaged or diseased tissues, potentially replacing damaged cells in various organs and offering hope for conditions ranging from heart disease to neurological disorders. They allow human diseases to be modelled: patient-derived stem cells, especially iPSCs, let researchers create "diseases in a dish," providing striking insight into disease mechanisms and potential treatments. They also serve as platforms for testing new drugs, since stem cell-derived tissues can be used for drug screening, potentially reducing the need for animal testing and improving the efficiency of drug development. Beyond this, iPSCs derived from individual patients open up possibilities for personalized therapy, including drug testing and cell replacement tailored to each patient's genetic makeup. Finally, embryonic and induced pluripotent stem cells advance our understanding of human development by providing windows into its earliest stages, offering insight into congenital disorders and developmental biology.

Ongoing research continues to characterize stem-cell types, properties, and potential applications. From the use of mesenchymal stem cells for their immunomodulatory properties to the development of organoids for studying complex tissue interactions, the field of stem cell biology is constantly expanding our understanding of human biology and disease.

The following sections define the main characteristics and classifications of stem cells, review established and investigational applications, and examine the technical, clinical, and regulatory limitations that determine how far individual approaches can be translated into practice.

2. Defining Stem Cells

Stem cells are undifferentiated or partially differentiated cells that possess two fundamental properties: the ability to self-renew through cell division and the capacity to differentiate into specialized cell types. These unique characteristics distinguish stem cells from other cell types and underpin their central roles in development, tissue homeostasis, and regeneration (Weissman, 2000).

2.1. Self-renewal

Self-renewal refers to the ability of stem cells to divide and produce identical daughter cells, thereby maintaining the stem cell pool. This process is critical for the long-term maintenance of stem cell populations and can occur through two main mechanisms.

Two modes of division underlie this capacity. In symmetric division, a stem cell divides into two identical daughter stem cells, a mode important for expanding the stem cell pool during development or in response to injury (Morrison & Kimble, 2006). In asymmetric division, a stem cell divides into one stem cell and one more differentiated progenitor cell, which allows the stem cell pool to be maintained while simultaneously producing cells that can differentiate to replenish tissues (Knoblich, 2008).

The balance between symmetric and asymmetric division is tightly regulated and can be influenced by various factors, including developmental stage, tissue demands, and environmental cues. For instance, during embryonic development, there is a predominance of symmetric divisions to expand the stem cell pool, while in adult tissues, asymmetric divisions are more common to maintain tissue homeostasis (He et al., 2009).

The molecular mechanisms underlying self-renewal are complex and involve numerous signalling pathways and transcription factors. Key players include:

Among the most important of these are the Wnt signalling pathway, which is important for maintaining stemness in various stem cell types, including intestinal and neural stem cells (Clevers et al., 2014), and the Notch signalling pathway, which governs stem cell fate decisions and self-renewal in many tissues (Koch et al., 2013). Transcription factors such as Oct4, Sox2, and Nanog form a core

regulatory network essential for maintaining pluripotency in embryonic stem cells (Young, 2011), while the Polycomb group (PcG) and Trithorax group (TrxG) proteins regulate chromatin structure and play key roles in maintaining stem cell identity (Richly et al., 2011).

Understanding and manipulating the mechanisms of self-renewal is essential for ex vivo expansion of stem cells for research and clinical applications. For example, the ability to expand haematopoietic stem cell ex vivo could greatly enhance their utility in bone marrow transplantation (Dahlberg et al., 2011).

2.2. Differentiation Potential

The differentiation potential, or potency, of stem cells refers to their capacity to give rise to different cell types. This property is what makes stem cell so valuable for regenerative medicine and tissue engineering. The potency of stem cells can be classified into several categories.

Totipotent cells are the most potent, capable of giving rise to all embryonic and extraembryonic tissues. The zygote and the first few cells resulting from its initial divisions, up to the 4-cell stage in humans, are considered totipotent, and they can generate an entire organism, including the placenta and other supporting tissues (Condic, 2014). Pluripotent stem cells can give rise to all cell types of the three germ layers, endoderm, mesoderm, and ectoderm , but cannot form extraembryonic tissues; embryonic stem cells (ESCs) derived from the inner cell mass of blastocysts and induced pluripotent stem cells (iPSCs) are examples, and they can potentially differentiate into any of the hundreds of cell types in the adult body (Romito & Cobellis, 2016). Multipotent stem cells differentiate into multiple cell types within a specific lineage or closely related family of cells: haematopoietic stem cells give rise to all types of blood cells (Seita & Weissman, 2010), neural stem cells differentiate into neurons, astrocytes, and oligodendrocytes (Gage, 2000), and mesenchymal stem cells differentiate into bone, cartilage, fat, and muscle cells (Uccelli et al., 2008). Oligopotent stem cells can differentiate into a few closely related cell types; lymphoid progenitor cells, for example, give rise to T cells, B cells, and natural killer cells (Ceredig et al., 2009). Unipotent stem cells, finally, produce only one cell type but retain the property of self-renewal, muscle satellite cells being a representative example (Yin et al., 2013).

The differentiation potential of stem cells is not fixed and can be influenced by various factors, including:

Epigenetic modifications are among the most important of these, since changes in DNA methylation and histone modifications can affect gene expression patterns and, consequently, differentiation potential (Reik, 2007). The expression of specific transcription factors can drive differentiation towards particular lineages, MyoD can induce muscle cell differentiation, while Runx2 promotes osteogenic differentiation (Davis et al., 1987; Komori, 2006) , and extracellular signals such as growth factors, cytokines, and other signalling molecules in the stem cell microenvironment likewise influence differentiation decisions (Watt & Hogan, 2000). Physical factors matter as well: mechanical forces and substrate stiffness can affect differentiation, particularly in mesenchymal stem cells (Engler et al., 2006).

Understanding and controlling the differentiation potential of stem cells is central for their application in regenerative medicine. Researchers are continually developing protocols to direct the differentiation of pluripotent stem cells into specific cell types for potential therapeutic use. For example, protocols have been developed to generate functional pancreatic β-cells from human pluripotent stem cells, offering hope for cell replacement therapy in diabetes (Pagliuca et al., 2014).

Moreover, recent advances in direct reprogramming or transdifferentiation, where one mature cell type is directly converted into another without going through a pluripotent state, have opened up new possibilities for generating specific cell types for therapy (Xu et al., 2015).

2.3. Stem Cell Markers

Identifying and isolating stem cells relies heavily on the expression of specific molecular markers. These markers can be cell surface proteins, transcription factors, or other molecules that are characteristically expressed by stem cells. The expression of these markers often varies depending on the type of stem cell and its state of differentiation.

For pluripotent stem cells (both embryonic stem cells and induced pluripotent stem cells), key markers include:

Oct4 (POU5F1) is a transcription factor important for maintaining pluripotency and is considered one of the master regulators of the pluripotent state (Nichols et al., 1998). Nanog is another transcription factor essential for pluripotency and self-renewal, working in concert with Oct4 and Sox2 to maintain the undifferentiated state (Mitsui et al., 2003), while Sox2 itself forms a core regulatory network with Oct4 and Nanog (Avilion et al., 2003). Among surface markers, SSEA-3 and SSEA-4 are cell surface glycoproteins highly expressed in human pluripotent stem cells (Adewumi et al., 2007), as are the keratan sulfate antigens TRA-1-60 and TRA-1-81 (Adewumi et al.,

2007). ALDH1A1, aldehyde dehydrogenase 1 family member A1, is an intracellular enzyme expressed in various stem cell populations (Tomita et al., 2016).

For adult stem cells, markers vary depending on the specific cell type. Haematopoietic stem cells are identified by CD34, a cell surface glycoprotein used for their identification and isolation (Sidney et al., 2014); by CD38, often used in combination with CD34, since CD34+/CD38- cells are more primitive (Bhatia et al., 1998); and by CD90, also known as Thy-1 (Baum et al., 1992). Mesenchymal stem cells are defined by the positive markers CD105, CD73, and CD90, together with the negative markers CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR (Dominici et al., 2006). Neural stem cells express nestin, an intermediate filament protein (Lendahl et al., 1990), Sox2, which is important for their maintenance (Ellis et al., 2004), and the surface marker CD133, or prominin-1 (Uchida et al., 2000). Intestinal stem cells are marked by Lgr5, a marker of the crypt base columnar cells (Barker et al., 2007), and by Bmi1, which marks a more quiescent population (Sangiorgi & Capecchi, 2008). Muscle satellite cells, finally, are identified by Pax7, a transcription factor marking quiescent cells (Seale et al., 2000), and by Myf5, which is expressed once they are activated (Kuang et al., 2007).

It's important to note that no single marker is typically sufficient to definitively identify a stem cell population. Instead, a combination of positive and negative markers is often used. Moreover, the expression of these markers can be dynamic, changing as stem cell transition between different states (e.g., from quiescence to activation).

The identification of specific stem cell markers has been key for isolating and characterising different stem cell populations. For example, the discovery of CD34 as a marker for haematopoietic stem cell substantially changed bone marrow transplantation by allowing for the enrichment of HSCs from donor samples (Civin et al., 1984).

Furthermore, the development of techniques like fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) has allowed researchers to isolate highly pure populations of stem cell based on their marker expression. This has been instrumental in studying the properties of different stem cell subpopulations and in developing cell-based therapies (Ratcliffe et al., 2011).

Recent advances in single-cell RNA sequencing technologies have further refined our understanding of stem cell heterogeneity and have led to the identification of new markers and stem cell subpopulations (Grün et al., 2015). This has revealed that many stem cell populations are more heterogeneous than previously thought, with different subpopulations possessing distinct molecular profiles and functional properties.

The continuing discovery and characterisation of stem cell markers not only enhances our fundamental understanding of stem cell biology but also has important practical implications for stem cell-based therapies. More precise identification and isolation of stem cell populations will improve the purity, consistency, and safety of cell-based products.

2.4. Stem Cell Niche

The stem cell niche is a specialized microenvironment that is central to regulating stem cell behaviour, including self-renewal, differentiation, and quiescence. This concept, first proposed by Ray Schofield in 1978, has become fundamental to our understanding of stem cell biology (Schofield, 1978).

The niche comprises several interacting components. Its cellular components include supporting cells that interact directly with stem cells; in the haematopoietic niche, for example, osteoblasts, endothelial cells, and mesenchymal stromal cells all play essential roles (Morrison & Scadden, 2014). The extracellular matrix provides structural support and acts as a reservoir for growth factors, and different matrix components influence stem cell behaviour; laminin-511, for instance, supports the self-renewal of embryonic stem cells (Rodin et al., 2010). Soluble factors, including various growth factors, cytokines, and morphogens, regulate stem cell fate; among them is stem cell factor (SCF), which is central for haematopoietic stem cell maintenance (Ding et al., 2012). Physical factors such as mechanical forces, substrate stiffness, and spatial organization also shape behaviour, with matrix elasticity shown to direct mesenchymal stem cell lineage specification (Engler et al., 2006). Finally, metabolic factors matter: the oxygen tension and nutrient availability of the niche significantly affect function, and many niches are characterised by low oxygen tension, which helps maintain stemness (Mohyeldin et al., 2010).

The niche serves several critical functions. It maintains stemness by providing signals that prevent differentiation and promote self-renewal; in the intestinal niche, Paneth cells secrete Wnt ligands that maintain stem cell identity (Sato et al., 2011). It regulates quiescence, since many adult stem cells are held in a quiescent state and activated only

when needed; in the hair follicle niche, BMP signalling from dermal papilla cells promotes this quiescence (Blanpain & Fuchs, 2014). It controls stem cell numbers through physical constraints that regulate the size of the pool, as in the Drosophila germline niche, where the number of cap cells determines how many stem cells can be maintained (Xie & Spradling, 2000). It directs differentiation by providing cues once stem cells begin to differentiate; in the neural niche, VEGF signalling from endothelial cells promotes neurogenesis (Tavazoie et al., 2008). And it protects stem cells from environmental insults: melanocyte stem cells in the hair follicle bulge, for instance, are shielded from UV-induced DNA damage by their location deep within the skin (Nishimura et al., 2002).

Understanding the stem cell niche has important implications for both basic science and clinical applications. In regenerative medicine, recreating aspects of the niche in vitro is important for maintaining and expanding stem cells for therapeutic use. For example, efforts to expand haematopoietic stem cell ex vivo have focused on mimicking key niche signals (Boitano et al., 2010).

Moreover, the niche plays a role in disease processes. Cancer stem cells can hijack normal niche signals to support their growth and survival. Understanding these processes could lead to new therapeutic strategies targeting the niche (Plaks et al., 2015).

Recent technological advances, such as intravital imaging and single-cell sequencing, are providing exceptional insights into the dynamics of stem cell-niche interactions in vivo. These advances reveal the heterogeneity within stem cell populations and the complexity of niche-mediated regulation (Krieger & Simons, 2015).

2.5. Epigenetic Regulation of Stem Cells

Epigenetic mechanisms are important in regulating stem-cell identity, self-renewal, and differentiation. These mechanisms alter gene expression without changing the underlying DNA sequence and include:

DNA methylation involves the addition of methyl groups to cytosine bases, typically at CpG dinucleotides; in embryonic stem cells the genome is generally hypomethylated, with key developmental genes bearing bivalent marks, both activating and repressive, that keep them poised for activation (Meissner, 2010). Histone modifications include various post-translational modifications of histone proteins, such as methylation, acetylation, and phosphorylation; H3K4me3 is associated with active transcription while H3K27me3 is repressive, and the presence of both marks, forming bivalent domains, on key developmental genes is a hallmark of pluripotent stem cells (Bernstein et al., 2006). Chromatin remodeling by ATP-dependent complexes alters the positioning of nucleosomes and thereby affects gene accessibility; the esBAF complex, for instance, is important for maintaining pluripotency in embryonic stem cells (Ho et al., 2009). Non-coding RNAs, including microRNAs and long non-coding RNAs, also play important regulatory roles, as in the case of the lncRNA HOTAIR, which regulates HOX genes and is involved in stem cell differentiation (Rinn et al., 2007).

Epigenetic regulation matters in stem cells for several reasons. In pluripotent cells, epigenetic mechanisms maintain the expression of pluripotency genes while keeping lineage-specific genes repressed but poised for activation (Boyer et al., 2006). During differentiation, epigenetic changes guide the activation of lineage-specific genes and the repression of pluripotency genes, a process involving extensive remodeling of the epigenome (Gifford et al., 2013). Epigenetic marks also provide a form of cellular memory that helps maintain cell identity through cell divisions, which is key for the stability of differentiated cell types (Probst et al., 2009), and they allow stem cells to respond to environmental cues and adapt their gene expression accordingly; particularly important in adult stem cells, which must respond to tissue demands (Ramalho-Santos et al., 2002). Finally, epigenetic changes accumulate with age and can affect stem cell function, so understanding them could provide insight into the age-related decline in tissue regeneration (Beerman et al., 2013).

Recent advances in epigenetic research have significant implications for stem cell biology and regenerative medicine.

The discovery that somatic cells can be reprogrammed to pluripotency, generating iPSCs through the overexpression of specific transcription factors, fundamentally relies on epigenetic remodeling (Takahashi & Yamanaka, 2006). Epigenetic manipulation can also facilitate direct reprogramming, converting one cell type to another without passing through a pluripotent state; fibroblasts, for example, can be converted directly to neurons through a combination of transcription factors and epigenetic modifiers (Ladewig et al., 2012). Understanding the epigenetic changes that occur during normal development further helps in designing more efficient protocols for directing differentiation in vitro (Xie et al., 2013), and drugs targeting epigenetic mechanisms, such as DNA methyltransferase inhibitors and histone deacetylase inhibitors, are being explored for their potential to manipulate stem cell

behaviour in vivo (Tough et al., 2016). Studying the epigenetic profiles of patient-derived iPSCs, finally, can provide insight into the epigenetic basis of various diseases, particularly those with a developmental origin (Rouhani et al., 2014).

Work on epigenetic regulation in stem cells continues to inform our view of development, disease and possible treatments. The dynamic nature of epigenetic modifications offers new possibilities for manipulating cell fate and function, with far-reaching implications for regenerative medicine and beyond.

3. Types of Stem Cells

Stem cells can be classified by origin, differentiation potential, and tissue source. These categories describe different biological properties and should be kept distinct when assessing a proposed treatment.

3.1. Classification Based on Origin

3.1.1 Embryonic stem cells (ESCs)

Embryonic stem cells are derived from the inner cell mass of blastocysts, typically 4-5 days post-fertilization. These cells are pluripotent, capable of differentiating into all cell types of the three germ layers: endoderm, mesoderm, and ectoderm (Thomson et al., 1998).

They express pluripotency markers such as Oct4, Nanog, and Sox2, exhibit indefinite self-renewal in vitro, form teratomas when injected into immunodeficient mice, and contribute to all tissues when injected into blastocysts.

ESCs have several unique properties that make them valuable for research and potential therapeutic applications. Their pluripotency, the ability to differentiate into any cell type of the body, makes them an ideal source for generating specific cell types for regenerative medicine (Murry & Keller, 2008). Their capacity for self-renewal allows them to proliferate indefinitely in culture while maintaining the pluripotent state, providing an unlimited source of cells for research and potential therapies (Thomson et al., 1998). They are also amenable to genetic modification, which allows the creation of transgenic cell lines and animal models (Capecchi, 2005), and human ESCs can be used to model early human development and study developmental disorders (Zhu & Huangfu, 2013).

The use of ESCs also presents several challenges. Their derivation involves the destruction of human embryos, which has led to significant ethical debate and to regulatory restrictions in many countries (Lo & Parham, 2009). Because they are derived from embryos, they are allogeneic to potential recipients, which raises concerns about immune rejection in clinical applications (Boyd et al., 2012). Their pluripotency also carries a risk of tumour formation, since undifferentiated cells can give rise to teratomas if transplanted (Lee et al., 2013).

Even so, ESCs remain a valuable tool in stem cell research and have contributed significantly to our understanding of early development and pluripotency. They have been used to generate a wide variety of cell types in vitro, including neurons, cardiomyocytes, and pancreatic β-cells, offering potential cell sources for regenerative medicine (Keller, 2005).

3.1.2 Adult (Somatic) Stem Cells

Adult stem cells, also known as somatic stem cells, are undifferentiated cells found in various tissues throughout the body. They are responsible for tissue homeostasis and repair. Unlike embryonic stem cells, adult stem cells are typically multipotent or unipotent, with a more restricted differentiation potential (Wagers & Weissman, 2004).

Adult stem cells are characterised by their capacity for self-renewal, their ability to differentiate into multiple cell types within a specific tissue or organ, and their relatively quiescent nature, being activated in response to injury or tissue turnover.

Adult stem cells can be found in various tissues.

a) Hematopoietic Stem Cells (HSCs)

HSCs are perhaps the best-characterised adult stem cell. They reside primarily in the bone marrow and give rise to all blood cell types (Seita & Weissman, 2010).

They are identified by markers such as CD34+ and CD38-, can reconstitute the entire blood system when transplanted, and are used clinically in bone marrow transplantation for treating blood disorders and cancers.

Recent research has revealed the heterogeneity within the HSC population, with distinct subpopulations showing different biases towards myeloid or lymphoid lineages (Copley et al., 2012).

b) Mesenchymal Stem Cells (MSCs)

MSCs are multipotent stromal cells that can differentiate into various cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes. They can be isolated from various tissues, including bone marrow, adipose tissue, and dental pulp (Uccelli et al., 2008).

They are plastic-adherent cells with a fibroblast-like morphology, express surface markers such as CD105, CD73, and CD90, and possess immunomodulatory properties.

MSCs have shown promise in various therapeutic applications, including tissue engineering for bone and cartilage repair, the treatment of graft-versus-host disease, and immunomodulation in autoimmune diseases.

Recent research has focused on the paracrine effects of MSCs, with their secreted factors showing therapeutic potential in various disease models (Caplan & Correa, 2011).

c) Neural Stem Cells (NSCs)

NSCs are self-renewing, multipotent cells that can generate neurons, astrocytes, and oligodendrocytes. They are primarily found in two regions of the adult brain: the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus (Gage, 2000).

They express markers such as nestin and Sox2, can be cultured as neurospheres in vitro, and play a role in adult neurogenesis and brain plasticity.

NSCs have potential applications in treating neurodegenerative diseases and brain injuries. Recent research has also highlighted the role of NSCs in learning and memory, as well as their potential involvement in psychiatric disorders (Zhao et al., 2008).

d) Epithelial Stem Cells

Epithelial stem cells are responsible for the continuous renewal of epithelial tissues, such as the skin, intestine, and cornea. They are typically unipotent or oligopotent, giving rise to the specific cell types within their respective tissues (Blanpain et al., 2007).

Examples include epidermal stem cells in the basal layer of the skin, intestinal stem cells at the base of the intestinal crypts, and limbal stem cells in the corneal limbus.

Recent research has revealed the importance of the stem cell niche in regulating epithelial stem cell function and the role of these cells in wound healing and cancer (Barker et al., 2010).

e) Cardiac Stem Cells

The existence of cardiac stem cells has been a subject of debate in recent years. While some studies have reported the isolation of c-kit+ cells with cardiogenic potential from the adult heart, more recent lineage tracing studies have questioned their significance in homeostasis and regeneration (van Berlo et al., 2014).

Current research is focusing on understanding the regenerative capacity of the heart and exploring strategies to enhance cardiac repair, including the use of other stem cell types or stimulation of cardiomyocyte proliferation (Eschenhagen et al., 2017).

3.1.3 Induced Pluripotent stem cells (iPSCs)

Induced pluripotent stem cells represent a breakthrough in stem cell research. First developed by Shinya Yamanaka and colleagues in 2006, iPSCs are created by reprogramming adult somatic cells to a pluripotent state through the introduction of specific transcription factors (Takahashi & Yamanaka, 2006).

They express pluripotency markers similar to those of ESCs, can differentiate into all cell types of the three germ layers, and exhibit similar epigenetic profiles. Crucially, they avoid the ethical concerns associated with ESCs and allow the creation of patient-specific stem cell lines.

The process of generating iPSCs typically involves the introduction of four key transcription factors - Oct4, Sox2, Klf4, and c-Myc (known as the Yamanaka factors) - into somatic cells. This induces a dramatic reprogramming of the cell's epigenetic state, reverting it to a pluripotent state similar to that of embryonic stem cell (Stadtfeld & Hochedlinger, 2010).

Since their initial discovery, significant advances have been made in iPSC technology. Improved reprogramming methods now include non-integrating approaches for delivering reprogramming factors, such as Sendai virus vectors, episomal plasmids, and mRNA transfection, which reduce the risk of insertional mutagenesis associated with earlier retroviral approaches (Schlaeger et al., 2015). Alternative reprogramming factors have also been identified: while the original Yamanaka factors remain widely used, other combinations can induce pluripotency, and Glis1, for instance, can replace c-Myc, potentially reducing the oncogenic risk (Maekawa et al., 2011). Chemical approaches using small molecules have been developed to replace some or all of the reprogramming factors, potentially simplifying the process and reducing variability (Hou et al., 2013), and building on iPSC technology, researchers have developed methods for converting one somatic cell type directly into another without passing through a pluripotent state, a process known as transdifferentiation (Xu et al., 2015).

The applications of iPSCs are correspondingly broad. Patient-specific iPSCs can be used to create “disease in a dish” models, allowing researchers to study disease mechanisms

and test potential therapies, an approach that has been particularly valuable for studying neurological disorders, cardiovascular diseases, and genetic conditions (Avior et al., 2016). iPSC-derived cells also provide a human-relevant platform for drug screening and toxicity testing, potentially improving the efficiency of drug development and reducing reliance on animal models (Engle & Puppala, 2013), and cells derived from individual patients could be used to predict drug responses and develop tailored therapies (Matsa et al., 2016). In regenerative medicine, although clinical applications remain at an early stage, iPSCs are being investigated for cell replacement therapies, and clinical trials using iPSC-derived cells are underway for conditions such as age-related macular degeneration and Parkinson's disease (Mandai et al., 2017; Barker et al., 2017).

Several challenges nonetheless require attention. iPSCs can acquire genetic and epigenetic abnormalities during reprogramming and culture, which must be carefully monitored (Liang & Zhang, 2013), and there can be significant variability between lines, even those derived from the same individual, so lines must be characterised and selected with care for research and clinical use (Kilpinen et al., 2017). Protocols for differentiating iPSCs into specific cell types often yield heterogeneous populations, requiring further purification for clinical applications (Murry & Keller, 2008). As with ESCs, finally, the pluripotency of iPSCs carries a risk of teratoma formation if undifferentiated cells are transplanted (Lee et al., 2013).

These difficulties notwithstanding, iPSCs have substantially changed stem cell research and continue to have investigational potential for both basic science and clinical applications.

3.1.4 Fetal Stem Cells

Fetal stem cells are derived from fetal tissues and possess intermediate characteristics between embryonic and adult stem cells. They can be isolated from various fetal tissues, including blood, liver, and bone marrow (O'Donoghue & Fisk, 2004).

They have a higher proliferative capacity than adult stem cells, a more diverse differentiation potential than most adult stem cells and are less likely to be rejected after transplantation than adult cells.

Several types of fetal stem cells are recognised. Fetal haematopoietic stem cells, found in fetal blood and liver, have a higher proliferative capacity and engraftment potential than adult HSCs (Rebel et al., 1996). Fetal mesenchymal stem cells, isolated from various fetal tissues including bone marrow, liver, and lung, show enhanced differentiation potential and immunomodulatory properties compared with adult MSCs (Götherström et al., 2004). Amniotic fluid stem cells, derived from amniotic fluid, exhibit properties of both embryonic and adult stem cells, can differentiate into cells of all three germ layers, and have shown promise in tissue engineering applications (De Coppi et al., 2007). Placental stem cells, isolated from various parts of the placenta including the amnion, chorion, and umbilical cord, have shown potential in treating disorders such as stroke and lung injury (Parolini et al., 2008).

Their potential applications include fetal tissue engineering, in which fetal stem cells are used to generate tissues for in utero repair of congenital defects (Flake & Zanjani, 1999), and the treatment of genetic disorders, since transplantation before birth can take advantage of the developing immune system's tolerance (Muench, 2005). Fetal neural stem cells have also shown promise in animal models of neurodegenerative disease, including MS (Aharonowiz et al., 2008; Nie et al., 2023), and have entered early clinical testing in progressive MS (Leone et al., 2023).

Ethical considerations:

The use of fetal stem cells raises ethical concerns like those associated with embryonic stem cells. However, some sources of fetal stem cells, such as amniotic fluid and placenta, can be obtained without terminating pregnancies, potentially mitigating some ethical issues (Antoniadou & David, 2016).

3.2. Classification Based on Differentiation Potential

3.2.1 Totipotent Stem Cells

Totipotent stem cells have the highest differentiation potential, capable of giving rise to all embryonic and extraembryonic tissues. In mammals, only the zygote and the first few cells resulting from its initial divisions (up to the 4-cell stage in humans) are considered totipotent (Condic, 2014).

Totipotent cells can generate an entire organism, including all embryonic tissues and extraembryonic tissues such as the placenta. They exist only transiently in early embryonic development and lose totipotency as cell fate becomes progressively restricted.

The concept of totipotency is fundamental to our understanding of early embryonic development, and recent research has provided insight into several of its aspects.

Totipotent cells undergo extensive epigenetic reprogramming, including global DNA demethylation and changes in histone modifications (Xu & Xie, 2018). The transition from maternal to zygotic control of gene expression, known as zygotic genome activation, is a critical event in early development and in the maintenance of totipotency (Lee et al., 2014). The first cell fate decision in mammalian development, which distinguishes the inner cell mass from the trophectoderm, marks the loss of totipotency (Chazaud & Yamanaka, 2016).

While natural totipotent cells exist only transiently, researchers are exploring ways to capture or induce a totipotent-like state in vitro. This could provide valuable insights into early development and potentially open new avenues for regenerative medicine (Ishiuchi & Torres-Padilla, 2013).

3.2.2 Pluripotent Stem Cells

Pluripotent stem cells can differentiate into all cell types of the three germ layers (endoderm, mesoderm, and ectoderm) but cannot form extraembryonic tissues. The main types of pluripotent stem cells are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) (Romito & Cobellis, 2016).

Pluripotent cells express pluripotency markers such as Oct4, Nanog, and Sox2, form teratomas containing tissues from all three germ layers when injected into immunodeficient mice and exhibit high telomerase activity together with indefinite self-renewal in vitro.

The pluripotent state is maintained by a complex network of transcription factors, epigenetic regulators, and signalling pathways. At its centre, the transcription factors Oct4, Sox2, and Nanog form the core regulatory network (Young, 2011). Two distinct pluripotent states have been identified in rodents, naïve and primed, with human PSCs generally exhibiting characteristics of the primed state; efforts are ongoing to capture naïve human PSCs, which may offer advantages for certain applications (Nichols & Smith, 2009). Beyond the core factors, many other genes contribute to pluripotency, including Klf4, c-Myc, Lin28, and Sall4 (Yeo & Ng, 2013), and various signalling pathways, among them LIF/STAT3, BMP, and Wnt, play essential roles in maintaining the pluripotent state (Weinberger et al., 2016).

Pluripotent stem cells have a wide range of applications. In developmental biology they provide a valuable tool for studying early human development and lineage specification (Zhu & Huangfu, 2013), while patient-specific iPSCs allow the creation of "disease in a dish" models that enable the study of disease mechanisms and drug screening (Avior et al., 2016). PSC-derived cells also provide a human-relevant platform for drug screening and toxicity testing (Engle & Puppala, 2013), and in regenerative medicine PSCs are being developed for cell replacement across a range of diseases, although clinical applications remain at an early stage (Trounson & DeWitt, 2016).

3.2.3 Multipotent Stem Cells

Multipotent stem cells can differentiate into multiple cell types within a specific lineage or closely related family of cells. Most adult stem cells are considered multipotent (Wagers & Weissman, 2004).

Examples of multipotent stem cells include haematopoietic stem cells, which give rise to all types of blood cells (Seita & Weissman, 2010); neural stem cells, which differentiate into neurons, astrocytes, and oligodendrocytes (Gage, 2000); mesenchymal stem cells, which differentiate into bone, cartilage, fat, and muscle cells (Uccelli et al., 2008); and intestinal stem cells, which generate all cell types of the intestinal epithelium (Barker, 2014).

These cells have a more restricted differentiation potential than pluripotent cells, are often tissue-specific and reside in specialized niches, and play central roles in tissue homeostasis and repair.

Recent research has revealed significant heterogeneity within populations of multipotent stem cells. Different subpopulations of HSCs show distinct lineage biases and self-renewal capacities (Copley et al., 2012), multiple types of neural stem cells have been identified in different regions of the brain with varying proliferation rates and differentiation potentials (Ming & Song, 2011), and studies have identified distinct subpopulations of MSCs with different differentiation propensities and immunomodulatory properties (Mo et al., 2016).

The clinical applications are correspondingly varied. HSCs are routinely used in bone marrow transplantation for treating blood disorders and cancers (Copelan, 2006), MSCs are being explored for tissue engineering applications including bone and cartilage repair (Ren et al., 2012), and NSCs warrant further investigation for treating neurodegenerative diseases and brain injuries (Lindvall & Kokaia, 2010). MSCs are also being investigated for their immunomodulatory properties in the treatment of autoimmune diseases and graft-versus-host disease (Le Blanc & Mougiakakos, 2012).

3.2.4 Oligopotent Stem Cells

Oligopotent stem cells can differentiate into a few closely related cell types. They have a more restricted differentiation potential than multipotent stem cell but can still give rise to multiple cell types within a specific lineage (Seaberg & van der Kooy, 2003).

Examples of oligopotent stem cells include lymphoid progenitors, which give rise to T cells, B cells, and natural killer cells (Ceredig et al., 2009); myeloid progenitors, which differentiate into granulocytes, monocytes, erythrocytes, and platelets (Akashi et al., 2000); neural crest stem cells, which generate various cell types including neurons, glial cells, melanocytes, and some mesenchymal derivatives (Shyamala et al., 2015); and hepatic oval cells, which can differentiate into hepatocytes and bile duct cells (Roskams et al., 2004).

They have a more restricted differentiation potential than multipotent stem cells, often serve as intermediate progenitors in lineage hierarchies, and play important roles in tissue-specific regeneration and homeostasis.

Recent research has provided insight into the biology of these cells. Many oligopotent progenitors exhibit lineage priming, expressing low levels of genes associated with multiple potential fates before committing to a specific lineage (Månsson et al., 2007). Some have shown unexpected plasticity under certain conditions, which challenges traditional lineage hierarchies (Yamamoto et al., 2013), and their behaviour is tightly regulated by their specific niches, which provide key signals for self-renewal and differentiation (Krieger & Simons, 2015).

Manipulation of lymphoid and myeloid progenitors could lead to new therapies for blood disorders and improve bone marrow transplantation outcomes (Doulatov et al., 2012). Understanding the biology of oligopotent progenitors in various tissues could also provide insight into cancer initiation and progression, potentially leading to new therapeutic strategies (Visvader & Lindeman, 2008), and these cells could be used for targeted regeneration of specific cell types within a tissue, potentially with fewer risks than more potent stem cells (Rezza et al., 2014).

3.2.5 Unipotent Stem Cells

Unipotent stem cells can only produce one cell type but retain the property of self-renewal. They are the most restricted in terms of differentiation potential but play important roles in maintaining and repairing specific tissues (Wagers & Weissman, 2004).

Examples of unipotent stem cells include muscle satellite cells, which give rise to mature muscle cells and contribute substantially to muscle repair and regeneration (Yin et al., 2013), epidermal stem cells, which produce keratinocytes and are responsible for the continual renewal of the skin epidermis (Blanpain & Fuchs, 2006); spermatogonial stem cells, which generate sperm cells throughout adult life (de Rooij & Russell, 2000); and hepatoblasts, which give rise to hepatocytes in the liver (Miyajima et al., 2014).

These cells are highly specialized and tissue-specific, maintain the ability to self-renew, and are often quiescent, becoming activated in response to injury or tissue turnover.

Recent research has provided new insight into the biology of unipotent stem cells. Some have shown unexpected plasticity under certain conditions; spermatogonial stem cells, for example, can be reprogrammed to pluripotency in vitro (Kanatsu-Shinohara et al., 2004). Functional heterogeneity exists even within unipotent populations, and different subpopulations of muscle satellite cells have been identified with varying propensities for self-renewal and differentiation (Kuang et al., 2007). Their behaviour is tightly controlled by their specific niches, and understanding these interactions is important for manipulating the cells therapeutically (Chakkalakal et al., 2012). Their function often declines with age, contributing to reduced tissue regeneration, so understanding the mechanisms of this decline could lead to strategies for maintaining tissue health in ageing populations (Sousa-Victor et al., 2015). Recent studies have also highlighted the importance of epigenetic mechanisms in maintaining their identity and function, with DNA methylation playing a key role in regulating the quiescence and activation of muscle satellite cells (Yue et al., 2017).

Unipotent stem cells offer the potential for highly targeted regeneration of specific tissues: muscle satellite cells, for instance, are being explored for treating muscular dystrophies and age-related muscle wasting (Tedesco et al., 2010). Epidermal stem cells are essential for skin wound healing, and understanding and manipulating them could lead to improved treatments for chronic wounds and burns (Mascré et al., 2012). Spermatogonial stem cells could be used to treat certain forms of male infertility, and techniques are being developed to isolate, expand, and transplant them (Valli et al., 2014), while hepatoblasts and other liver progenitor cells are being studied for their potential in treating liver diseases and promoting regeneration after injury (Miyajima et al., 2014).

Several obstacles persist. Developing methods to reliably isolate and expand unipotent stem cells while maintaining their properties is still difficult for many cell types, and finding ways to activate and direct endogenous unipotent cells in vivo could lead to novel regenerative therapies. Understanding the factors that maintain unipotent identity could also inform strategies for cellular reprogramming and regeneration, and elucidating the mechanisms of unipotent stem cell ageing, together with developing interventions to maintain their function throughout the lifespan, remains an important area of ongoing research.

3.3. Classification Based on Tissue of Origin

Stem cells can also be classified based on the specific tissues or organs from which they are derived. This classification is particularly relevant for adult stem cells, which are found in various tissues throughout the body. Understanding the properties and potentials of stem cells from different tissue sources is central for developing targeted therapies and regenerative medicine approaches.

3.3.1 Bone Marrow-Derived Stem Cells

Bone marrow is a rich source of several types of stem cells. Haematopoietic stem cells are the best-characterised and most clinically used adult stem cells; they give rise to all blood cell lineages and are routinely used in bone marrow transplantation for treating blood disorders and cancers (Copelan, 2006). They are identified by markers such as CD34+, CD38-, and CD90+, can reconstitute the entire blood system when transplanted, and are regulated by a complex bone marrow niche involving multiple cell types and signalling molecules (Morrison & Scadden, 2014). Mesenchymal stem cells, also known as multipotent stromal cells, can differentiate into various cell types including osteoblasts, chondrocytes, adipocytes, and myocytes (Uccelli et al., 2008); they are plastic-adherent cells with a fibroblast-like morphology, express markers such as CD105, CD73, and CD90, and possess immunomodulatory properties. Endothelial progenitor cells, the third population, contribute to the formation of new blood vessels and have potential applications in treating cardiovascular diseases (Asahara et al., 1997).

Recent advances in this area include the development of ex vivo expansion protocols for HSCs to increase the number of transplantable cells (Dahlberg et al., 2011), the exploration of MSCs for their immunomodulatory properties in treating autoimmune diseases and graft-versus-host disease (Le Blanc & Mougiakakos, 2012), and the investigation of bone marrow-derived stem cells for treating ischemic diseases and promoting tissue repair (Raval & Losordo, 2013).

3.3.2 Adipose-Derived Stem Cells (ADSCs)

Adipose tissue is an abundant and accessible source of mesenchymal stem cell. ADSCs have gained significant attention in recent years due to their ease of isolation and multipotent differentiation potential (Zuk et al., 2002).

ADSCs can differentiate into adipogenic, osteogenic, chondrogenic, and myogenic lineages, express surface markers similar to those of bone marrow-derived MSCs and possess immunomodulatory and anti-inflammatory properties.

Recent advances include the development of optimised isolation and culture protocols for clinical-grade ADSCs (Bourin et al., 2013), the exploration of these cells for soft tissue reconstruction and wound healing (Hassan et al., 2014), and the investigation of ADSC-derived exosomes for their therapeutic potential in various diseases (Katsuda et al., 2013).

3.3.3 Neural stem cells (NSCs)

Neural stem cells are self-renewing, multipotent cells that can generate neurons, astrocytes, and oligodendrocytes. In the adult brain, NSCs are primarily found in two regions: the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus (Gage, 2000).

NSCs express markers such as nestin and Sox2, can be cultured as neurospheres in vitro, and play a role in adult neurogenesis and brain plasticity.

Recent advances include the identification of diverse subtypes of neural stem cell with distinct molecular profiles and differentiation potentials (Llorens-Bobadilla et al., 2015), the development of protocols for generating region-specific neural organoids from pluripotent stem cells (Lancaster & Knoblich, 2014), and the exploration of NSC-based therapies for neurodegenerative diseases and brain injuries (Lindvall & Kokaia, 2010).

3.3.4 Dental Pulp Stem Cells (DPSCs)

Dental pulp stem cells are mesenchymal stem cells found in the dental pulp of adult teeth. They have gained attention due to their accessibility and potential applications in regenerative dentistry and beyond (Gronthos et al., 2000).

DPSCs can differentiate into odontoblasts, osteoblasts, chondrocytes, and neuronal cells, express markers similar

to those of bone marrow-derived MSCs and possess immunomodulatory properties.

Recent advances include the exploration of DPSCs for dental tissue engineering, including regeneration of the dentin-pulp complex and periodontal tissues (Huang et al., 2009), their investigation for neural tissue engineering and potential applications in treating neurological disorders (Martens et al., 2014), and the development of DPSC banking services for potential future autologous use (Hilkens et al., 2013).

3.3.5 Liver Stem Cells

The liver has a considerable regenerative capacity, and several populations of stem and progenitor cells have been identified in this organ. Hepatic stellate cells can be activated to become myofibroblasts in response to liver injury but have also shown stem cell-like properties (Kordes et al., 2013). Oval cells are bipotential progenitors that can give rise to both hepatocytes and biliary epithelial cells, and they are activated in certain types of liver injury when hepatocyte proliferation is impaired (Roskams et al., 2004). Hepatic progenitor cells, finally, form a heterogeneous population with varying degrees of differentiation towards the hepatocyte or biliary lineages (Dollé et al., 2010).

Recent advances include the development of protocols for expanding liver stem cells in vitro while maintaining their bipotential differentiation capacity (Huch et al., 2015), the exploration of these cells for cell therapy in various liver diseases (Forbes et al., 2015), and the investigation of their role in liver cancer together with potential therapeutic targeting strategies (Marquardt et al., 2015).

3.3.6 Intestinal Stem Cells

Intestinal stem cells are responsible for the continuous renewal of the intestinal epithelium, one of the most rapid self-renewing tissues in the body, and two main populations have been identified (Barker, 2014). Crypt base columnar cells are fast-cycling stem cells marked by Lgr5 expression and located at the base of the intestinal crypts, while +4 cells are more quiescent stem cells located at the +4 position from the crypt base and marked by Bmi1 expression.

Recent advances include the development of protocols for long-term culture of intestinal organoids from single Lgr5+ stem cells (Sato et al., 2009), the exploration of intestinal stem cells for modelling intestinal diseases and drug screening (Clevers, 2016), and the investigation of the interplay between different intestinal stem cell populations in homeostasis and injury repair (Tian et al., 2011).

3.3.7 Skin Stem Cells

The skin contains several populations of stem cells that contribute to its continuous renewal and repair. Epidermal stem cells are in the basal layer of the epidermis and are responsible for its constant renewal (Blanpain & Fuchs, 2006). Hair follicle stem cells reside in the bulge region of hair follicles and contribute to hair regeneration and wound healing (Cotsarelis et al., 1990), while melanocyte stem cells, also located in the bulge, are responsible for generating pigment-producing melanocytes (Nishimura et al., 2002).

Recent advances include the development of 3D skin equivalents using epidermal stem cells for drug testing and disease modelling (Mathes et al., 2014), the exploration of hair follicle stem cells for hair regeneration therapies (Ohyama & Veraitch, 2013), and the investigation of the role of various skin stem cell populations in skin cancer initiation and progression (White & Lowry, 2015).

3.3.8 Limbal Stem Cells

Limbal stem cells are in the limbal region of the cornea and are responsible for maintaining the corneal epithelium. They are key determinants of corneal wound healing and transparency (Cotsarelis et al., 1989).

Limbal stem cells express markers such as ABCG2 and ΔNp63α, and they can be expanded in vitro and used for corneal regeneration.

Recent advances include the development of optimised culture methods for the ex vivo expansion of limbal stem cells (Pellegrini et al., 2013), the successful clinical application of cultured limbal stem cell transplantation for treating limbal stem cell deficiency (Rama et al., 2010), and the exploration of alternative cell sources, such as oral mucosal epithelial cells, for treating the same condition.

Understanding the unique properties and potentials of stem cells from different tissue sources is important for developing targeted therapies and regenerative medicine approaches. Each type of tissue-specific stem cell offers unique advantages and challenges, and their appropriate use depends on the specific research or clinical context. As our knowledge of tissue-specific stem cell biology continues to grow, we are likely to uncover new sources of stem cells and novel applications for existing ones, further expanding the potential of stem cell-based therapies.

4. Clinical Applications of Stem Cells

Clinical uses range from established haematopoietic transplantation to experimental tissue-repair strategies. Evidence from one cell type or indication cannot be transferred automatically to another.

4.1. Hematological Disorders

Haematopoietic stem cell transplantation (HSCT) is the most well-established stem cell therapy, used for over 50 years to treat various blood disorders (Copelan, 2006).

Applications include leukemia and lymphoma, multiple myeloma, severe aplastic anemia, sickle cell disease, and thalassemia.

For example, allogeneic HSCT has become a standard treatment for many types of leukemia, offering a potential cure for otherwise fatal diseases (Passweg et al., 2016). Recent advances in HSCT include:

Several developments have extended the reach of the procedure. Reduced-intensity conditioning regimens, being less toxic preparative regimens, have expanded the use of HSCT to older patients and to those with comorbidities. Advances in graft manipulation and post-transplant immunosuppression have made haploidentical transplantation from half-matched family donors a viable option, expanding donor availability (Luznik et al., 2012), while the use of umbilical cord blood as a source of haematopoietic stem cells has provided an alternative for patients without matched donors (Ballen et al., 2013). Combining HSCT with gene therapy approaches has also shown promise for treating genetic blood disorders such as sickle cell disease and beta-thalassemia (Cavazzana-Calvo et al., 2010).

4.2. Autoimmune Diseases

Stem cell therapies, particularly mesenchymal stem cells (MSCs), are being investigated for their immunomodulatory properties in treating autoimmune diseases (Wang & Jokerst, 2016).

Conditions under investigation include multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and Crohn's disease.

In the case of Multiple Sclerosis, several clinical trials have shown early findings using autologous haematopoietic stem cell transplantation (aHSCT) to "reset" the immune system. This approach has demonstrated significant improvements in relapsing-remitting MS patients, particularly those who have failed conventional therapies (Muraro et al., 2017).

Recent advances in stem cell therapies for autoimmune diseases include optimised culture conditions, since researchers have developed methods to enhance the immunomodulatory properties of MSCs through preconditioning with inflammatory cytokines (Krampera et al., 2013). Studies are also exploring the potential of combining stem cell therapies with other immunomodulatory treatments for enhanced efficacy (Swart et al., 2017). Beyond MSCs, tissue-specific stem cells are being investigated for organ-specific autoimmune diseases; neural stem cells, for example, are being explored for treating MS (Pluchino et al., 2003). MSC-derived extracellular vesicles, finally, have shown immunomodulatory effects like those of whole cells, offering a potential cell-free alternative (Lai et al., 2010). The cellular and molecular mechanisms through which stem cells act in multiple sclerosis, immunomodulation, myelin regeneration, and neuroprotection , are examined in detail in Section 4.4.

4.3. Cardiovascular Diseases

Stem cell therapies are being explored for heart repair and regeneration after myocardial infarction and in chronic heart failure (Nguyen et al., 2016).

Approaches include the injection of bone marrow-derived stem cells, the use of cardiac progenitor cells, and the application of iPSC-derived cardiomyocytes.

While results have been mixed, some studies have shown improvements in cardiac function and reduction in scar size. Recent advances include:

Several strategies are being pursued to improve on these results. Combining different types of stem cell, or adding growth factors, has shown enhanced efficacy in preclinical studies, and the use of hydrogels and other biomaterials to improve cell retention and survival after transplantation is under investigation (Seif-Naraghi et al., 2013). Rather than transplanting cells at all, some researchers are focusing on the beneficial effects of factors secreted by stem cells (Gnecchi et al., 2008), while in situ reprogramming of cardiac fibroblasts into cardiomyocytes is being explored as a further alternative (Song et al., 2012).

4.4. Neurological Disorders

Stem cells are being investigated to treat various neurological conditions, including Parkinson's disease, Alzheimer's disease, spinal cord injury, stroke, and amyotrophic lateral sclerosis.

For instance, clinical trials are underway using human embryonic stem cell-derived dopaminergic neurons for

treating Parkinson's Disease, with early results showing promise (Barker et al., 2017). Recent advances in stem cell therapies for neurological disorders include:

Research in this area has advanced on several fronts. Improved differentiation protocols now allow the generation of more authentic and functional neural cell types from pluripotent stem cells, including region-specific neurons and oligodendrocytes (Kirkeby et al., 2017), and in vivo reprogramming of glial cells into neurons is being explored as a potential strategy for brain repair (Guo et al., 2014). The use of biomaterial scaffolds to support transplanted cells and guide axon regeneration is showing promise in spinal cord injury models (Führmann et al., 2017), while stem cell-derived exosomes have shown neuroprotective and regenerative effects in various disease models, offering a potential cell-free alternative (Zhang et al., 2019). Brain organoids derived from patient-specific iPSCs, finally, are providing new insight into disease mechanisms and could be used for drug screening (Quadrato et al., 2016).

4.5. Orthopedic Applications

Mesenchymal stem cells are being used in orthopedics for cartilage repair in osteoarthritis, for bone regeneration, and for the treatment of tendon injuries.

Several clinical trials have demonstrated the safety and potential efficacy of MSC injections for knee osteoarthritis, although larger, controlled studies are needed to confirm long-term benefits (Jevotovsky et al., 2018). Recent developments include:

Several approaches are being developed to build on these findings. Researchers are exploring 3D bioprinting techniques to create custom scaffolds seeded with stem cells for cartilage and bone repair (Di Bella et al., 2018), and genetic modification of MSCs to enhance their therapeutic properties, such as improved cartilage formation or anti-inflammatory effects, is under investigation (Glass et al., 2014). MSC-derived extracellular vesicles have shown promise in promoting cartilage repair and reducing inflammation in osteoarthritis models (Cosenza et al., 2017), and the use of MSCs in combination with platelet-rich plasma or growth factors is being explored to enhance therapeutic efficacy (Andia & Maffulli, 2013).

4.6. Diabetes

Stem cell approaches for diabetes include the differentiation of pluripotent stem cells into insulin-producing β-cells and the use of MSCs to modulate the immune response in type 1 diabetes.

Clinical trials are ongoing to evaluate the safety and efficacy of stem cell-derived β-cells for treating type 1 diabetes. Recent advances include:

Several lines of work are advancing in this field. Improved differentiation protocols now allow the generation of more mature and functional β-cells from pluripotent stem cells (Pagliuca et al., 2014), and various encapsulation technologies are being developed to protect transplanted β-cells from immune rejection (Vegas et al., 2016). Direct reprogramming of pancreatic exocrine cells into β-cells in vivo is being explored as an alternative to cell transplantation (Zhou et al., 2008), and clinical trials are investigating the use of MSCs to preserve residual β-cell function and modulate autoimmunity in early-stage type 1 diabetes (Carlsson et al., 2015).

4.7. Liver Diseases

Stem cell therapies are being investigated for various liver diseases, including cirrhosis, acute liver failure, and genetic liver disorders.

The approaches under study include transplantation of hepatocytes derived from pluripotent stem cells, the use of MSCs for their anti-fibrotic and immunomodulatory effects, and the activation of endogenous liver progenitor cells.

Recent advances in this field include improved differentiation protocols for generating more mature and functional hepatocytes from pluripotent stem cells (Carpentier et al., 2014), and the exploration of stem cell-derived hepatocytes for use in bioartificial liver devices to support patients with acute liver failure (Nibourg et al., 2012). Combining stem cell therapy with gene editing technologies offers potential for treating genetic liver disorders (Omer et al., 2017), while liver organoids derived from patient-specific iPSCs are providing new platforms for disease modelling and drug screening (Takebe et al., 2013).

4.8. Ocular Diseases

Stem cell therapies warrant further investigation for treating various eye disorders, including age-related macular degeneration, corneal disorders, and glaucoma.

For example, clinical trials using human embryonic stem cell-derived retinal pigment epithelial (RPE) cells for treating AMD have shown encouraging early results (Schwartz et al., 2015). Other recent developments include:

Work in this area has progressed along several lines. Cultured limbal epithelial stem cell transplantation has become an established treatment for limbal stem cell

deficiency (Rama et al., 2010), and the development of 3D retinal organoids from pluripotent stem cells is providing new insight into retinal development and disease (Zhong et al., 2014). Cell sheet engineering techniques for transplanting intact sheets of RPE cells are being developed to improve the integration and function of transplanted cells (Kamao et al., 2014), and stem cell-derived factors are being explored for their neuroprotective effects in glaucoma and other optic neuropathies (Mead et al., 2015).

4.9. Wound Healing and Skin Disorders

Stem cells, particularly MSCs and adipose-derived stem cells, are being investigated for promoting wound healing and treating various skin disorders, with applications in chronic wound healing, burns, scar reduction, and skin rejuvenation.

Recent advances in this field include:

Recent advances in this field include 3D bioprinting techniques for creating complex skin constructs incorporating multiple cell types (Ng et al., 2018), and exosomes from various stem cell types, which have shown promise in promoting wound healing and reducing scarring (Wang et al., 2017). Genetic modification of stem cells to enhance their wound healing properties, such as improved angiogenesis or anti-inflammatory effects, is also being explored (Liu et al., 2022), as is the use of stem cells in combination with advanced wound dressings and biomaterials (Vig et al., 2017).

4.10. Graft-versus-Host Disease (GvHD)

Mesenchymal stem cells have shown significant promise in treating steroid-refractory acute GvHD, a severe complication of allogeneic haematopoietic stem cell transplantation. MSCs are now approved for this indication in several countries (Le Blanc et al., 2008). Recent developments include:

Several developments are shaping this application. Researchers are developing standardised, off-the-shelf MSC products for more consistent and readily available treatment (Galipeau & Sensébé, 2018), and studies are exploring the potential of combining MSCs with other immunomodulatory treatments for enhanced efficacy in GvHD (Ball et al., 2013). MSC-derived extracellular vesicles are being investigated as a cell-free alternative (Kordelas et al., 2014), and efforts are underway to identify biomarkers that can predict patient response to MSC therapy in this setting (Galleu et al., 2017).

These diverse clinical applications highlight the broad potential of stem cell therapies in modern medicine. While many difficulties remain, ongoing research and clinical trials continue to advance our understanding and improve the efficacy of stem cell-based treatments across a wide range of diseases and conditions.

5. Stem Cells and Technology: The Intersection with Artificial Intelligence

The integration of stem cell research with artificial intelligence (AI) and machine learning (ML) technologies is creating new opportunities for advancing our understanding of stem cell biology and improving stem cell-based therapies. This intersection is driving innovation in several key areas.

5.1. High-Throughput Screening and Analysis

AI and ML algorithms are being used to analyse the large datasets generated by high-throughput screening of stem cells. Machine learning models can predict the differentiation potential of stem cells from their molecular profiles, helping to optimise cell selection for specific applications (Waisman et al., 2019), and AI algorithms can analyse complex datasets to identify optimal culture conditions for maintaining stemness or directing differentiation (Kanda et al., 2022). In drug discovery, machine learning approaches predict the effects of small molecules on stem cell behaviour, accelerating the identification of compounds that can modulate stem cell function (Jing et al., 2018).

5.2. Image Analysis and Cell Characterization

Advanced image analysis techniques powered by AI are enhancing our ability to characterize stem cells and their derivatives. Deep learning algorithms accurately identify and classify different cell types in mixed populations, improving the efficiency of stem cell characterisation (Buggenthin et al., 2017), and AI-powered image analysis can detect subtle morphological changes that provide insight into cell state and function (Wakui et al., 2017).

5.3. Predictive Modeling of Stem Cell Behavior

AI and ML are also being used to develop predictive models of stem cell behaviour. Machine learning models can predict differentiation trajectories from single-cell transcriptomic data, providing insight into developmental processes (Jumde et al., 2024), and AI algorithms are being used to predict optimal combinations of factors for cellular reprogramming, potentially improving the efficiency of iPSC generation (Rackham et al., 2016). Machine learning

approaches are likewise being applied to understand and predict the effects of ageing on stem cell function (Kimmel et al., 2020).

5.4. Optimizing Stem Cell Manufacturing

AI and automation are being integrated into stem cell manufacturing processes to improve efficiency and consistency. Machine learning algorithms can analyse real-time data from bioreactors to optimise culture conditions and improve stem cell yield and quality (Arefin et al., 2023), and AI-powered image analysis can automate the detection of abnormal or contaminated cultures, enhancing quality control (Mamaeva et al., 2022).

5.5. Personalized Medicine Approaches

The combination of stem cell technology and AI is enabling more personalised approaches to medicine. Machine learning algorithms can analyse patient-derived iPSC models to predict individual drug responses and disease progression, aiding personalised treatment strategies (Coronnello & Francipane, 2022).

5.6. Ethical Considerations

The integration of AI with stem cell research also raises ethical considerations. The use of large-scale genetic and clinical data in AI applications raises important privacy concerns, which necessitates stringent data protection measures, and AI algorithms must be designed and evaluated so as to avoid perpetuating or exacerbating existing biases in healthcare (Zou & Schiebinger, 2018).

6. Challenges and Future Directions

The main translational problems concern cell identity, differentiation, manufacturing consistency, delivery, and long-term safety.

6.1. Improving Differentiation Efficiency and Fidelity

Challenge: Current protocols for differentiating pluripotent stem cells into specific cell types often yield heterogeneous populations and may not produce fully mature, functional cells.

Future work is directed at developing more precise control over the signalling pathways involved in differentiation (Loh et al., 2016), exploring the use of 3D culture systems and organoids to better mimic developmental processes (Lancaster & Knoblich, 2014), investigating the role of mechanical forces and substrate properties in directing differentiation (Engler et al., 2006), and using single-cell technologies to understand and manipulate cell fate decisions at higher resolution (Tritschler et al., 2019).

6.2. Overcoming Immune Rejection

Challenge: Allogeneic stem cell transplants face the risk of immune rejection, limiting their therapeutic potential.

Future directions include developing universal donor stem cell lines through genetic engineering (Deuse et al., 2019), exploring novel immunomodulatory approaches to induce tolerance to transplanted cells (Lei & Schaffer, 2013), advancing autologous iPSC-based therapies to avoid immune compatibility issues altogether (Mandai et al., 2017), and investigating the use of stem cell-derived extracellular vesicles as a cell-free alternative (Phinney & Pittenger, 2017).

6.3. Addressing Tumorigenicity Concerns

Challenge: The pluripotency of stem cells, particularly ESCs and iPSCs, carries a risk of teratoma formation if undifferentiated cells are transplanted.

Work in this area is focused on developing more efficient purification methods to eliminate undifferentiated cells (Wang, 2023), exploring genetic safeguards that selectively eliminate tumorigenic cells (Lee et al., 2013), investigating direct reprogramming approaches that bypass the pluripotent state (Xu et al., 2015), and advancing our understanding of the molecular mechanisms underlying tumourigenicity in stem cell populations (Ben-David & Benvenisty, 2011).

6.4. Improving Cell Survival and Integration

Challenge: Transplanted stem cells often show poor survival and integration in the host tissue, limiting their therapeutic efficacy.

Future directions include developing advanced biomaterial scaffolds to support transplanted cells (Moroni et al., 2018), exploring preconditioning strategies to enhance cell survival and function (Yu et al., 2013), investigating the use of gene modification to improve survival and integration (Cai et al., 2022), and advancing our understanding of the host tissue microenvironment in order to optimise engraftment (Ballios et al., 2015).

6.5. Scaling Up Manufacturing

Challenge: Large-scale production of clinical-grade stem cells remains a significant hurdle for widespread therapeutic application.

Future directions include developing automated, closed-system bioreactors for stem cell expansion (Panchalingam et al., 2015), exploring novel methods for xeno-free,

chemically defined culture conditions (Chen et al., 2011), advancing cryopreservation techniques for long-term storage of clinical-grade cells (Hunt, 2011), and implementing quality control systems based on advanced analytics and artificial intelligence (Garmany & Terzic, 2024).

6.6. Navigating Regulatory Challenges

Challenge: The complex and novel nature of stem cell therapies presents unique regulatory challenges.

Future directions include developing standardised protocols and quality control measures for stem cell production and characterisation (Andrews et al., 2015), establishing clear guidelines for preclinical testing and clinical trial design specific to stem cell-based therapies (Fink, 2009), implementing adaptive licensing approaches that facilitate earlier access to promising therapies while long-term safety data are gathered (Corbett et al., 2017), and fostering international collaboration to harmonize regulatory approaches across countries (Rosemann et al., 2016).

6.7. Addressing Ethical Concerns

Challenge: Stem cell research, particularly involving embryonic stem cells, continues to raise ethical questions.

Future directions include advancing alternative sources of pluripotent stem cells, such as induced pluripotent stem cells and direct reprogramming approaches (Takahashi & Yamanaka, 2016), developing clear ethical guidelines for emerging technologies such as human-animal chimeras and synthetic embryos (Hyun et al., 2020), promoting public engagement and education to foster informed discussion of the ethical implications (Kamenova & Caulfield, 2015), and exploring the ethical implications of gene editing in stem cells, particularly in the context of heritable genetic modifications (Bosley et al., 2015).

6.8. Enhancing In Vivo Tracking and Monitoring

Challenge: Long-term tracking of transplanted stem cells in vivo remains difficult, limiting our understanding of their fate and mechanism of action.

Future directions include developing advanced imaging techniques for non-invasive tracking of stem cells in vivo (Wang & Jokerst, 2016), exploring novel biomarkers for monitoring engraftment and function, investigating the use of reporter genes and genetic barcoding for long-term cell tracking (Nguyen et al., 2016), and advancing single-cell sequencing technologies for high-resolution analysis of stem cell fate in vivo (Tritschler et al., 2019).

6.9. Understanding Stem Cell Heterogeneity

Challenge: Stem cell populations, even those considered "homogeneous," often display significant functional heterogeneity.

Future directions include using single-cell technologies to characterize stem cell heterogeneity at unusual resolution (Trapnell, 2015), developing methods to isolate and expand specific subpopulations with desired properties (Notta et al., 2016), investigating the molecular mechanisms underlying heterogeneity and its implications for therapeutic applications (Li et al., 2023), and exploring the use of artificial intelligence and machine learning to analyse complex single-cell datasets and predict stem cell behaviour (Coronnello & Francipane, 2022).

6.10. Advancing Tissue Engineering and Organoid Technologies

Challenge: Creating complex, functional tissues and organs from stem cells remains a significant challenge.

Future directions include developing advanced bioprinting technologies for creating complex, multi-cellular tissues (Zhan et al., 2024), exploring methods to vascularize engineered tissues and organoids for improved survival and function (Takebe et al., 2017), investigating the use of developmental biology principles to guide the self-organization of stem cells into complex structures (Little & Combes, 2019), and advancing organoid technologies so that they better recapitulate the complexity and functionality of native organs (Lancaster & Huch, 2019).

6.11. Integrating Stem Cells with Gene Editing Technologies

Challenge: Combining stem cell therapy with precise genetic modifications offers new possibilities but also raises technical and ethical challenges.

Future directions include developing more efficient and precise gene editing techniques for use in stem cells (Hockemeyer & Jaenisch, 2016), exploring the potential of gene editing to create universal donor stem cells (Simpson et al., 2023), investigating the use of gene editing to enhance the therapeutic properties of stem cells (Farag, 2025), and addressing the ethical and regulatory challenges associated with genetically modified stem cells, particularly in the context of germline editing (National Academies of Sciences, Engineering, and Medicine, 2017).

6.12. Elucidating Stem Cell–Niche Interactions

Challenge: The complex interactions between stem cells and their niche are not fully understood, limiting our ability to manipulate stem cell behaviour in vivo.

Future directions include developing advanced in vitro models that better recapitulate the stem cell niche (Ranga et al., 2014), using intravital imaging techniques to study stem cell-niche interactions in real time in vivo (Ritsma et al., 2014), exploring the role of mechanical forces and extracellular matrix properties in regulating stem cell behaviour (Vining & Mooney, 2017), and investigating the potential of targeting the niche to enhance endogenous stem cell function in various diseases (Lane et al., 2014).

6.13. Advancing Stem Cell-Based Disease Modeling

Challenge: While stem cell-derived disease models have provided valuable insights, they often fail to fully recapitulate the complexity of human diseases.

Future directions include developing more sophisticated organoid models that incorporate multiple cell types and tissue structures (Yao et al., 2024), exploring the use of microfluidic organ-on-a-chip technologies to model complex physiological systems (Low et al., 2021), integrating patient-specific genetic and epigenetic information into stem cell-based disease models (Soldner & Jaenisch, 2018), and advancing high-throughput screening technologies for drug discovery using such models (Ryoo et al., 2024).

These challenges and future directions highlight the dynamic and multidisciplinary nature of stem cell research. Addressing these challenges will require continued collaboration between stem cell biologists, bioengineers, clinicians, ethicists, and regulatory bodies. The field may yet reshape parts of human biology and widen the range of treatable disease.

7. Conclusion

Stem-cell research now spans established haematopoietic transplantation, induced pluripotent stem-cell systems, organoid models, and experimental regenerative products. These approaches have expanded the study of cellular plasticity, development, and tissue repair, although their clinical maturity varies widely.

Self-renewal and differentiation define stem-cell biology, but neither property alone predicts clinical usefulness. The source, potency, and composition of the administered product determine which mechanisms and risks need to be tested.

The diverse types of stem cells, ranging from totipotent zygotes to unipotent tissue-specific stem cells, offer a wide array of possibilities for research and therapy. Embryonic stem cells continue to provide valuable insights into early development, while adult stem cells play important roles in tissue homeostasis and repair. The advent of induced pluripotent stem cells has substantially changed the field, providing a source of patient-specific pluripotent cells that bypass many of the ethical concerns associated with embryonic stem cells.

The clinical applications of stem cells have expanded dramatically in recent years. Haematopoietic stem cell transplantation, long established as a treatment for blood disorders, has provided a basis for exploring stem cell therapies in a wide range of conditions, from neurological disorders to heart disease. While many of these applications are still in experimental stages, early clinical trials have shown early findings in several areas.

The integration of stem cell research with advanced technologies, particularly artificial intelligence and machine learning, is opening new avenues for discovery and application. From predicting cell fate decisions to optimising manufacturing processes, these technologies are accelerating progress in the field and enabling more personalised approaches to stem cell therapy.

However, significant problems remain. These include improving the efficiency and fidelity of stem cell differentiation, overcoming immune rejection in allogeneic transplantation, addressing safety concerns related to tumourigenicity, and scaling up manufacturing processes for clinical applications. Ethical and regulatory challenges also persist, particularly as new technologies such as gene editing are integrated with stem cell research.

Current stem-cell research is focused on improving biological models, manufacturing, safety, and clinical translation. Advances in organoid technology are providing increasingly sophisticated models of human development and disease. The combination of stem cell therapy with gene editing offers new possibilities for treating genetic disorders. Improved understanding of the stem cell niche may lead to novel approaches for activating and directing endogenous stem cells for tissue repair.

As we continue to unravel the complexities of stem cell biology, we edge closer to realizing the full potential of these cells in regenerative medicine, disease modelling, and drug discovery. The path from bench to bedside is often long

and challenging, but the progress made in stem cell research over the past few decades gives reason for optimism.

Stem cell research provides experimental systems for studying development, disease mechanisms, and tissue repair, and it has also generated candidate therapies for selected disorders. Clinical translation depends on reproducible manufacturing, appropriate patient selection, long-term safety assessment, and ethical oversight; efficacy must be established separately for each cell product and indication.

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4.2. SOURCES OF STEM CELLS FOR THERAPY

A. TYPES OF STEM CELLS

1. Embryonic stem cells (ESCs)

Embryonic stem cells are derived from early-stage embryos, typically from blastocysts. They are pluripotent, capable of differentiating into any cell type, possess an indefinite capacity for self-renewal, and express specific markers such as Oct4, Sox2, and Nanog. Their applications lie in regenerative medicine for a range of diseases, in tissue engineering and organ development, and in disease modelling and drug screening. Their use raises ethical considerations, since the derivation of these cells from in vitro fertilized embryos raises moral concerns and has generated continuing debate about the moral status of embryos. The sources in current therapeutic use, and their practical differences, are compared in Figure 4.2.1.

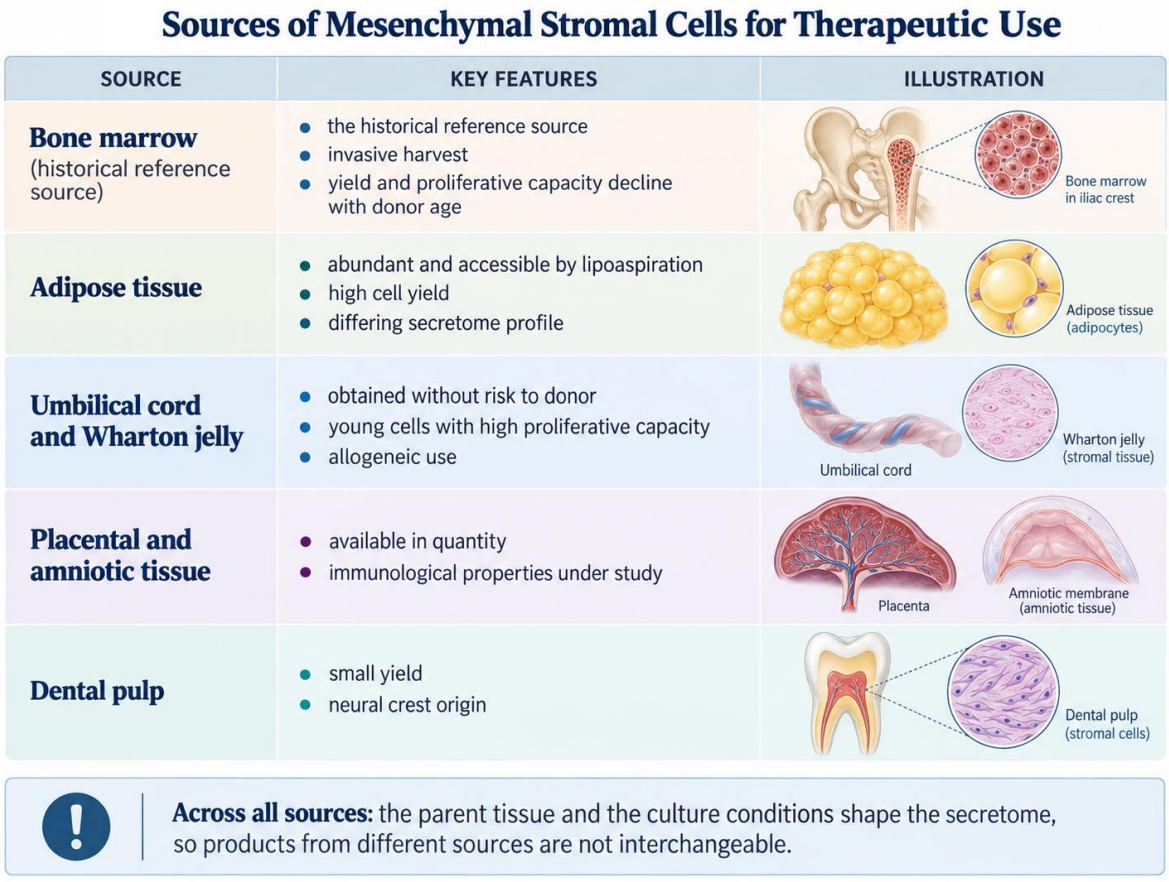


Figure 4.2.1. Sources of mesenchymal stromal cells for therapeutic use. Parent tissue and culture conditions shape the secretome, so products from different sources are not interchangeable.

Human embryonic stem cells (hESCs) represent the gold standard in stem cell research due to their pluripotency and unlimited self-renewal capacity (Thomson et al., 1998). These cells, derived from the inner cell mass of blastocysts, possess the unique ability to differentiate into any cell type in the body, making them invaluable for cell replacement therapies and regenerative medicine applications (Jiang et al., 2013).

The pluripotency of hESCs is maintained by a complex network of transcription factors, including OCT4, SOX2, and NANOG, which work in concert to preserve the cells' undifferentiated state while retaining their capacity to form all three germ layers (Boyer et al., 2005). This exceptional property has positioned hESCs as a powerful tool for studying early human development and modelling various diseases.

In 1998, Thomson et al. successfully derived human embryonic stem cell lines from blastocysts, demonstrating their pluripotency and potential to differentiate into various cell types. These cell lines exhibited normal karyotypes, high telomerase activity, and expressed markers characteristic of primate embryonic stem cells. This notable work laid the foundation for advancements in human developmental biology, drug discovery, and transplantation

medicine (Thomson et al., 1998). For instance, considerable progress has been made in deriving neural progenitor cells from hESCs, supporting the development of potential treatments for neurodegenerative diseases and spinal cord injuries (Tabar & Studer, 2014).

One of the most promising applications of hESCs is in the field of regenerative cardiology. Researchers have successfully differentiated hESCs into functional cardiomyocytes, which have shown potential in repairing damaged heart tissue in animal models of myocardial infarction (Shiba et al., 2012). These hESC-derived cardiomyocytes not only survive and integrate into the host myocardium but also improve cardiac function, offering hope for patients with heart failure.

Furthermore, hESCs have shown promise in treating retinal degenerative diseases. Clinical trials using hESC-derived retinal pigment epithelial (RPE) cells for the treatment of age-related macular degeneration (AMD) and Stargardt's macular dystrophy have demonstrated the safety and potential efficacy of this approach (Schwartz et al., 2015). These studies represent a significant milestone in the clinical application of hESC-based therapies.

However, the use of hESCs is not without controversy. Ethical concerns surrounding the destruction of human embryos in the process of deriving these cells have led to significant debate and regulatory challenges (Lo & Parham, 2009). These ethical considerations have prompted researchers to explore alternative sources of pluripotent stem cells, supporting the development of induced pluripotent stem cells and other adult stem cell sources.

Moreover, the clinical application of hESCs faces several technical challenges, including the risk of teratoma formation due to the presence of undifferentiated cells in transplanted populations (Hentze et al., 2009). To address this issue, researchers have developed various strategies, such as genetic modification of hESCs to express suicide genes or the use of cell sorting techniques to purify differentiated cell populations (Lee et al., 2013).

Another significant challenge in hESC research is the potential for immune rejection in allogeneic transplantation settings. While hESCs were initially thought to be immune-privileged, subsequent studies have shown that they can elicit an immune response upon differentiation and transplantation (Zhao et al., 2011). Researchers are exploring various approaches to overcome this challenge, including the creation of HLA-matched hESC lines and the development of immunomodulatory strategies to promote graft tolerance (Taylor et al., 2012).

Against this, hESCs remain a valuable tool in stem cell research and regenerative medicine. Their unparalleled differentiation potential and capacity for self-renewal continue to drive scientific discoveries and therapeutic innovations. As researchers develop new techniques to address the ethical and technical challenges associated with hESCs, their potential for clinical applications continues to expand.

2. Adult stem cells (ASCs)

2.1. Hematopoietic stem cells (HSCs)

Haematopoietic stem cells are obtained from bone marrow, peripheral blood, and umbilical cord blood. They are multipotent, developing into various blood cell types, have a self-renewing capacity, and are characterised by a CD34+CD38− phenotype. Clinically they are used in bone marrow transplants, in the treatment of blood disorders and cancers, and in immune system reconstitution.

Haematopoietic stem cells (HSCs) are multipotent stem cells capable of generating all blood and immune cell types (Weissman & Shizuru, 2008). These cells, typically characterised by markers such as CD34+, CD38-, and CD90+, are found in bone marrow, peripheral blood, and cord blood (Till & McCulloch, 1961).

HSC transplantation has also been effective in treating hereditary blood disorders and immune deficiencies. Lucarelli et al. (1990) demonstrated that HSC transplantation could cure beta-thalassemia in children, while Gennery et al. (2010) showed its efficacy in treating severe combined immunodeficiency (SCID).

Advanced applications of HSCs include gene therapy for genetic disorders. Cartier et al. (2009) demonstrated that genetically corrected HSCs could halt disease progression in patients with X-linked adrenoleukodystrophy.

2.2. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells can be obtained from bone marrow, adipose tissue, umbilical cord tissue, and dental pulp. They are multipotent, differentiating into bone, cartilage, fat, and muscle cells, possess immunomodulatory properties, and express CD73, CD90, and CD105 markers. They are applied in regenerative medicine, in orthopedic treatments, and in immunomodulation therapies.

Mesenchymal stromal cells are studied in regenerative medicine because they can be isolated from several tissues and

exhibit immunomodulatory and trophic properties (Zhidu et al., 2024). Sources include bone marrow, adipose tissue, umbilical-cord-derived tissues, and dental pulp, although cells from different sources are not biologically interchangeable.

The therapeutic potential of MSCs extends to a wide range of medical conditions, including neurological disorders, cardiovascular diseases, and autoimmune conditions (Chang et al., 2014). Their ability to differentiate into various cell types, including osteoblasts, chondrocytes, and adipocytes, makes them particularly valuable for tissue engineering and regenerative medicine applications (Pittenger et al., 1999).

One of the most promising sources of MSCs is bone marrow. Bone marrow-derived MSCs (BM-MSCs) have been extensively studied and are considered the gold standard for MSC-based therapies. These cells have shown efficacy in treating graft-versus-host disease, promoting bone regeneration, and supporting hematopoiesis. The ability of BM-MSCs to modulate the immune system has also made them attractive candidates for treating autoimmune disorders such as multiple sclerosis and rheumatoid arthritis (Uccelli et al., 2008).

Adipose tissue has developed into another valuable source of MSCs. Adipose-derived stem cell (ADSCs) can be easily obtained through minimally invasive procedures and are available in large quantities (Zuk et al., 2002). Chang et al. (2014) highlighted the therapeutic potential of human ADSCs in treating neurological disorders, demonstrating their ability to differentiate into neural-like cells and secrete neurotrophic factors that promote neuroprotection and regeneration. ADSCs have also shown promise in cardiac repair, wound healing, and treating metabolic disorders (Gimble et al., 2007).

The immunomodulatory properties of MSCs have made them attractive candidates for treating autoimmune and inflammatory conditions. These cells have been shown to suppress T-cell proliferation, modulate B-cell function, and inhibit dendritic cell maturation, contributing to their therapeutic effects in conditions such as graft-versus-host disease and multiple sclerosis (Le Blanc & Mougiakakos, 2012). The ability of MSCs to secrete anti-inflammatory cytokines and promote regulatory T-cell function has led to their exploration in treating inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis (Molendijk et al., 2015).

Recent research has focused on enhancing the therapeutic efficacy of MSCs through genetic modification or preconditioning strategies. For instance, overexpression of specific factors, such as CXCR4, has been shown to improve the homing and engraftment of MSCs to sites of injury (Lien et al., 2009). This approach has demonstrated enhanced therapeutic effects in animal models of myocardial infarction and ischemic stroke (Szydlak, 2023). Additionally, preconditioning MSCs with hypoxia or inflammatory cytokines has been demonstrated to enhance their survival and therapeutic potential in various disease models (Hu & Li, 2018).

2.3. Neural stem cells (NSCs)

Neural stem cells are found in the brain and spinal cord. They are multipotent, differentiating into neurons, astrocytes, and oligodendrocytes, have a self-renewing capacity, and express nestin and Sox2 markers. Their applications include the treatment of neurological disease, spinal cord injury repair, and brain tumour therapy.

Neural stem cells (NSCs) have come to be seen as a promising source for cell-based therapies targeting neurological disorders. These cells are capable of self-renewal and can differentiate into neurons, astrocytes, and oligodendrocytes, making them particularly valuable for treating conditions affecting the central nervous system (Gage, 2000).

NSCs can be isolated from various regions of the developing and adult brain, including the subventricular zone and hippocampus. They can also be derived from pluripotent stem cells, such as embryonic stem cells or induced pluripotent stem cells (Temple, 2001). NSCs are typically characterised by the expression of markers such as Nestin, Sox2, and Musashi-1 (Kaneko et al., 2000).

The therapeutic potential of NSCs has been demonstrated in various neurological conditions. In animal models of Parkinson's disease, transplantation of NSCs has shown improvements in motor function and dopamine production (Lindvall & Kokaia, 2010). Studies have also shown that NSC transplantation can promote functional recovery in animal models of stroke by replacing lost neurons and secreting neuroprotective factors (Abe, 2000).

NSCs have demonstrated the ability to promote axonal regeneration and improve functional outcomes in preclinical models of spinal cord injury (Teng et al., 2002). Additionally, NSC transplantation has shown potential in promoting remyelination and reducing inflammation in animal models of multiple sclerosis (Pluchino et al., 2003).

Recent advancements in NSC research have focused on enhancing their therapeutic efficacy. Researchers are exploring the use of gene editing technologies, such as CRISPR-Cas9, to enhance the therapeutic properties of NSCs or correct genetic defects in patient-derived cells (Heman-Ackah et al., 2016). The integration of NSCs with advanced biomaterials and scaffolds has shown promise in enhancing cell survival and integration in the host tissue (Meng et al., 2014).

2.4. Epithelial Stem Cells

Epithelial stem cells are found in the skin, the gastrointestinal tract, and the corneal limbus. They are multipotent, differentiating into various epithelial cells, have a high proliferative capacity, and express markers specific to their tissue of origin. They are applied in skin regeneration, in the treatment of gastrointestinal diseases, and in corneal surface reconstruction.

Epithelial stem cells are closely involved in maintaining the integrity and function of various epithelial tissues throughout the body. These cells are responsible for the continuous renewal of epithelial surfaces, which is essential for tissue homeostasis and repair (Blanpain et al., 2007).

In the skin, epidermal stem cells reside in the basal layer of the epidermis and in hair follicles. These cells are responsible for maintaining the epidermis and hair follicles throughout life. Skin epithelial stem cells have been extensively studied for their potential in wound healing and skin regeneration (Fuchs, 2008). Recent advancements in culturing techniques have allowed for the development of skin grafts from autologous epidermal stem cells, which have shown promise in treating severe burns and chronic wounds (Green, 2008).

The gastrointestinal tract contains a population of epithelial stem cells located at the base of intestinal crypts. These cells, known as crypt base columnar (CBC) cells, are responsible for the continuous renewal of the intestinal epithelium (Barker et al., 2007). Gastrointestinal epithelial stem cells have been studied for their potential in treating inflammatory bowel diseases and promoting intestinal regeneration after injury (Sato & Clevers, 2013).

Limbal stem cells (LSCs) are a specialized population of epithelial stem cells found in the limbal region of the cornea. These cells are essential for maintaining the transparency and function of the corneal surface. LSCs have been successfully used in the treatment of limbal stem cell deficiency, a condition that can lead to vision loss (Pellegrini et al., 1997). Cultivated limbal epithelial transplantation (CLET) has become a promising technique for restoring the corneal surface in patients with limbal stem cell deficiency (Rama et al., 2010).

2.5. Dental Pulp Stem Cells (DPSCs)

Dental pulp stem cells are obtained from the dental pulp of extracted teeth. They are multipotent, differentiating into odontoblasts, osteoblasts, chondrocytes, and neural-like cells, have a high proliferative capacity, and express markers such as CD73, CD90, and CD105. Their applications include dental regeneration, craniofacial reconstruction, and neuroregeneration.

Dental pulp stem cells (DPSCs) have attracted attention as a promising source of adult stem cells for regenerative medicine applications. First isolated by Gronthos et al. in 2000, these cells have gained significant attention due to their accessibility, multilineage differentiation potential, and neurotrophic properties.

DPSCs are typically isolated from the pulp tissue of extracted teeth, particularly wisdom teeth or other healthy teeth extracted for orthodontic reasons. These cells exhibit characteristics similar to mesenchymal stem cells (MSCs) and express markers such as CD73, CD90, and CD105, while lacking haematopoietic markers like CD45 and CD34 (Gronthos et al., 2000). DPSCs have demonstrated the ability to differentiate into various cell types, including odontoblasts, osteoblasts, chondrocytes, adipocytes, and neural-like cells (Ledesma-Martínez et al., 2016).

The therapeutic potential of DPSCs has been explored in various fields. In dental and craniofacial regeneration, studies have demonstrated their ability to regenerate dentin-pulp complex, periodontal ligament, and alveolar bone (Nakashima et al., 2009). This potential could be applied to treating dental caries, periodontal diseases, and craniofacial defects.

DPSCs have also been investigated in preclinical models of neurological disorders. Their ability to differentiate into neural-like cells and secrete neurotrophic factors makes them attractive candidates for treating conditions such as Parkinson's disease, Alzheimer's disease, and spinal cord injury (Martens et al., 2014). The neural crest origin of DPSCs may contribute to their enhanced neuroregenerative properties compared to other MSC sources.

Recent advancements in DPSC research have focused on enhancing their therapeutic efficacy. The integration of DPSCs with advanced biomaterials and scaffolds has opened new possibilities in tissue engineering. For example, DPSC-seeded scaffolds have shown promise in bone

and dental tissue engineering, providing structural support for cell growth and differentiation (Galler et al., 2018).

The immunomodulatory properties of DPSCs are being investigated for potential applications in treating autoimmune and inflammatory conditions. Their ability to suppress T-cell proliferation and modulate the immune response could be valuable in treating conditions such as graft-versus-host disease and autoimmune disorders (Pierdomenico et al., 2005).

2.6. Limbal Stem Cells (LSCs)

Limbal stem cells are located in the limbus region of the eye. They are essential for corneal regeneration and repair, express markers such as ABCG2, ΔNp63α, and ABCB5, and have a high proliferative capacity. They are used in corneal surface reconstruction, in the treatment of limbal stem cell deficiency, and in the management of ocular surface disorders.

Limbal stem cells (LSCs) are a specialized population of epithelial stem cells located in the limbal region of the cornea, at the border between the cornea and the conjunctiva. These cells are important for maintaining the transparency and integrity of the corneal surface by continuously replenishing the corneal epithelium (Cotsarelis et al., 1989).

LSCs are characterised by their expression of specific markers, including ABCG2, ΔNp63α, and ABCB5, which distinguish them from the more differentiated corneal epithelial cells (Pellegrini et al., 2001; Schlötzer-Schrehardt & Kruse, 2005). These cells possess a high proliferative capacity and are essential for corneal wound healing and regeneration.

The therapeutic potential of LSCs has been extensively studied, particularly in the treatment of limbal stem cell deficiency (LSCD), a condition characterised by the loss or dysfunction of LSCs. LSCD can result from various causes, including chemical burns, Stevens-Johnson syndrome, and congenital disorders, leading to corneal opacity, vascularization, and ultimately, vision loss (Dua et al., 2000).

Cultivated limbal epithelial transplantation (CLET) has been investigated as a promising technique for treating LSCD. This approach involves the isolation and ex vivo expansion of LSCs from a small biopsy of healthy limbal tissue, followed by transplantation onto the damaged corneal surface. Rama et al. (2010) demonstrated the long-term efficacy of this technique in a clinical study, showing successful corneal regeneration and vision improvement in patients with LSCD.

Recent advancements in LSC research have focused on improving cultivation techniques and developing alternative cell sources for patients with bilateral LSCD. Rama et al. (2001) reported the successful use of autologous cultured LSCs for treating burn-related corneal destruction, demonstrating the clinical application of this stem cell therapy.

Furthermore, researchers are exploring the use of bioengineered scaffolds and alternative cell sources, such as oral mucosal epithelial cells, to enhance the efficacy of LSC transplantation and expand its applicability to a wider range of patients (Nishida et al., 2004; Kolli et al., 2010).

2.7. Testicular (Spermatogonial) Stem Cells (SSCs)

Spermatogonial stem cells are found in the seminiferous tubules of the testes. They are unipotent, giving rise to sperm cells, although they have the potential to generate various cell types when reprogrammed, and they express markers such as PLZF, GFRα1, and ID4. Their applications include infertility treatment, the preservation of fertility in cancer patients, and use as a potential source of pluripotent stem cells.

Testicular stem cells, also known as spermatogonial stem cells (SSCs), are responsible for maintaining spermatogenesis throughout adult life. These cells reside in the seminiferous tubules of the testes and have the unique ability to self-renew and differentiate into sperm cells (de Rooij & Russell, 2000).

SSCs are characterised by the expression of specific markers, including PLZF, GFRα1, and ID4, which are essential for maintaining their stem cell properties (Oatley & Brinster, 2008). While SSCs are typically unipotent, giving rise only to the male germ cell lineage, recent studies have demonstrated their potential for generating various cell types when reprogrammed under specific conditions (Kanatsu-Shinohara et al., 2004).

The therapeutic potential of SSCs has been primarily explored in the context of male infertility treatments. Transplantation of SSCs has shown promise in restoring fertility in animal models of infertility and has potential applications in human reproductive medicine (Hermann et al., 2010). This approach could be particularly valuable for preserving fertility in young male cancer patients undergoing gonadotoxic treatments (Valli et al., 2014).

Recent research has also focused on the potential of SSCs as a source of pluripotent stem cells. Kanatsu-Shinohara et al. (2004) demonstrated that SSCs could be reprogrammed

to exhibit pluripotent characteristics similar to embryonic stem cells. This discovery opens up new possibilities for using SSCs in regenerative medicine applications beyond reproductive medicine.

Furthermore, advances in in vitro spermatogenesis techniques using SSCs have shown promise for treating certain forms of male infertility and for studying germ cell development (Sato et al., 2011). These techniques could allow for the generation of functional sperm cells from SSCs in vitro, offering new options for assisted reproductive technologies.

2.8. Ovarian Stem Cells

Ovarian stem cells are thought to reside in the ovarian surface epithelium or cortex, although both their existence and their properties remain controversial. They may have the potential to differentiate into oocytes and express markers such as DDX4 and OCT4. Their potential applications lie in fertility treatment, in regenerative medicine for ovarian disorders, and in the study of ovarian biology and ageing.

The existence and properties of ovarian stem cells have been a subject of intense debate and research in recent years. Traditionally, it was believed that females are born with a fixed number of oocytes that gradually decline throughout their reproductive lifespan. However, recent studies have challenged this dogma, suggesting the presence of stem cells in the adult ovary capable of generating new oocytes (Johnson et al., 2004).

Several research groups have reported the isolation of cells from the ovarian surface epithelium or cortex that exhibit stem cell-like properties and the potential to differentiate into oocytes. These cells have been shown to express markers associated with germline stem cells, such as DDX4 and OCT4 (White et al., 2012; Zou et al., 2009).

The therapeutic potential of ovarian stem cells, if their existence is conclusively proven, could have significant implications for fertility treatments and regenerative medicine approaches for ovarian disorders. These cells could be used to generate new oocytes for women with diminished ovarian reserve or premature ovarian failure (Virant-Klun et al., 2013).

However, the field remains controversial, with conflicting reports on the existence and properties of ovarian stem cells. Some researchers argue that the observed stem cell-like cells may be misidentified or that their contribution to oogenesis in vivo is minimal (Zhang et al., 2012).

2.9. Urine-Derived Stem Cells: A Novel and Non-Invasive Source

Urine-derived stem cells are obtained from human urine. They are multipotent, differentiating into various cell types, express markers such as CD29, CD44, and CD73, and show high proliferation rates. Their applications include urological regenerative medicine, cardiovascular tissue engineering, and neurological disease modelling.

Urine-derived stem cells have been investigated as a non-invasive cell source because samples can be obtained repeatedly without tissue biopsy. Their proposed therapeutic value depends on reproducible phenotyping, functional characterisation, and disease-specific evidence rather than ease of collection alone.

Lin et al. (2019) developed a protocol for isolating and culturing multipotent progenitor cells from human urine, demonstrating the feasibility of using this readily available biological fluid as a source of stem cells for regenerative medicine. The process typically involves collecting midstream urine samples, isolating cells through centrifugation, and culturing them under specific conditions to promote the growth of stem cell populations.

Urine-derived cells can express markers associated with progenitor states and can show differentiation capacity under selected culture conditions. Marker expression alone, including OCT4 or SSEA-4, does not establish functional pluripotency or the ability to generate all three germ layers. Identity and potency require validation through preparation-specific characterisation.

The therapeutic potential of USCs extends to various fields of regenerative medicine. Given their origin, they have shown particular promise in urological conditions: Bharadwaj et al. (2013) demonstrated the ability of USCs to differentiate into smooth muscle cells and urothelial cells, suggesting their potential use in reconstructive urology and in regenerative medicine applications for the urinary tract, which could be particularly valuable for treating stress urinary incontinence, vesicoureteral reflux, and bladder augmentation. In cardiovascular regeneration, studies have shown that USCs can be differentiated into endothelial cells for vascular tissue engineering and the treatment of cardiovascular diseases (Liu et al., 2018); these cells have demonstrated the ability to form functional blood vessels when implanted in vivo, offering potential applications in ischemic disease and in promoting angiogenesis in damaged tissues. USCs have also shown promise in neural regeneration, since studies have demonstrated their ability to

differentiate into neural progenitor cells and mature neurons, suggesting potential use in neurodegenerative diseases and spinal cord injuries (Guan et al., 2014). In orthopedic applications, research has shown that USCs can be differentiated into osteoblasts and chondrocytes (Kang et al., 2015), which could prove valuable for osteoarthritis, bone fractures, and cartilage defects. Perhaps the most striking development concerns induced pluripotent stem cells: Zhou et al. (2012) successfully reprogrammed USCs into iPSCs using a non-integrating episomal vector system, offering a non-invasive method for generating patient-specific iPSCs for disease modelling, drug screening, and personalised cell therapies.

The use of USCs offers several unique advantages. Collection is non-invasive, since unlike other sources of adult stem cells that require invasive procedures such as bone marrow aspiration, urine can be collected easily and painlessly. The supply is abundant, as urine is a renewable source that allows repeated collection from the same individual over time, and the cells can be used autologously, being harvested from a patient's own urine and thereby minimising the risk of immune rejection. Ethically, their use avoids the concerns associated with embryonic stem cells, and the simple collection and isolation procedures make them a potentially cost-effective source of cells for research and clinical applications.

Current research and future directions for USCs cover several areas. Researchers are exploring their use in 3D bioprinting applications to create complex tissue structures (Zhang et al., 2022), which could ultimately lead to bioengineered organs for transplantation, and they are being investigated as a tool for high-throughput drug screening, particularly for testing the nephrotoxicity of compounds (Bento et al., 2020), an application that could accelerate drug discovery and reduce reliance on animal models. Patient-derived USCs are also being used to create in vitro disease models, allowing the study of disease mechanisms and the testing of potential therapies (Yin et al., 2024), while work continues on advanced biomaterials and scaffolds that can enhance the survival, differentiation, and integration of transplanted cells in target tissues (Wu et al., 2011). A further direction concerns exosomes, the lipid bilayer vesicles released by various cell types, which are emerging as promising cell-free therapeutics in dermatologic disease; they play key roles in intercellular communication, influencing scar formation, burns, ageing, and wound healing, and their ability to carry bioactive cargos including lipids, nucleic acids, and proteins reflects their parent cells and makes them attractive for therapeutic applications; studies have confirmed their significant role in modulating these dermatologic conditions, suggesting their potential as innovative treatments (Shi et al., 2021).

Several challenges nonetheless need to be addressed. Developing standardised protocols for the isolation, culture, and characterisation of USCs is essential for ensuring consistency across research and clinical applications and improving methods for large-scale expansion while maintaining stem cell properties is essential for clinical translation. More extensive studies are needed to evaluate the long-term safety and efficacy of USC-based therapies, particularly with respect to genetic stability and potential tumourigenicity; enhancing the efficiency and completeness of differentiation into desired cell types remains an ongoing area of research; and advancing these therapies through rigorous clinical trials is necessary to establish their safety and efficacy in human patients.

The continued exploration of their potential, combined with advancements in tissue engineering and cell therapy technologies, could lead to significant breakthroughs in the treatment of various diseases and injuries.

2.10. Adipose-Derived Stem Cells (ADSCs)

Adipose-derived stem cells are obtained from adipose tissue. They are multipotent, differentiating into various cell types, express markers such as CD73, CD90, and CD105, and are easily accessible and abundant. They are applied in regenerative medicine, tissue engineering, cosmetic and reconstructive surgery, and the treatment of inflammatory and autoimmune diseases.

Adipose-derived stem cells (ADSCs) have come to be seen as a promising source of adult stem cells for regenerative medicine applications. These cells, first isolated and characterised by Zuk et al. in 2001, have gained significant attention due to their abundance, accessibility, and multilineage differentiation potential.

ADSCs are typically isolated from lipoaspirates, the by-product of liposuction procedures, making them readily accessible and abundantly available (Zuk et al., 2002). The isolation process involves enzymatic digestion of adipose tissue, followed by centrifugation to separate the stromal vascular fraction (SVF) containing the stem cells.

These cells possess many of the same properties as bone marrow-derived mesenchymal stem cells (BM-MSCs), including multi-lineage differentiation potential and immunomodulatory effects, but with the added advantage of being easier to obtain in large quantities. ADSCs express

typical mesenchymal stem cell markers such as CD90, CD73, and CD105, while lacking haematopoietic markers like CD45 and CD34 (Bourin et al., 2013).

The therapeutic potential of ADSCs has been demonstrated in various fields. In orthopedics they have shown promise in promoting bone regeneration and cartilage repair: Pak and colleagues (2017) described improvements in pain and function after ADSC therapy for knee osteoarthritis in an uncontrolled setting, in which a placebo contribution cannot be excluded, and their ability to differentiate into osteoblasts and chondrocytes makes them valuable for bone defects, osteoarthritis, and other musculoskeletal disorders (Spasovski et al., 2018). In cardiology, Perin et al. (2014) reported the PRECISE trial, a small randomised study of autologous adipose-derived regenerative cells in ischemic cardiomyopathy that primarily assessed safety and feasibility; proposed cardioprotective effects have been attributed to the ability of ADSCs to promote angiogenesis, reduce inflammation, and potentially differentiate into cardiomyocytes (Madonna et al., 2016). Their neuroprotective and neuroregenerative properties have been studied extensively, and Chang et al. (2014) highlighted their potential in stroke, Parkinson's disease, and spinal cord injury, since these cells secrete neurotrophic factors, promote neurogenesis, and modulate neuroinflammation (Huang et al., 2014). In wound healing and soft tissue reconstruction, their capacity to differentiate into various cell types and secrete growth factors promotes tissue repair and angiogenesis, and in plastic and reconstructive surgery they have been used to enhance fat grafting procedures, improving graft retention and aesthetic outcomes in breast reconstruction and facial rejuvenation (Kokai et al., 2014). Finally, their immunomodulatory properties have led to exploration in autoimmune and inflammatory conditions, with early findings in graft-versus-host disease, Crohn's disease, and systemic lupus erythematosus (Wang et al., 2013), effects attributed to their ability to suppress T-cell proliferation and modulate the immune response.

Recent advances in ADSC research have focused on enhancing therapeutic efficacy through several strategies. Genetic modification is being explored to improve their therapeutic properties: overexpression of specific factors such as brain-derived neurotrophic factor has been shown to enhance their neuroprotective effects in models of neurodegenerative disease. Preconditioning with hypoxia or inflammatory cytokines has been demonstrated to improve survival and therapeutic potential in various disease models (Hu & Li, 2018), an approach that aims to prime the cells for the harsh microenvironment they encounter upon transplantation. Integrating ADSCs with advanced biomaterials and scaffolds has opened new possibilities in tissue engineering, and ADSC-seeded scaffolds have shown promise in bone tissue engineering by providing structural support for cell growth and differentiation (Zanetti et al., 2013). There is also growing interest in ADSC-derived exosomes, extracellular vesicles containing bioactive molecules that can mediate the paracrine effects of the cells and offer a cell-free alternative that may overcome some of the challenges of cell transplantation. The use of ADSCs in 3D bioprinting is likewise being explored for creating complex tissue constructs, combining their regenerative potential with the precision of 3D printing (Gruene et al., 2011).

Despite this promise, several challenges need to be addressed. Developing standardised protocols for isolation, culture, and characterisation is central for consistency across research and clinical applications, and the heterogeneous nature of ADSC populations can lead to variability in therapeutic outcomes, which makes the isolation and characterisation of specific subpopulations with enhanced therapeutic properties an ongoing area of research. More extensive long-term studies are needed to evaluate safety, particularly with respect to potential tumorigenic risk; improving cell delivery techniques to enhance the survival and integration of transplanted cells is important for maximizing efficacy; and navigating the regulatory environment, particularly with regard to minimally manipulated versus culture-expanded cells, remains a challenge for clinical translation.

Their abundance, accessibility, and versatile therapeutic potential make them a valuable tool in the development of novel treatments for a wide range of diseases and injuries. Continued research and clinical trials will be essential in fully realizing the potential of ADSCs and translating these promising therapies into clinical practice.

2.11. Endothelial Progenitor Cells (EPCs)

Endothelial progenitor cells are obtained from bone marrow, peripheral blood, and umbilical cord blood. They can differentiate into endothelial cells, express markers such as CD34, CD133, and VEGFR-2, and contribute to neovascularization and vascular repair. Their applications include the treatment of cardiovascular disease, wound healing, tissue engineering, and cancer therapy.

Endothelial Progenitor Cells (EPCs) are a unique population of cells that drives vascular biology and regeneration. First identified by Asahara et al. (1997), EPCs are

precursor cells capable of differentiating into endothelial cells that line blood vessels. These cells, typically expressing markers such as CD34, CD133, and VEGFR-2, have shown promise in various therapeutic applications (Hristov et al., 2003).

Endothelial progenitor cells originate primarily from bone marrow and can also be detected in peripheral and umbilical-cord blood. They can migrate toward vascular injury and participate in vasculogenesis and angiogenic repair (Urbich & Dimmeler, 2004).

The therapeutic potential of EPCs has been demonstrated in various fields. In cardiovascular disease they have shown potential for improving cardiac function after myocardial infarction: a clinical study by Assmus et al. (2002) showed that EPC transplantation increased left ventricular ejection fraction in heart attack patients, and for peripheral artery disease Losordo et al. (2012) reported lower amputation rates after autologous CD34+ cell therapy in a small randomised pilot study in critical limb ischemia. In pulmonary hypertension, recent studies have shown that EPC therapy can alleviate the condition in animal models by improving endothelial function, reducing inflammation, and reversing vascular remodeling (Guo & Wang, 2024). Their role in wound healing has also been studied, and Asai et al. (2006) showed in mice that dibutyryl cAMP influences the recruitment of EPCs during wound neovascularization; their capacity to promote angiogenesis and tissue regeneration has prompted interest in their use for chronic wounds and burns. In tissue engineering, EPCs have been used to bioengineer artificial blood vessels, and Melero-Martin et al. (2008) demonstrated their ability to form functional vessels when combined with appropriate scaffolds, opening possibilities for vascularized tissue constructs. They have also shown potential in cancer therapy: while EPCs can contribute to tumour angiogenesis, Nolan et al. (2007) showed that bone marrow-derived EPCs are a major determinant of nascent tumour neovascularization, which has prompted interest both in targeting EPCs and in exploiting their tumour-homing properties for drug delivery.

Several obstacles persist in the clinical translation of EPC-based therapies. Standardizing methods for isolating and characterising these cells is important for consistency in research and clinical applications, an area complicated by the heterogeneity of EPC populations and the lack of consensus on definitive markers (Fadini et al., 2012). Improving the survival and engraftment of transplanted cells in target tissues remains a significant challenge, and strategies such as genetic modification and preconditioning are being explored to enhance their function and survival in vivo (Chavakis et al., 2008). The number and function of EPCs can be significantly reduced in aged individuals and in those with chronic diseases, which potentially limits the efficacy of autologous therapies in these populations (Heiss et al., 2005). Developing effective delivery methods is likewise key for maximizing therapeutic potential, and research continues into various routes and techniques to enhance homing and retention (Yoder, 2012). Ensuring long-term safety, particularly in terms of potential pro-angiogenic effects in occult tumours, is a further important consideration for clinical translation (Urbich & Dimmeler, 2004).

Future research directions in EPC biology and therapeutics include elucidating the mechanisms of mobilization, homing, and differentiation in order to develop strategies for enhancing regenerative potential; exploring the use of allogeneic EPCs or EPC-derived products such as exosomes to overcome the limitations of autologous cell therapies; investigating the potential of combining EPCs with other cell types or biomaterials to enhance therapeutic efficacy in tissue engineering and regenerative medicine; developing novel strategies for ex vivo expansion and modification to improve functionality; and advancing our understanding of their role in various pathological conditions in order to identify new therapeutic targets and applications.

Their unique properties and potential for promoting vascular repair and regeneration make them valuable tools in developing novel therapies for a wide range of vascular and ischemic diseases.

3. Induced Pluripotent stem cells (iPSCs)

Induced pluripotent stem cells are generated from adult somatic cells such as skin fibroblasts or blood cells. They are produced by reprogramming adult cells to an embryonic-like state, are pluripotent in a manner similar to ESCs, and express pluripotency markers such as Oct4, Sox2, and Nanog. They are used in regenerative medicine, disease modelling, drug testing and toxicology studies, and personalised medicine.

The discovery of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka and his team in 2006 substantially changed the field of stem cell research (Takahashi & Yamanaka, 2006). iPSCs are generated by reprogramming adult somatic cells to a pluripotent state through the introduction of specific transcription factors, typically OCT4, SOX2, KLF4, and c-MYC (collectively known as the

Yamanaka factors). This breakthrough has opened up new possibilities for patient-specific cell therapies and disease modelling, circumventing many of the ethical issues associated with embryonic stem cells (Son et al., 2016).

The development of iPSC technology has led to numerous advancements in regenerative medicine and disease modelling. For instance, researchers have generated iPSCs from patients with various genetic disorders, allowing for the study of disease mechanisms in vitro and the development of personalised drug screening platforms (Avior et al., 2016). This approach has been particularly valuable in modelling neurodegenerative diseases such as Alzheimer's and Parkinson's, where access to affected human brain tissue is limited (Yagi et al., 2011).

One of the most significant advantages of iPSCs is their potential for autologous transplantation, which could theoretically eliminate the need for immunosuppression in cell replacement therapies. This approach has shown promise in preclinical studies for various conditions, including macular degeneration, Parkinson's disease, and spinal cord injury (Okano et al., 2013).

Recent advancements in iPSC technology have focused on improving the efficiency and safety of the reprogramming process. For instance, Liao et al. (2013) demonstrated that inhibition of the PTEN tumour suppressor promotes the generation of iPSCs, potentially enhancing the production of patient-specific stem cells for transplantation therapy. Furthermore, the development of integration-free reprogramming methods, such as the use of synthetic mRNAs or episomal vectors, has addressed concerns about genomic instability and potential oncogenicity associated with earlier iPSC generation techniques (Okita et al., 2011; Warren et al., 2010).

The versatility of iPSCs extends to their potential use in treating autoimmune diseases. Son et al. (2016) successfully generated and characterised integration-free iPSCs from patients with various autoimmune conditions, highlighting the possibility of developing personalised cell therapies for these complex disorders. This approach not only offers a potential treatment avenue but also provides valuable tools for studying the underlying mechanisms of autoimmune diseases in vitro.

In addition to their therapeutic potential, iPSCs have become invaluable tools in drug discovery and toxicology screening. By generating iPSC-derived cell types relevant to specific diseases or organs, researchers can create more physiologically relevant models for testing drug efficacy and toxicity (Sayed et al., 2016). This approach has the potential to improve the efficiency of drug development processes and reduce reliance on animal models.

One of the most promising applications of iPSCs is in the field of regenerative medicine for age-related macular degeneration (AMD). In a notable clinical trial, researchers in Japan successfully transplanted retinal pigment epithelial (RPE) cells derived from autologous iPSCs into a patient with AMD, demonstrating the feasibility and safety of this approach (Mandai et al., 2017). This landmark study provided a basis for future clinical applications of iPSC-derived therapies in treating various degenerative diseases.

The potential of iPSCs in treating cardiovascular diseases has also been extensively explored. Researchers have successfully differentiated iPSCs into functional cardiomyocytes, endothelial cells, and smooth muscle cells, opening up possibilities for cardiac repair and vascular regeneration (Yoshida & Yamanaka, 2017). Preclinical studies have shown early findings in using iPSC-derived cardiac cells to improve heart function in animal models of myocardial infarction and heart failure (Kawamura et al., 2012).

Furthermore, iPSCs have shown promise in the field of hematology, particularly in the treatment of blood disorders and haematological malignancies. Researchers have developed protocols for generating haematopoietic stem cells (HSCs) from iPSCs, which could provide an unlimited source of HSCs for transplantation in patients with leukemia or other blood disorders (Sugimura et al., 2017).

The use of iPSCs in regenerative medicine extends to the field of diabetes research as well. Scientists have made significant progress in differentiating iPSCs into insulin-producing beta cells, offering hope for a cell-based therapy for type 1 diabetes (Millman et al., 2016). These iPSC-derived beta cells have shown the ability to respond to glucose stimulation and secrete insulin in vitro and in animal models, bringing us closer to a potential cure for diabetes.

Despite the enormous potential of iPSCs, several difficulties remain to be addressed before their widespread clinical application. These include optimising differentiation protocols to generate pure populations of desired cell types, ensuring the genomic stability of iPSC lines over long-term culture, and developing strategies to overcome potential immune rejection in allogeneic transplantation settings (Yoshihara et al., 2017).

One of the major concerns in iPSC-based therapies is the potential for tumourigenicity due to residual undifferentiated cells or genetic instability acquired during the

reprogramming process. To address this issue, researchers are developing sophisticated purification techniques and employing genome editing tools to enhance the safety of iPSC-derived cell products (Heslop et al., 2015).

Another challenge in the clinical translation of iPSC technology is the time and cost associated with generating patient-specific iPSC lines. To overcome this limitation, researchers are exploring the concept of HLA-matched iPSC banks, which could provide off-the-shelf cell products for a large portion of the population (Taylor et al., 2012). This approach could significantly reduce the time and cost of iPSC-based therapies while still minimising the risk of immune rejection.

New applications and technologies continue to emerge from iPSC research. For instance, the combination of iPSC technology with gene editing tools like CRISPR-Cas9 has opened new possibilities for correcting genetic defects in patient-specific cells before transplantation (Hockemeyer & Jaenisch, 2016). This approach promises to treat a wide range of genetic disorders, from monogenic diseases to complex polygenic conditions.

Moreover, the development of organoid technologies using iPSCs has provided new insights into human development and disease processes. These three-dimensional tissue structures recapitulate many aspects of organ development and function, offering powerful tools for studying disease mechanisms, drug screening, and potentially even organ replacement (Lancaster & Knoblich, 2014).

4. Perinatal Stem Cells

Perinatal stem cells are derived from perinatal tissues, including the placenta, umbilical cord blood, amniotic fluid, and Wharton's jelly. They are multipotent, differentiating into multiple cell types, exhibit properties intermediate between those of embryonic and adult stem cells, and show low immunogenicity. Their applications include regenerative medicine, tissue repair, immunomodulation, and the treatment of haematological disorders.

Perinatal stem cells, derived from tissues associated with childbirth, have become a valuable source for regenerative medicine. These cells combine the advantages of embryonic-like plasticity with the non-controversial nature of adult stem cells. Perinatal stem cells can be isolated from various sources, including the placenta, umbilical cord blood, amniotic fluid, and Wharton's jelly of the umbilical cord.

Amniotic fluid-derived stem cells (AFSCs) possess characteristics intermediate between embryonic and adult stem cells, exhibiting broad differentiation potential and high proliferative capacity (De Coppi et al., 2007). These cells can be easily obtained during routine amniocentesis or at birth, providing an ethically acceptable and readily available source of stem cells for autologous or allogeneic use.

AFSCs have shown promise in various therapeutic applications, including tissue engineering, cardiovascular repair, and treatment of neurological disorders (Antonucci et al., 2011). Their ability to differentiate into cells from all three germ layers (ectoderm, mesoderm, and endoderm) makes them versatile tools for regenerative medicine.

Recent studies have demonstrated the potential of AFSCs in treating various congenital anomalies, such as spina bifida and diaphragmatic hernia (Kunisaki, 2018). In animal models, prenatal transplantation of AFSCs has shown promise in ameliorating the effects of these conditions, offering hope for future fetal therapies. This approach, known as in utero stem cell therapy, has the potential to treat congenital disorders before birth, potentially preventing or reducing the severity of symptoms (Troeger et al., 2007).

The immunomodulatory properties of AFSCs have also been investigated, with studies showing their ability to suppress T-cell proliferation and modulate inflammatory responses (Di Trapani et al., 2015). This characteristic makes AFSCs potential candidates for treating autoimmune disorders and inflammatory conditions.

In the field of cardiovascular medicine, AFSCs have demonstrated the ability to differentiate into cardiomyocytes and endothelial cells, suggesting their potential use in cardiac repair and regeneration (Bollini et al., 2011). Also, in their 2018 review, Bollini et al. discuss the potential of fetal and perinatal stem cells, particularly those derived from the placenta, in cardiac repair and regeneration. They highlight the regenerative capacity, high self-renewal capability, and ease of collection of these cells from clinical waste material. The review covers various therapeutic strategies, including cell transplantation, paracrine therapy, reprogramming, and tissue engineering approaches, emphasizing the promise of these cells in addressing cardiovascular diseases (Bollini et al., 2018). Preclinical studies have shown that transplantation of AFSCs can improve cardiac function and reduce scar formation following myocardial infarction (Yeh et al., 2010).

Researchers have also explored the use of AFSCs in neurological applications. Studies have shown that these cells can differentiate into neural lineages and secrete neurotrophic

factors, promoting neuroprotection and regeneration in models of neurodegenerative diseases and spinal cord injury (Loukogeorgakis & De Coppi, 2016). The ability of AFSCs to cross the blood-brain barrier makes them particularly attractive for treating central nervous system disorders.

The placenta contains a heterogeneous population of stem cells, including haematopoietic, mesenchymal, and epithelial stem cells (Parolini et al., 2008). Placenta-derived stem cells have demonstrated potential in treating various conditions, including liver fibrosis, myocardial infarction, and inflammatory diseases (Oliveira & Barreto-Filho, 2015).

One of the significant advantages of placenta-derived stem cells is their abundance and ease of isolation. The placenta, typically discarded after birth, provides a large quantity of stem cells without the need for invasive procedures. This makes placental stem cells an attractive option for large-scale cell therapy applications.

Placental mesenchymal stem cells (P-MSCs) have garnered particular interest due to their potent immunomodulatory properties. Studies have shown that P-MSCs have enhanced immunosuppressive capabilities compared to MSCs derived from other sources, making them particularly attractive for treating inflammatory and autoimmune conditions (Bárcia et al., 2015). Clinical trials are currently underway to evaluate the safety and efficacy of P-MSCs in treating conditions such as graft-versus-host disease, Crohn's disease, and multiple sclerosis (Trounson & McDonald, 2015).

Placenta-derived mesenchymal stem cells (PMSCs) have been studied in preclinical cardiovascular models. Research indicates that PMSCs can promote angiogenesis, reduce inflammation, and improve cardiac function in animal models of myocardial infarction and heart failure. The implication is that PMSCs may serve as an investigational therapeutic approach for cardiac repair and regeneration (Wang et al., 2022). The ability of placental stem cells to secrete pro-angiogenic factors and differentiate into endothelial-like cells makes them candidates for vascular regeneration therapies.

Recent research has focused on harnessing the potential of placental stem cells for treating pregnancy-related complications. For example, studies have explored the use of placenta-derived MSCs in treating preeclampsia, a potentially life-threatening hypertensive disorder of pregnancy (H. Shi et al., 2024). These cells have shown promise in modulating the immune response and promoting vascular repair, offering a potential therapeutic avenue for this complex condition.

The multi-lineage differentiation potential of amniotic fluid and placental stem cells has also been exploited for tissue engineering applications. Researchers have successfully used these cells to generate various tissue types, including bone, cartilage, and even complex organs such as the kidney (Loukogeorgakis & De Coppi, 2016). This approach could address the shortage of donor organs for transplantation and developing personalised tissue-engineered constructs for regenerative medicine.

Amniotic-fluid and placenta-derived stem cells are being studied in regenerative medicine, but several translational problems remain unresolved. Developing consistent methods for isolating and characterising these cells is essential for ensuring reproducibility and comparability across studies and improving techniques for large-scale expansion while maintaining stem cell properties is essential for clinical application. More extensive preclinical and clinical studies are needed to evaluate long-term safety and efficacy; although these cells are generally considered to have low immunogenicity, further research is required to understand and mitigate potential immune responses in allogeneic transplantation settings; and improving cell delivery techniques to enhance engraftment and survival in target tissues is central for maximizing therapeutic efficacy.

Future directions in this field include combining stem cell therapy with gene editing, since integrating technologies such as CRISPR-Cas9 with amniotic fluid and placental stem cells could enhance their therapeutic potential and enable the correction of genetic defects. Exosomes and other extracellular vesicles derived from these cells are being investigated as cell-free alternatives for regenerative medicine, and more physiologically relevant in vitro models are being created for drug screening and disease modelling through advanced 3D culture systems. Researchers are also studying the unique epigenetic profiles of these cells in order to understand their enhanced pluripotency and differentiation potential and are further developing in utero and early postnatal therapies for treating congenital disorders and developmental abnormalities.

5. Parthenogenetic Stem Cells

Parthenogenetic stem cells are derived from unfertilized oocytes stimulated to divide. They are pluripotent in a manner like ESCs, express pluripotency markers such as Oct4, Sox2, and Nanog, and are genetically and

epigenetically distinct from fertilized embryos. They are used in regenerative medicine and research and offer potential for generating HLA-matched cell lines. Ethically, they avoid the use of fertilized embryos, although potential concerns related to egg donation remain.

Parthenogenetic stem cells (PSCs) represent a unique and ethically less controversial source of pluripotent stem cells. These cells are derived from unfertilized oocytes that are artificially activated to undergo cell division without fertilization by sperm (Revazova et al., 2007). This process, known as parthenogenesis, occurs naturally in some invertebrates and reptiles but is not a normal part of mammalian development.

The generation of human parthenogenetic stem cells was first reported by Revazova et al. in 2007. These cells exhibit many characteristics like embryonic stem cells (ESCs), including the expression of pluripotency markers such as Oct4, Sox2, and Nanog, and the ability to differentiate into cell types from all three germ layers (Kim et al., 2007).

One of the key advantages of PSCs is that they avoid the ethical concerns associated with the destruction of fertilized embryos, which has been a major point of contention in ESC research. This makes PSCs a potentially more acceptable source of pluripotent stem cells for both research and clinical applications.

The genetic and epigenetic characteristics of PSCs are distinct from those of fertilized embryos. PSCs are typically homozygous in the regions close to the centromeres due to the retention of sister chromatids during the activation process. This unique genetic makeup has both advantages and potential limitations in terms of their application (Daughtry & Mitalipov, 2014).

In terms of applications, PSCs have shown promise in various areas of regenerative medicine. Studies have demonstrated the ability of PSCs to differentiate into functional cell types, including cardiomyocytes, neurons, and hepatocytes (Harness et al., 2011). This potential could be harnessed for treating a wide range of diseases, from cardiovascular disorders to neurodegenerative conditions.

One particularly interesting application of PSCs is the potential for generating HLA-matched cell lines for transplantation. By carefully selecting oocyte donors based on their HLA type, it may be possible to create banks of PSC lines that could provide immunologically matched tissues for a large portion of the population (Kim et al., 2007). This approach could significantly reduce the risk of immune rejection in cell transplantation therapies.

PSCs have also proven valuable as research tools. Their unique genetic and epigenetic characteristics make them useful for studying genomic imprinting, X-chromosome inactivation, and other epigenetic phenomena (Stelzer et al., 2011). Additionally, PSCs can serve as a platform for drug screening and toxicology studies, potentially reducing the need for animal testing in pharmaceutical development.

Despite their potential, several challenges and considerations are associated with PSC research and application. The process of generating PSCs is still relatively inefficient, and further research is needed to optimise protocols for consistent and reliable derivation. Long-term culture and expansion may lead to genetic abnormalities, as observed with other pluripotent stem cell types, so careful monitoring and characterisation of PSC lines are necessary to ensure their safety for clinical use. While PSCs have demonstrated the ability to form various cell types, some studies suggest that their differentiation potential may be more limited than that of ESCs or iPSCs, particularly in forming certain extraembryonic tissues. Although they avoid the use of fertilized embryos, ethical considerations remain in relation to egg donation and the potential commodification of human oocytes for research purposes, and their unique nature may present regulatory challenges in terms of classification and approval, so that clear guidelines specific to PSCs will need to be developed to facilitate clinical translation.

As research in this field progresses, parthenogenetic stem cells may offer a valuable alternative or complement to other pluripotent stem cell sources, potentially expanding the range of options available for regenerative medicine and drug development while addressing some of the ethical concerns associated with embryonic stem cell research.

6. Somatic Cell Nuclear Transfer (SCNT)

Somatic cell nuclear transfer involves transferring the nucleus of a somatic cell into an enucleated egg cell. The technique creates pluripotent stem cells that are genetically identical to the somatic cell donor and exhibit properties comparable to those of embryonic stem cells. Its applications include therapeutic cloning, regenerative medicine, disease modelling, and the study of nuclear reprogramming. It raises several ethical considerations, including concerns related to cloning, the ethics of egg donation, and the potential for reproductive cloning.

Somatic Cell Nuclear Transfer (SCNT) is a technique that involves transferring the nucleus of a somatic cell into an

enucleated oocyte (an egg cell from which the nucleus has been removed). This process results in the creation of a cell that, when activated, can develop into an embryo genetically identical to the somatic cell donor. When used for the purpose of generating stem cells, this process is often referred to as "therapeutic cloning" (Wilmut et al., 1997).

The first successful SCNT in mammals was reported in 1997 with the birth of Dolly the sheep (Wilmut et al., 1997). However, it wasn't until 2013 that human embryonic stem cells were successfully derived using SCNT (Tachibana et al., 2013). This breakthrough opened up new possibilities for generating patient-specific pluripotent stem cells for research and potential therapeutic applications.

SCNT-derived stem cells share many characteristics with embryonic stem cells (ESCs), including pluripotency and the ability to differentiate into all cell types of the body. However, they have the unique advantage of being genetically identical to the somatic cell donor, potentially allowing for the creation of patient-specific stem cell lines for personalised medicine applications (Chung et al., 2014).

The applications of SCNT in stem cell research and regenerative medicine are diverse. Its primary goal in regenerative medicine is therapeutic cloning: creating patient-specific pluripotent stem cells that can be used to generate tissues or organs for transplantation without the risk of immune rejection (Chung et al., 2014). The technique can also generate stem cell lines from patients with specific genetic disorders, allowing researchers to study disease mechanisms and test potential treatments in a genetically relevant context (Tachibana et al., 2013), and SCNT-derived cells can serve as a platform for drug screening and toxicity testing, potentially improving the efficiency of pharmaceutical development (Matoba & Zhang, 2018). Beyond these therapeutic uses, SCNT provides a unique system for studying nuclear reprogramming itself, which could yield insight into cellular plasticity and improved methods for generating pluripotent stem cells (Jullien et al., 2011).

Despite its potential, SCNT faces several technical and ethical challenges. The process is technically demanding and has a low success rate, particularly in humans, so optimising protocols to improve efficiency remains an ongoing area of research (Chung et al., 2014). SCNT-derived cells contain mitochondrial DNA from the oocyte donor, which may have implications for cell function and potential clinical applications (Tachibana et al., 2013), and the reprogramming process may not fully reset all epigenetic marks, potentially leading to abnormalities in gene expression or development (Matoba & Zhang, 2018). Ethically, the technique raises issues related to cloning, the status of the created embryos, and the source of human oocytes for research, concerns that have led to regulatory restrictions in many countries (Ishii et al., 2013). The need for human oocytes raises further questions about egg donation, including informed consent, potential coercion, and the commodification of human biological materials (Lomax & Trounson, 2013), and while the intent of SCNT in stem cell research is therapeutic, the technique could theoretically be used for reproductive cloning, which is widely considered unethical and is banned in many countries (Ishii et al., 2013).

However, the emergence of induced pluripotent stem cells (iPSCs) as a more ethically acceptable and technically simpler alternative has shifted some focus away from SCNT in recent years. Nonetheless, SCNT remains an important tool in stem cell research, particularly for studying nuclear reprogramming and mitochondrial diseases.

The future of SCNT in stem cell research and regenerative medicine will likely depend on overcoming technical challenges, addressing ethical concerns, and demonstrating clear advantages over other pluripotent stem cell sources.

7. Cancer Stem Cells (CSCs)

Cancer stem cells are found in several types of tumours. They possess a self-renewal capacity, the ability to differentiate into heterogeneous cancer cell populations, expression of specific surface markers such as CD44 and CD133, and enhanced resistance to conventional therapies. Their implications are considerable, since they contribute to tumor initiation and progression, to metastasis, and to therapy resistance and cancer recurrence. Therapeutic approaches under investigation include targeting CSC-specific pathways, combination therapies, and immunotherapy directed against these cells.

Cancer stem cells (CSCs), also known as tumour-initiating cells, represent a subpopulation of cells within tumours that possess stem cell-like properties. The concept of CSCs was first demonstrated experimentally in the 1990s and has since substantially changed our understanding of cancer biology and treatment (Lapidot et al., 1994; Bonnet & Dick, 1997).

CSCs are characterised by their ability to self-renew and differentiate into various cell types within a tumour, contributing to tumour heterogeneity. They express specific surface markers, which can vary depending on the cancer type, but commonly include CD44, CD133, and ALDH1

(Al-Hajj et al., 2003; Singh et al., 2004; Ginestier et al., 2007).

The implications of CSCs in cancer biology are significant. They are thought to be responsible for tumour initiation and sustained growth, since they can give rise to all cell types found in a particular cancer sample and thereby drive tumour heterogeneity (Reya et al., 2001). They have also been implicated in metastasis through their ability to initiate tumour growth at distant sites (Hermann et al., 2007), and they often exhibit enhanced resistance to conventional therapies including chemotherapy and radiation, a resistance attributed to increased DNA repair capacity, the expression of drug efflux pumps, and quiescence (Dean et al., 2005). The survival of CSCs following initial treatment is believed to be a major factor in cancer recurrence and relapse (Clevers, 2011).

The discovery of CSCs has led to new therapeutic approaches in cancer treatment. Researchers are developing therapies that specifically target signalling pathways important for CSC maintenance and function, and inhibitors of the Notch, Wnt, and Hedgehog pathways are being investigated in clinical trials (Takebe et al., 2015). Strategies combining conventional therapies with CSC-targeted treatments are being explored in order to eliminate both bulk tumour cells and CSCs (Dragu et al., 2015), and immunotherapy directed against cancer stem cells is an active and promising area, encompassing monoclonal antibodies and CAR-T cells designed to recognize and eliminate CSCs on the basis of their unique surface markers; such targeted immunotherapies aim to eradicate the subpopulation of cancer cells responsible for tumour initiation, metastasis, and recurrence, thereby improving long-term treatment outcomes and reducing the risk of relapse (Yi et al., 2024). Inducing CSCs to differentiate into non-stem cancer cells could make them more susceptible to conventional therapies (Visvader & Lindeman, 2008), and therapies aimed at disrupting the tumour microenvironment that supports CSCs are likewise under investigation (Plaks et al., 2015).

Despite the promise of CSC-targeted therapies, several questions remain open. CSCs are heterogeneous, with different markers and properties across cancer types and even within the same tumour, a diversity that complicates the development of universal CSC-targeted therapies (Meacham & Morrison, 2013). Non-CSCs can acquire CSC properties under certain conditions, which suggests a dynamic equilibrium between the two populations and poses difficulties for therapies aimed solely at CSCs (Chaffer et al., 2011). Accurately identifying and isolating these cells also remains challenging, which hinders both research and the development of targeted therapies (Medema, 2013). Finally, many pathways important for CSC function are equally key for normal stem cell maintenance, so ensuring the specificity of CSC-targeted therapies in order to minimise toxicity to normal stem cells is a significant challenge (Pattabiraman & Weinberg, 2014).

Future directions in CSC research include the use of single-cell sequencing technologies to better understand CSC heterogeneity and evolution (Lawson et al., 2018), the development of more specific markers and improved isolation techniques (Abbaszadegan et al., 2017), investigation of the role of epigenetics in CSC function and plasticity (Wainwright & Scaffidi, 2017), exploration of the metabolic dependencies of these cells as potential therapeutic targets (Snyder et al., 2018), and the development of nanotechnology-based approaches for targeted drug delivery (Qi et al., 2016).

The ultimate goal is to develop therapies that can effectively eliminate CSCs while sparing normal stem cells, potentially leading to more durable responses and reduced rates of cancer recurrence.

B. STEM CELL THERAPY: CURRENT APPLICATIONS AND FUTURE PROSPECTS

Current applications of stem cell therapy include haematopoietic stem cell transplantation, skin grafts for burns, corneal regeneration, and cartilage repair, while emerging applications extend to neurodegenerative diseases, cardiovascular disorders, diabetes, and autoimmune diseases. Several problems remain, among them cell survival and integration, immune rejection, the risk of tumour formation, and questions of scalability and cost. Future prospects include personalised medicine, combination with gene therapy, 3D bioprinting of tissues and organs, and the development of off-the-shelf stem cell products.

Stem cell therapy has attracted attention as an investigational approach in regenerative medicine, offering potential treatments for a wide range of diseases and injuries. While some stem cell therapies are already in clinical use, many others are still in various stages of research and development.

Among current applications, haematopoietic stem cell transplantation is the most well-established, and is used to treat various blood cancers, genetic blood disorders, and immune system disorders; Copelan (2006) provides a review of its clinical applications. Epithelial stem cells have

been used successfully to create skin grafts for severe burn victims, and Green (2008) discusses the development and application of cultured epithelial autografts in burn treatment. Limbal stem cells are used to treat corneal damage and limbal stem cell deficiency, and Rama et al. (2010) demonstrated the long-term effectiveness of this therapy in restoring vision in patients with severe corneal damage. Mesenchymal stem cells have shown promise in repairing cartilage damage in conditions such as osteoarthritis, and Pers et al. (2016) conducted a clinical trial using adipose-derived MSCs for knee osteoarthritis, showing improvements in pain and function.

Among emerging applications, stem cell therapies are being investigated for neurodegenerative conditions such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis; Lindvall and Kokaia (2010) review their potential in neurological disorders. Various cell types are being explored for heart failure, myocardial infarction, and peripheral artery disease, and Sanganalmath and Bolli (2013) provide an overview of cell therapy approaches in cardiovascular medicine. Research is ongoing to develop stem cell-based treatments for both type 1 and type 2 diabetes, with Sneddon et al. (2018) discussing recent advances in generating functional beta cells from pluripotent stem cells. Mesenchymal stem cells are likewise being investigated for their immunomodulatory properties in conditions such as multiple sclerosis, rheumatoid arthritis, and Crohn's disease, and Wang et al. (2013) review their therapeutic potential in autoimmune disease. Which of the cell sources described above is best suited to multiple sclerosis, and by what mechanisms these cells act in that disease, are considered in Section 4.4.

Several challenges shape this field. Ensuring the survival and functional integration of transplanted cells remains a significant difficulty and improving delivery methods together with developing supportive biomaterials are active areas of research (Mao & Mooney, 2015). For allogeneic therapies, overcoming immune rejection is essential, and strategies such as creating universal donor stem cells through genetic engineering are being explored (Deuse et al., 2019). Especially for pluripotent stem cell-based therapies, the risk of teratoma formation is a major safety concern, which makes it essential to develop methods for eliminating undifferentiated cells and ensuring the purity of transplanted populations (Lee et al., 2013). Finally, developing cost-effective methods for the large-scale production of clinical-grade cells is central for widespread application (Jenkins & Farid, 2015).

Looking ahead, the combination of induced pluripotent stem cell technology with gene editing tools such as CRISPR-Cas9 opens possibilities for personalised cell therapies and disease modelling (Hockemeyer & Jaenisch, 2016), and integrating gene therapy with stem cell approaches could enhance therapeutic efficacy and enable the treatment of genetic disorders (Naldini, 2015). Advances in 3D bioprinting, combined with stem cell biology, may enable the creation of complex tissues and even whole organs for transplantation (Murphy & Atala, 2014), while the development of allogeneic therapies that can be used as off-the-shelf products could increase accessibility and reduce costs (Trounson & McDonald, 2015).

However, addressing the challenges of safety, efficacy, and scalability will be important for realizing the full potential of stem cell-based treatments. Continued research, rigorous clinical trials, and interdisciplinary collaboration will be essential in translating the promise of stem cell therapy into clinical reality.

C. ETHICAL AND REGULATORY CONSIDERATIONS IN STEM CELL RESEARCH

The ethical issues raised by this field include embryonic stem cell research, egg donation for research purposes, informed consent, and the commercialization of human tissues. Regulatory frameworks vary internationally and encompass clinical trial regulations as well as the problem of stem cell tourism. Emerging ethical challenges include gene editing in stem cells, chimera research, and the ethics of organoids. Public perception and education form a further dimension, requiring efforts to address misconceptions, engage stakeholders, and improve science communication.

Stem cell research and therapy, while holding investigational potential for treating various diseases, also raise significant ethical and regulatory challenges. These considerations have shaped the field's development and continue to influence its trajectory.

Among the ethical issues, the use of human embryos for deriving embryonic stem cells has been the subject of intense debate, with the main concern revolving around the moral status of the embryo and whether destroying embryos for research purposes is ethically justifiable; Lo and Parham (2009) provide a detailed review of these issues. The need for human oocytes in some forms of research, such as somatic cell nuclear transfer, raises concerns about the risks to and potential exploitation of egg donors, and Haimes et al. (2012) discuss the ethical implications of egg

provision for stem cell research. Ensuring proper informed consent from tissue donors is important, particularly in the context of creating immortalized cell lines or induced pluripotent stem cells, since concerns arise about privacy, genetic information, and the long-term use of donated tissues (Lomax & Trounson, 2013). Finally, the potential for commercial exploitation of human tissues and stem cell lines raises questions about ownership, benefit-sharing, and the commodification of the human body (Sleeboom-Faulkner et al., 2016).

Regulatory frameworks vary significantly between countries, which creates challenges for international collaboration and raises concerns about stem cell tourism (Caulfield et al., 2009). As these therapies move into clinical trials, ensuring appropriate oversight and patient protection becomes key, and the International Society for Stem Cell Research has published guidelines for stem cell research and clinical translation (Daley et al., 2016). The proliferation of unproven treatments offered in countries with lax regulations poses risks to patients and challenges the credibility of the field, and regulators and scientists are working to address this problem (Sipp et al., 2017).

Several ethical challenges are emerging. The advent of CRISPR-Cas9 and other gene editing technologies has raised new questions, particularly regarding germline editing, and the potential for creating genetically modified embryos has sparked intense debate (Doudna, 2020). The creation of human-animal chimeras for research purposes, such as growing human organs in animals, raises concerns about blurring species boundaries (Hyun, 2016), and as organoid technology advances, new questions arise about the moral status of these tissue structures, especially brain organoids (Munsie et al., 2017).

Public understanding of stem cell research is often limited, which leads to misconceptions and makes improved science communication essential for informed public debate (Rachul, 2011). Involving various stakeholders, including patients, ethicists, and policymakers, in discussions about the research and its implications is important for developing balanced policies (Illes et al., 2010), and researchers themselves have a responsibility to communicate their work accurately and engage with the public about the potential benefits and risks (Kamenova & Caulfield, 2015).

The ethical and regulatory landscape of stem cell research continues to evolve as science advances. Striking a balance between promoting scientific progress and ensuring ethical conduct remains a key challenge. International cooperation in developing ethical guidelines and regulatory frameworks is central for the responsible advancement of stem cell research and therapy.

Ethical questions will continue to evolve with gene editing, organoid research, and combinations of stem-cell methods with other technologies. Governance should develop alongside the science, with attention to consent, provenance of biological material, proportionality of risk, and transparent public oversight.

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4.3. ISOLATION AND CULTURE OF STEM CELLS

1. Introduction

Isolation and culture determine the identity and behaviour of a stem-cell preparation (Ahmed et al., 2020; Bhatia et al., 2014). Reproducible clinical manufacturing requires control of donor material, expansion conditions, differentiation, and release testing. Figure 4.3.1 traces this pathway from tissue collection to the administered product.

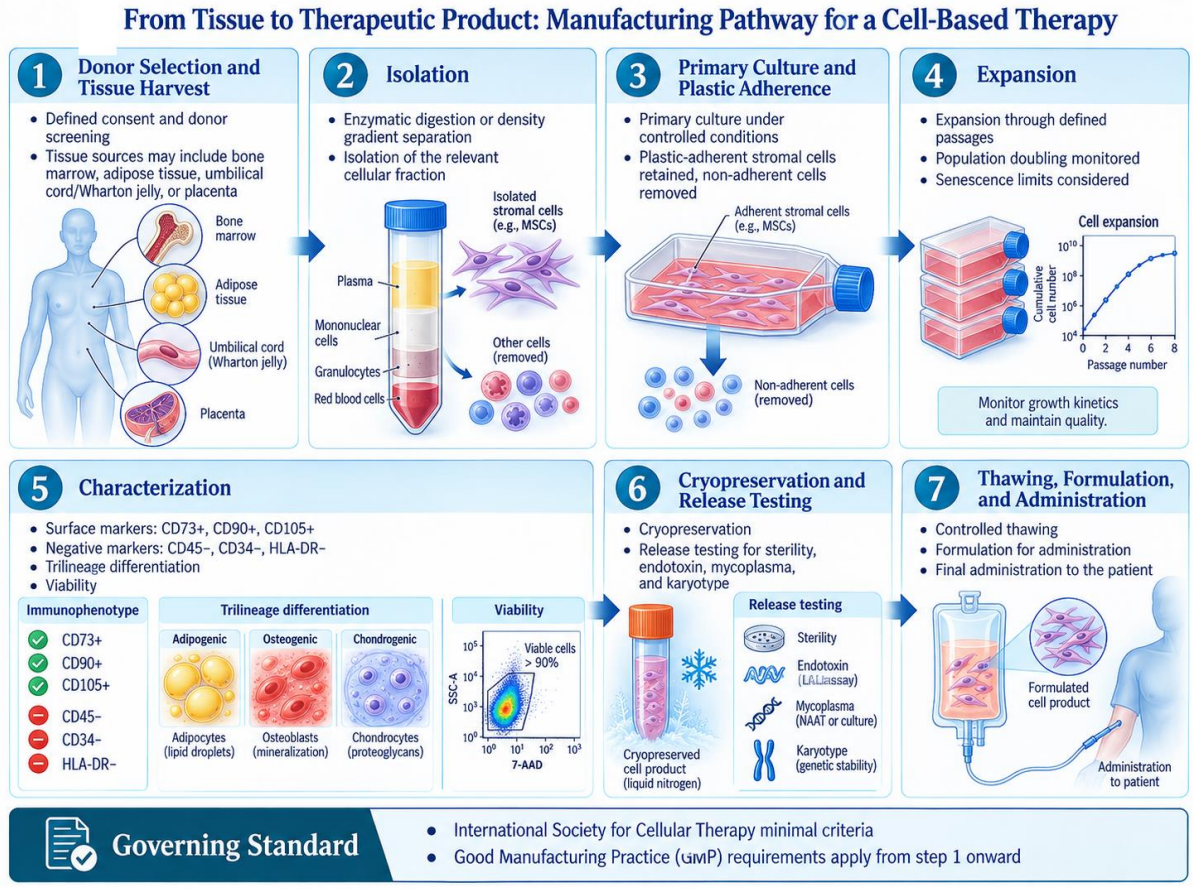


Figure 4.3.1. Manufacturing pathway for an MSC-based cell therapy, from donor selection to administration. The identity criteria shown are MSC-specific and follow the International Society for Cellular Therapy definition; GMP requirements apply from the first step onward.

The significance of stem cells lies in their capacity to self-renew and differentiate into various cell types. These properties make them invaluable tools for regenerative medicine, disease modelling, drug discovery, and developmental biology studies (Zakrzewski et al., 2019). However, the successful utilization of stem cells in research and clinical applications hinges on our ability to isolate them from their native tissues and maintain them in culture while preserving their unique characteristics.

Techniques for isolation and culture have developed in step with stem cell biology itself. From traditional methods of mechanical dissociation and basic culture media to current microfluidic devices and bioengineered 3D culture systems, the field has seen substantial advances. These developments have not only improved the efficiency and specificity of stem cell isolation but have also enhanced our ability to maintain stemness, direct differentiation, and scale up production for clinical applications (Azarin & Palecek, 2010).

Isolation methods vary with tissue source. Culture conditions then influence cell survival, expansion, and differentiation, so the two stages need to be evaluated together.

Furthermore, we will address the challenges that persist in the field, from maintaining genetic stability in long-term cultures to scaling up production for clinical use. We will also look ahead to the future of stem cell research, discussing emerging trends and technologies that promise to advance the field.

For clinical translation, the practical question is whether a method produces a defined, reproducible preparation with evidence of the intended biological activity.

2. Stem Cell Isolation: Techniques and Considerations

Stem cell research usually begins with the critical step of isolation. This process involves separating stem cells from their native tissue environment, a task that requires both precision and innovation. The choice of isolation method can significantly impact the purity, viability, and functionality of the resulting stem cell population, making it an important consideration for researchers (Ahmed et al., 2020).

2.1. Tissue Dissociation and Single-Cell Suspension

One of the primary steps in stem cell isolation is the dissociation of tissue to obtain a single-cell suspension. This process typically involves enzymatic or mechanical methods to break down the extracellular matrix and separate individual cells (Ahmed et al., 2020). The choice of dissociation method depends on various factors, including the tissue type, the specific stem cell population of interest, and the downstream applications.

2.1.1. Enzymatic Dissociation

Enzymatic dissociation is widely used due to its efficiency in breaking down extracellular matrix components. Common enzymes used include:

Several enzymes are used for this purpose. Collagenase is particularly effective for tissues rich in collagen, such as adipose tissue and cartilage (Estes et al., 2010). Trypsin, a proteolytic enzyme, is commonly used to dissociate epithelial tissues by cleaving the proteins responsible for cell adhesion, although its application can be harsh on certain cell types and may affect cell viability and function; prolonged exposure has been associated with decreased viability and with alterations in cell surface markers, so optimising digestion time and considering alternative enzymes such as Accutase are recommended in order to preserve cell integrity (Trish et al., 2006). Dispase is gentler than trypsin and is useful for isolating skin stem cells and other delicate tissues (Guo et al., 2013), while Accutase, a commercially available enzyme mix, is less damaging to cell surface proteins than trypsin (Bajpai et al., 2008).

The choice of enzyme, its concentration, and the duration of treatment must be optimised for each tissue type to maximize cell yield while minimising damage to the stem cells. For instance, Estes et al. (2010) published a detailed protocol for isolating adipose-derived stem cells by collagenase digestion and inducing their chondrogenic differentiation.

2.1.2. Mechanical Dissociation

Mechanical dissociation methods are often used in conjunction with or as an alternative to enzymatic dissociation. These methods include:

Common mechanical approaches include mincing, in which the tissue is cut into small pieces using scalpels or scissors (Vishnubalaji et al., 2012); trituration, in which the tissue is repeatedly passed through a pipette tip or needle (Reynolds & Weiss, 1992); and filtration, in which the dissociated tissue is passed through cell strainers of varying pore sizes (Greenwell-Wild et al., 2021). Automated systems such as the gentleMACS Dissociator combine mechanical and enzymatic dissociation in a single device (Greenwell-Wild et al., 2021).

Mechanical methods can be gentler on cells and avoid potential enzymatic damage to cell surface proteins. However, they may result in lower cell yields compared to enzymatic methods; Aronowitz et al. (2015) compared mechanical and enzymatic isolation of stromal vascular fraction cells from adipose tissue and found that the isolation method substantially affected cell yield.

2.2. Considerations for Specific Tissues

Different tissues require tailored approaches for optimal stem cell isolation. Neural tissue, because of its delicate nature, often requires a combination of gentle enzymatic treatment with an agent such as papain and careful mechanical dissociation, with single-cell suspensions typically obtained through trituration using fire-polished glass pipettes (Reynolds & Weiss, 1992). Isolating haematopoietic stem cells from bone marrow often involves flushing the marrow cavity with a syringe, followed by density gradient centrifugation to separate mononuclear cells (Bøyum & Borgstrøm, 1968). Lipoaspirates from adipose tissue are typically treated with collagenase and then centrifuged to separate the stromal vascular fraction containing adipose-derived stem cells (Estes et al., 2010). Embryonic stem cell isolation involves careful microdissection of the inner cell mass from blastocysts, followed by enzymatic or mechanical disaggregation (Thomson et al., 1998), while for dental pulp, mincing of the tissue followed by enzymatic digestion with collagenase and dispase has proved effective (Gronthos et al., 2000).

2.3. Cell Sorting and Enrichment

Following tissue dissociation, the next challenge is to isolate and enrich the desired stem cell population from the heterogeneous mixture of cells. Various techniques have been developed to achieve this, each with its own advantages and limitations:

2.3.1. Fluorescence-Activated Cell Sorting (FACS)

FACS is a powerful technique that allows stem cells to be isolated on the basis of specific surface markers or reporter gene expression. The process involves labeling cells with fluorescent antibodies directed against specific surface markers, passing the labeled cells through a laser beam in a single-cell stream, analysing the fluorescence and light scattering properties of each cell, and then sorting the cells according to predetermined criteria.

FACS offers high purity and the ability to isolate rare cell populations. For example, Nowak and Fuchs (2009) used FACS to isolate epithelial stem cells based on α6 integrin and CD34 expression, enabling close analysis of these cells using various culture assays.

Recent advances in FACS technology have further enhanced its capabilities. Modern instruments can analyse up to 18 parameters simultaneously, which allows more precise identification of stem cell populations (Perfetto et al., 2004), and index sorting allows researchers to track the sorting decision made for each cell, enabling retrospective analysis of cell properties (Osborne, 2011). Microfluidic-based FACS systems offer gentler cell handling and the ability to sort small sample volumes, which is particularly useful for rare stem cell populations (Shields et al., 2015).

However, FACS has some limitations, including potential cell damage due to high-pressure sorting, the need for large initial cell numbers, and the time-consuming nature of the process.

2.3.2. Magnetic-Activated Cell Sorting (MACS)

MACS is another widely used technique for stem cell isolation. Cells are labeled with magnetic beads conjugated to antibodies against specific surface markers, the labeled suspension is passed through a column placed in a strong magnetic field, the magnetically labeled cells are retained in the column while unlabeled cells pass through, and the magnetic field is then removed so that the labeled cells can be collected.

MACS is generally faster and gentler than FACS, making it suitable for processing large numbers of cells and for enriching rare stem cell populations, such as those present in amniotic fluid, for which Phermthai et al. (2010) described a derivation method intended for therapeutic purposes.

Recent developments in MACS technology include MultiSort technology, which allows sequential sorting using multiple markers and thereby enhances the specificity of isolation (Plouffe et al., 2015), and automated systems such as the AutoMACS Pro Separator, which offer high-throughput, standardised cell separation (Plouffe et al., 2015). Integrating MACS with microfluidic devices has also enabled continuous-flow magnetic separation, offering higher throughput and efficiency (Xia et al., 2006).

While MACS is gentler on cells compared to FACS, it may result in lower purity, especially for rare cell populations.

2.3.3. Density Gradient Centrifugation

This technique separates cells according to their density and is often used as a preliminary step before more specific isolation methods. A cell suspension is layered over a density gradient medium such as Ficoll or Percoll, the sample is centrifuged so that cells separate according to density, and the layer containing the cells of interest is then collected.

Density gradient centrifugation has been particularly useful in isolating haematopoietic stem cells from bone marrow and peripheral blood. Recent refinements include customized gradients developed specifically for isolating stem cell populations (Pertoft, 2000), and the combination of this technique with other methods, such as selective lysis of red blood cells, in order to enhance the purity of the isolated cells (Jaatinen & Laine, 2007).

While simple and cost-effective, density gradient centrifugation alone often does not provide the high purity required for many applications.

2.3.4. Adherence-Based Selection

Some stem cells, particularly mesenchymal stem cells, can be isolated on the basis of their ability to adhere to plastic culture surfaces. The heterogeneous cell suspension is plated on tissue culture plastic, cells are allowed to adhere for a specified period, usually 24 to 48 hours, non-adherent cells are washed away, and the adherent cells are then expanded through subsequent passages.

This method is simple and widely used for isolating MSCs from various tissues, including bone marrow, adipose tissue, and dental pulp (Pittenger et al., 1999). However, it

results in a heterogeneous population of cells and may not be suitable for all stem cell types.

Recent improvements in adherence-based selection include the development of specialized surfaces that enhance the selective adherence of stem cells (Curran et al., 2006) and the creation of specific micropatterns on culture surfaces to guide stem cell adhesion and organization (McBeath et al., 2004).

2.3.5. Microfluidic Devices

Emerging microfluidic technologies are offering new possibilities for stem cell isolation, since these devices can sort cells by size, deformability, or specific markers, potentially offering higher throughput and gentler handling (Shields et al., 2015). Inertial microfluidics uses the inertial forces generated in curved microchannels to separate cells by size and deformability (Di Carlo, 2009), acoustophoresis uses sound waves to separate cells according to their physical properties (Laurell et al., 2007), and dielectrophoresis exploits the dielectric properties of cells for separation (Pethig, 2010). Antibody-coated microchannels, meanwhile, allow the specific capture of stem cells expressing particular surface markers (Stott et al., 2010).

Microfluidic devices offer the potential for high-throughput, automated stem cell isolation with minimal cell manipulation. For instance, Nagrath et al. (2007) developed a microfluidic device capable of isolating rare circulating tumour cells from blood, a technology that could be adapted for isolating rare stem cell populations.

2.4. Tissue-Specific Considerations

Stem-cell isolation differs by tissue source, with tissue-specific constraints affecting yield, purity, phenotype, and downstream use:

2.4.1. Neural Stem Cells

Isolating neural stem cells (NSCs) requires careful consideration, given the complexity and delicacy of neural tissue. Different regions of the brain contain distinct NSC populations, and the subventricular zone of the lateral ventricles together with the dentate gyrus of the hippocampus are rich sources of adult NSCs (Doetsch et al., 1999; Seri et al., 2001). The abundance and properties of these cells vary with developmental stage, since embryonic and fetal tissues generally yield more abundant and more plastic NSCs than adult tissue (Temple, 2001). Neural tissue is particularly sensitive to harsh dissociation, so a combination of gentle enzymatic treatment with papain or Accutase and mechanical trituration is often used (Reynolds & Weiss, 1992). The neurosphere assay is a common method for isolating and expanding NSCs, in which dissociated cells are cultured in suspension to form floating spheres of stem cells and progenitors (Reynolds & Weiss, 1992), and markers such as CD133, SSEA-1, and CD15 have been used to enrich for NSCs by FACS or MACS (Uchida et al., 2000; Capela & Temple, 2002).

A recent advance in NSC isolation is single-cell RNA sequencing, which has revealed the heterogeneity of NSC populations and helped identify new markers for isolation (Llorens-Bobadilla et al., 2015).

2.4.2. Mesenchymal Stem Cells

Mesenchymal stem cells can be isolated from various tissues, including bone marrow, adipose tissue, and umbilical cord, and several factors shape the outcome. The choice of tissue source affects both yield and cell properties: adipose tissue typically yields more MSCs per gram of tissue than bone marrow (Zuk et al., 2002). While plastic adherence is commonly used for isolation, more specific methods have been developed. Density gradient centrifugation is often employed as an initial step to separate the mononuclear cell fraction containing MSCs (Pittenger et al., 1999), and FACS or MACS can yield purer populations through positive selection using markers such as CD105, CD73, and CD90, or through negative selection to remove haematopoietic cells (Dominici et al., 2006). Microfluidic devices offer a further option: in their 2018 study, Yin et al. used a spiral micro-channel device to separate culture-expanded human bone marrow-derived MSCs into five subgroups by cell size, and found that the medium-sized subpopulation of 17 to 21 μm exhibited a significantly higher proliferation rate and chondrogenic capacity than the others; by repeatedly isolating this subpopulation during culture expansion they maintained chondrogenic potential over extended periods, so that this size-based, label-free selection method offers an investigational approach to enhancing MSC efficacy in cartilage regeneration (Yin et al., 2018). Beyond the isolation method itself, the age, health status, and genetic background of the donor can significantly affect both the quantity and the quality of isolated MSCs (Siegel et al., 2013); the time between tissue harvesting and isolation affects viability and yield, so rapid processing is generally preferred (Sotiropoulou et al., 2006); and the initial plating density together with culture medium composition can influence the proliferation and characteristics of the isolated cells (Both et al., 2007).

Recent advances in MSC isolation include novel 3D culture systems that mimic the in vivo niche, which have been explored for more efficient isolation and expansion (Cesarz & Tamama, 2016), and exosome-based approaches, in which MSC-derived exosomes are used as markers for the indirect isolation of cells with specific functional properties.

Challenges in MSC isolation include maintaining homogeneity, as MSC populations can be heterogeneous even after purification. Additionally, there's ongoing debate about the true identity and potency of MSCs isolated from different tissues (Caplan, 2017).

2.4.3. Hematopoietic stem cells (HSCs)

Haematopoietic stem cells are typically isolated from bone marrow, peripheral blood, or umbilical cord blood, and each source has its own protocol. Bone marrow isolation usually involves aspiration followed by density gradient separation (Bøyum & Borgstrøm, 1968); peripheral blood often requires mobilization of HSCs from the marrow using agents such as G-CSF before collection by apheresis (To et al., 1997); and cord blood is collected immediately after birth and processed to isolate the mononuclear cell fraction (Ballen et al., 2013). HSCs are typically identified and isolated on the basis of CD34 expression together with the absence of lineage markers, and more refined populations can be obtained using additional markers such as CD38, CD90, and CD45RA (Notta et al., 2011). Functional confirmation relies on colony-forming unit assays and long-term culture-initiating cell assays (Sutherland et al., 1989), and many protocols involve cryopreservation of the isolated cells for future use, which requires careful optimisation to maintain viability and function (Woods et al., 2009).

Recent advances in HSC isolation include microfluidic devices developed for label-free isolation based on biophysical properties (Shields et al., 2015), antibody-free methods using synthetic substrates that mimic the bone marrow niche to capture HSCs, and single-cell transcriptomics, which has enabled better characterisation of HSC heterogeneity and the identification of new markers for isolation (Laurenti & Göttgens, 2018).

Challenges in HSC isolation include maintaining stemness during ex vivo manipulation and achieving sufficient yields for clinical applications, especially from cord blood samples.

2.4.4. Embryonic stem cells (ESCs)

Embryonic stem cells are derived from the inner cell mass of blastocyst-stage embryos, and several considerations apply. ESCs are typically derived from surplus embryos from in vitro fertilization clinics, which raises ethical considerations (Thomson et al., 1998). Three isolation techniques are used: immunosurgery, in which the trophectoderm is selectively lysed leaving the inner cell mass intact (Solter & Knowles, 1975); mechanical dissection, in which the inner cell mass is physically separated from the trophectoderm using micromanipulators (Amit & Itskovitz-Eldor, 2002); and laser-assisted isolation, in which laser pulses create a precise opening in the zona pellucida for extracting the inner cell mass (Tanaka et al., 2006). Traditionally, ESCs are cultured on mitotically inactivated mouse embryonic fibroblasts, although feeder-free systems have been developed (Xu et al., 2001), and the isolated cells must be thoroughly characterised for pluripotency markers such as OCT4 and NANOG as well as for differentiation potential (Adewumi et al., 2007).

Recent advances in ESC isolation include single-blastomere extraction, which allows ESCs to be derived without destroying the embryo (Klimanskaya et al., 2006), fully defined xeno-free culture systems established for deriving and maintaining clinical-grade ESCs (Chen et al., 2011), and non-integrating reprogramming methods for generating induced pluripotent stem cells without genomic integration, which provide an alternative to traditional ESCs (Okita et al., 2008).

Challenges in ESC isolation include ethical concerns, the need for highly skilled technicians, and variability in derivation efficiency.

2.4.5. Induced Pluripotent Stem Cells (iPSCs)

While not isolated in the traditional sense, the generation of iPSCs involves reprogramming somatic cells, and several factors govern the process. Various starting cell types can be reprogrammed, with fibroblasts, peripheral blood cells, and urine-derived cells being common choices (Takahashi et al., 2007; Staerk et al., 2010; Zhou et al., 2011). Reprogramming can be delivered by viral vectors, since retroviruses and lentiviruses are commonly used to deliver the factors but carry a risk of genomic integration (Takahashi & Yamanaka, 2006), or by non-integrating methods including episomal vectors, Sendai virus, mRNA transfection, and protein transduction (Okita et al., 2008; FUSAKI et al., 2009; Warren et al., 2010; Kim et al., 2009). The original OSKM factors, namely OCT4, SOX2, KLF4, and c-

MYC, remain the most common, although alternative combinations have been explored (Takahashi & Yamanaka, 2006; Yu et al., 2007). The choice of culture medium, substrate, and environmental conditions can significantly affect reprogramming efficiency (Chen et al., 2011), and iPSC colonies are typically selected on the basis of morphology and then thoroughly characterised for pluripotency markers and differentiation potential (Martí et al., 2013).

Recent advances in iPSC generation include small molecule reprogramming, in which cocktails of small molecules replace some or all of the genetic factors (Hou et al., 2013); microfluidic reprogramming, in which microfluidic devices enhance efficiency and enable high-throughput generation (Luni et al., 2016); and direct reprogramming, which converts one somatic cell type into another without passing through a pluripotent state (Vierbuchen et al., 2010).

Challenges in iPSC generation include variability in reprogramming efficiency, the potential for genetic and epigenetic abnormalities, and the time-consuming nature of the process.

2.4.6. Tissue-Specific Stem Cells

Various other tissue-specific stem cells require unique isolation approaches. Intestinal stem cells are typically isolated from intestinal crypts using EDTA-based chelation methods (Sato et al., 2009), with markers such as LGR5 used for FACS-based isolation (Barker et al., 2007); recent advances include organoid culture systems that maintain and expand these cells in vitro (Sato et al., 2011). Neural crest stem cells can be isolated from various tissues including gut, skin, and dental pulp (Achilleos & Trainor, 2012), using explant culture, FACS sorting based on markers such as p75 and HNK-1, or derivation from induced pluripotent stem cells (Lee et al., 2007). Cardiac stem cells are isolated from heart tissue using markers such as c-kit, Sca-1, or Isl1 (Beltrami et al., 2003; Oh et al., 2003; Laugwitz et al., 2005), although recent studies have questioned the existence of true adult cardiac stem cells, which underlines the importance of rigorous characterisation (van Berlo et al., 2014). Hepatic progenitor cells are typically isolated on the basis of markers such as EpCAM and CD133 (Schmelzer et al., 2007), and recent work has identified hybrid hepatocyte-biliary cells as potential liver stem cells (Font-Burgada et al., 2015). Skeletal muscle stem cells, or satellite cells, are isolated according to their anatomical location beneath the basal lamina of muscle fibres or using markers such as Pax7 (Mauro, 1961; Seale et al., 2000), with recent techniques employing genetic reporter mice and advanced imaging methods for in situ identification and isolation (Sambasivan et al., 2011).

Each of these tissue-specific stem cell types presents unique challenges in isolation, often requiring a combination of mechanical dissociation, enzymatic treatment, and cell sorting techniques. The choice of isolation method can significantly impact the yield, purity, and functional properties of the isolated stem cells.

3. Challenges and Future Directions in Stem Cell Isolation

Despite significant advances, several obstacles persist in the field of stem cell isolation. The process of isolation can alter the properties of the cells, so developing gentler methods that better preserve their in vivo characteristics is an ongoing challenge (Hines et al., 2014). Some populations are extremely rare, which makes their isolation technically demanding and makes improving the sensitivity and specificity of techniques for these populations important (Pang et al., 2011). Even purified populations can exhibit functional heterogeneity, so methods for isolating functionally homogeneous subpopulations remain a significant challenge (Göttgens, 2015). Many current techniques work well at laboratory scale but are difficult to scale up for clinical application, which makes GMP-compliant, scalable methods essential for translational research (Schallmoser et al., 2007). Finally, the lack of standardised protocols can lead to variability in results between laboratories, so establishing consensus protocols and quality control measures is key for advancing the field.

Future directions in stem cell isolation are likely to focus on addressing these challenges. Advanced microfluidic devices capable of isolating cells on the basis of multiple biophysical and biochemical properties simultaneously are likely to become more prevalent (Shields et al., 2015), and artificial intelligence and machine learning could be applied to image analysis and flow cytometry data in order to improve the accuracy and efficiency of identification and isolation (Doan et al., 2020). Developing methods to identify and isolate stem cells within their native niche, without removing them from the tissue, could provide valuable insight into stem cell biology (Yin et al., 2016), while advances in label-free techniques based on intrinsic cell properties could reduce the need for specific markers and minimise cell manipulation (González-González et al., 2011). The integration of single-cell genomics, transcriptomics,

and proteomics could enable more precise identification and isolation of subpopulations (Stuart & Satija, 2019), and isolation platforms built from materials that mimic the properties of the stem cell niche could improve the maintenance of stem cell properties during isolation (Choi et al., 2015).

Stem cell isolation techniques have come a long way, but there is still significant room for improvement. Isolation methods will continue to improve as the underlying biology becomes clearer, which in turn supports both research and clinical use.

4. Stem Cell Culture: Maintaining Stemness and Directing Differentiation

Once isolated, the cultivation of stem cells presents its own set of challenges and opportunities. The primary goals of stem cell culture are to maintain the cells' self-renewal capacity and multipotency (or pluripotency) while providing the flexibility to induce differentiation when desired. Achieving these goals requires careful control of the culture environment, including media composition, growth factors, and physical culture conditions.

4.1. Culture Media and Supplements

The composition of culture media is critical for maintaining stem cell properties and supporting their growth. Serum-free media supplemented with specific growth factors and cytokines are often preferred to maintain stemness and avoid unwanted differentiation (Chen et al., 2011). The exact formulation can vary depending on the type of stem cell being cultured.

4.1.1. Basic Media Components

The basic components of culture media include the basal medium, with common choices such as DMEM/F12, RPMI-1640, and αMEM providing essential nutrients and buffering capacity (Xu et al., 2001); serum alternatives, since defined supplements such as N2 and B27 are often used to avoid the variability associated with serum (Brewer et al., 1993); proteins and hormones, with insulin, transferrin, and albumin commonly added to support cell survival and proliferation (Chase et al., 2010); and trace elements such as selenium, copper, and zinc, which are essential for various cellular processes (Eagle et al., 1955).

4.1.2. Growth Factors and Cytokines

The choice of growth factors and cytokines can significantly influence stem cell behaviour. FGF2, also known as bFGF, is essential for maintaining pluripotency in human ESCs and iPSCs (Levenstein et al., 2006), while leukemia inhibitory factor is essential for maintaining mouse ESC pluripotency (Williams et al., 1988) and TGF-β/Activin A is important for maintaining human ESC and iPSC pluripotency (James et al., 2005). BMP4 can promote self-renewal in mouse ESCs when used in combination with LIF but induces differentiation in human ESCs (Ying et al., 2003; Xu et al., 2002). Epidermal growth factor is often used in neural stem cell cultures to promote proliferation (Reynolds & Weiss, 1992), and platelet-derived growth factor is important for maintaining neural stem cells and oligodendrocyte precursor cells (Jackson et al., 2006). Stem cell factor is central for haematopoietic stem cell maintenance and expansion (Wohrer et al., 2014), and Wnt proteins can promote self-renewal in various stem cell types, including intestinal stem cells (Sato et al., 2011).

The precise combination and concentration of growth factors can significantly impact stem cell behaviour. For example, Ng et al. (2005) showed that forced aggregation of defined numbers of human ESCs into embryoid bodies, in the presence of defined growth factors, fostered robust and reproducible haematopoietic differentiation, demonstrating how culture format and growth factors together direct stem cell fate.

4.1.3. Small Molecules

Small molecule compounds have gained popularity in stem cell culture because of their stability, cost-effectiveness, and reversible action. CHIR99021, a GSK3β inhibitor that promotes Wnt signalling, is used in various culture protocols (Ying et al., 2008), and PD0325901, a MEK inhibitor, is often combined with it to maintain mouse ESC pluripotency (Ying et al., 2008). The ROCK inhibitor Y-27632 enhances the survival of dissociated human ESCs and iPSCs (Watanabe et al., 2007), valproic acid, a histone deacetylase inhibitor, can enhance reprogramming efficiency and maintain pluripotency (Huangfu et al., 2008), and thiazovivin improves the survival and self-renewal of human pluripotent stem cells (Xu et al., 2010).

The use of small molecules in stem cell culture has led to the development of chemically defined culture systems, such as the "3i" (three inhibitor) and "2i" (two inhibitor) systems for mouse ESCs, which maintain a more homogeneous pluripotent state (Ying et al., 2008).

4.2. Physical Culture Conditions

The physical environment in which stem cells are cultured can profoundly affect their behaviour and fate.

4.2.1. Substrate and Extracellular Matrix

Several substrate options are available. Feeder cells, traditionally mouse embryonic fibroblasts, were used as feeder layers for ESCs, although feeder-free systems have since been developed to reduce variability and xenogenic components (Xu et al., 2001). Matrigel, a complex mixture of extracellular matrix proteins derived from Engelbreth-Holm-Swarm mouse sarcoma, is widely used for human pluripotent stem cell culture (Xu et al., 2001). Defined substrates using recombinant proteins such as laminin and vitronectin, or engineered peptides, have also been developed (Rodin et al., 2010; Chen et al., 2011), as have fully synthetic substrates such as polymer coatings, which offer a more defined and reproducible culture surface (Villa-Diaz et al., 2010).

The choice of substrate can influence stem cell adhesion, proliferation, and differentiation. For instance, Brafman et al. (2013) showed that integrin–extracellular matrix interactions regulate the endodermal differentiation of human embryonic stem cells.

4.2.2. Oxygen Tension

The oxygen concentration in standard cell culture incubators, at 21%, is much higher than the physiological levels most stem cells experience in vivo, and lowering it has several effects. Hypoxic conditions of 1% to 5% oxygen can promote the maintenance of pluripotency in ESCs and iPSCs (Ezashi et al., 2005; Yoshida et al., 2009), and low oxygen tension can enhance the efficiency of iPSC generation (Yoshida et al., 2009). The optimal concentration varies with cell type and native niche: haematopoietic stem cells, for example, are typically maintained at 1% to 5% oxygen in order to mimic bone marrow conditions (Eliasson & Jönsson, 2010).

4.2.3. Mechanical Forces

Mechanical stimuli can significantly influence stem cell behaviour. The elasticity of the culture substrate can direct lineage specification, with soft substrates promoting neuronal differentiation and stiff substrates promoting osteogenic differentiation of MSCs (Engler et al., 2006). Fluid shear stress can affect differentiation and function, and promotes endothelial differentiation of ESCs (Yamamoto et al., 2005), while cyclic tensile strain can influence cell alignment and differentiation, which is particularly relevant for musculoskeletal tissue engineering (Mouw et al., 2007). Three-dimensional culture environments provide more physiologically relevant mechanical cues than 2D cultures (Baker & Chen, 2012).

4.3. Advanced Culture Systems

Culture systems designed to mimic the in vivo niche have grown more sophisticated alongside that biology.

4.3.1. 3D Culture Systems

Three-dimensional culture systems offer several advantages over traditional 2D cultures. Stem cells can form 3D aggregates or spheroids, which can better maintain stemness or promote specific differentiation pathways; neural stem cells cultured as neurospheres, for instance, can maintain their multipotency (Reynolds & Weiss, 1992). Natural or synthetic hydrogels provide a 3D environment that can be tailored to mimic specific tissue properties, and hyaluronic acid-based hydrogels have been used to culture neural stem cells (Seidlits et al., 2010). Porous scaffolds made from materials such as polylactic acid or polycaprolactone can provide structural support for 3D cultures (Zonari et al., 2012), and stem cells can self-organize into organoids, complex 3D structures that mimic organ development and function, as has been demonstrated for intestine, brain, and kidney (Lancaster & Knoblich, 2014; Takasato et al., 2015).

4.3.2. Microfluidic Systems

Microfluidic devices offer precise control over the stem cell microenvironment. They can generate stable gradients of growth factors or small molecules in order to study their effects on cell behaviour (Chung et al., 2005), and microfluidic platforms enable parallel testing of multiple culture conditions or drug candidates (Occhetta et al., 2015). Organ-on-a-chip devices combine microfluidics with 3D cell culture to create miniature organ models, which are useful for studying stem cell behaviour in a more physiological context (Huh et al., 2010).

4.3.3. Bioreactors

Bioreactors provide a controlled environment for large-scale stem cell culture. Stirred-tank bioreactors are useful for scaling up the production of stem cells or their derivatives and have been used for the large-scale production of cardiomyocytes from pluripotent stem cells (Kropp et al., 2017). Rotating wall vessel bioreactors provide a low-shear environment that can support 3D cell growth and differentiation (Gerecht-Nir et al., 2004), and perfusion bioreactors allow continuous nutrient supply and waste removal,

which is beneficial for long-term 3D cultures (Wendt et al., 2009).

5. Challenges in Stem Cell Culture

Despite significant advances, several challenges persist in stem cell culture. Even under well-defined conditions, populations can exhibit heterogeneity in their molecular profiles and functional properties (Kumar et al., 2014), and maintaining pluripotency over extended periods can be difficult, since some cells in culture differentiate spontaneously (Allegrucci & Young, 2007). Long-term culture can lead to the accumulation of genetic and epigenetic changes, potentially affecting both stem cell properties and safety for clinical use (Lund et al., 2012). Translating small-scale protocols to larger, clinically relevant scales while maintaining cell quality is a further significant challenge (Kropp et al., 2017), and variations in reagents, handling techniques, and environmental factors can produce inconsistent results between laboratories (Hines et al., 2014). Developing fully defined animal component-free culture systems is important for clinical application but technically demanding (Chen et al., 2011), and many stem cell-derived tissues, such as neurons and cardiomyocytes, exhibit immature phenotypes that limit their utility for disease modelling and cell therapy (Jiang et al., 2018).

6. Future Directions in Stem Cell Culture

Several emerging trends are shaping the future of stem cell culture. Artificial intelligence and machine learning are being applied to optimise culture conditions, predict stem cell behaviour, and automate the monitoring of cultures (Waisman et al., 2019), while precise genetic modification through CRISPR-Cas9 is becoming increasingly feasible and opens new avenues for disease modelling and cell therapy (Hotta & Yamanaka, 2015). Advances in single-cell technologies are providing marked insight into heterogeneity and differentiation trajectories (Tritschler et al., 2017), and 3D bioprinting is enabling the creation of complex, multi-cellular tissue constructs incorporating stem cells (Murphy & Atala, 2014). Biomimetic materials are being developed as culture substrates and scaffolds that more closely mimic the natural niche (Tian et al., 2015), techniques for manipulating cell fate in vivo are being explored as alternatives to traditional transplantation approaches (Heinrich et al., 2015), and methods to introduce functional vasculature into organoid cultures are being developed to enhance their maturation and complexity (Wimmer et al., 2019).

Overall, stem cell culture techniques have advanced significantly in recent years, driven by our growing understanding of stem cell biology and the development of new technologies. However, difficulties remain in maintaining stem cell potency, controlling differentiation, and scaling up production for clinical applications. As the field continues to evolve, we can expect to see more sophisticated, physiologically relevant culture systems that better recapitulate the in vivo stem cell niche and enable new applications in regenerative medicine, disease modelling, and drug discovery.

7. Applications of Stem Cells

Stem cells are used across several areas of biomedical research and clinical development. Their applications differ in biological rationale, technical maturity, and the strength of evidence supporting clinical use:

7.1. Disease Modeling

Stem cells, and particularly induced pluripotent stem cells, have substantially changed our ability to model human disease in vitro. Patient-specific models can be created by deriving iPSCs from individuals with genetic disorders and differentiating them into relevant cell types, which allows researchers to study disease mechanisms in a patient-specific context (Takahashi et al., 2007). They also enable the study of developmental disorders from their earliest stages, providing insight that would be impossible to obtain from patient samples alone (Lancaster et al., 2013). Disease models based on stem cells can be used for high-throughput screening of potential therapeutic compounds (Ebert et al., 2009), and patient-derived models can be used to predict individual responses to treatment, supporting the development of personalised medicine (Matsa et al., 2016).

Successful examples span several disease areas. In neurological disorders, iPSC-derived neurons have been used to model Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, revealing new insight into disease mechanisms (Israel et al., 2012; Soldner et al., 2009; Dimos et al., 2008). In cardiac disease, iPSC-derived cardiomyocytes have been used to model various genetic cardiomyopathies and arrhythmias, enabling the study of disease progression and drug responses (Moretti et al., 2010; Sun et al., 2012). Hepatocytes derived from patient iPSCs have been used to model inherited metabolic disorders of the liver (Rashid et al., 2010), and cancer stem cell models have provided insight into tumour initiation, progression, and drug resistance (Medema, 2013).

Despite these successes, questions remain open in creating fully mature and physiologically relevant disease models. Ongoing research focuses on improving the fidelity of stem cell-derived tissues and incorporating multiple cell types to better recapitulate complex disease environments (Jiang et al., 2018).

7.2. Drug Discovery and Toxicity Testing

Stem cells offer several advantages for drug discovery and toxicity testing. Stem cell-derived tissues often provide more accurate models of human physiology than traditional cell lines or animal models (Avior et al., 2016), and large numbers of compounds can be screened using stem cell-based assays, which accelerate the discovery process (Desbordes et al., 2008). Cardiomyocytes derived from human pluripotent stem cells are increasingly used to assess the cardiotoxicity of new drugs, addressing a major cause of attrition in clinical trials (Braam et al., 2010), while stem cell-derived hepatocytes provide a renewable source of human liver cells for toxicity testing and may reduce reliance on animal models (Sirenko et al., 2014). Neural cells derived from human pluripotent stem cells can likewise be used to assess neurotoxic potential, which is particularly relevant for developmental neurotoxicity studies (Breier et al., 2010).

Recent advances in this area include organ-on-a-chip devices, in which microfluidic systems incorporating stem cell-derived tissues model complex organ functions and drug responses (Huh et al., 2010); 3D organoids, complex self-organizing structures that provide more physiologically relevant models for drug screening (Takasato et al., 2015); and high-content imaging, in which advanced imaging combined with stem cell-derived tissues enables detailed analysis of drug effects on cellular morphology and function (Sirenko et al., 2014).

While stem cell-based models offer many advantages, problems remain in standardising these models and validating their predictive power for human drug responses.

7.3. Regenerative Medicine

Perhaps the most anticipated application of stem cells is in regenerative medicine, where they may allow repairing or replacing damaged tissues and organs. Haematopoietic stem cell transplantation is already well established for treating blood disorders and certain cancers (Copelan, 2006), and epithelial stem cells have been used to generate skin grafts for burn victims (Pellegrini et al., 1999). Limbal stem cells have been used successfully to treat corneal damage and restore vision (Rama et al., 2010), and mesenchymal stem cells warrant further investigation for cartilage regeneration in osteoarthritis (Gupta et al., 2012). Neural stem cells are being explored for conditions such as Parkinson's disease, spinal cord injury, and stroke (Lindvall & Kokaia, 2010); their application in multiple sclerosis, together with the mechanisms involved, is treated separately in Section 4.4. Various stem cell approaches are being investigated for cardiac repair after myocardial infarction (Menasché et al., 2015), and stem cell-derived insulin-producing cells warrant further investigation for treating type 1 diabetes (Pagliuca et al., 2014).

Recent advances and ongoing clinical trials illustrate this progress. Trials using human embryonic stem cell-derived retinal pigment epithelium for macular degeneration have shown early findings (Schwartz et al., 2015), trials are underway using human embryonic stem cell-derived dopaminergic neurons for Parkinson's disease (Barker et al., 2017), and neural stem cell transplantation for spinal cord injury has shown early findings in early-stage clinical trials (Curtis et al., 2018).

Despite these advances, significant obstacles persist in translating stem cell therapies to the clinic, including ensuring long-term safety, achieving functional integration of transplanted cells, and scaling up production to clinically relevant quantities.

7.4. Developmental Biology

Stem cells provide invaluable tools for studying human development. They enable researchers to track the development of specific cell lineages during embryogenesis (Kretzschmar & Watt, 2012), and stem cell-derived organoids recapitulate key aspects of organ development, providing insight into morphogenesis and patterning (Lancaster & Knoblich, 2014). iPSCs and human embryonic stem cells allow the study of human-specific developmental processes that may differ from those in animal models (Nakamura et al., 2016), and they provide a platform for studying epigenetic changes during development and cellular differentiation (Gifford et al., 2013).

Recent advances in this area include gastruloids, 3D aggregates of mouse or human embryonic stem cells that model aspects of gastrulation and provide insight into early embryo patterning (Beccari et al., 2018); blastoid models, stem cell-derived models of the blastocyst-stage embryo that enable the study of early lineage decisions (Rivron et al., 2018); and human embryo models, in which recent work has used human pluripotent stem cells to model

aspects of early human development, raising both further options and ethical questions (Warmflash et al., 2014).

8. Ethical Considerations in Stem Cell Research

Stem cell research, particularly involving human embryonic stem cells and certain applications of induced pluripotent stem cells, raises significant ethical considerations.

8.1. Human Embryonic Stem Cells

The derivation of human embryonic stem cells involves the destruction of human embryos, which raises significant ethical concerns. There is ongoing debate about the moral status of early human embryos and whether their use in research can be ethically justified (Robertson, 2001). Most hESCs are derived from surplus embryos from IVF clinics, and the ethical implications of creating embryos specifically for research are hotly debated (Hyun, 2010). Ensuring proper informed consent from embryo donors is important and has been a source of controversy in some cases (Robertson, 2001), and efforts to derive ESCs without destroying embryos, such as single-cell biopsy, have been explored but raise ethical questions of their own (Chung et al., 2008).

8.2. Induced Pluripotent Stem Cells

While iPSCs avoid many of the ethical issues associated with hESCs, they raise new considerations. The use of integrating vectors in early protocols raised concerns about genetic modification; non-integrating methods have largely addressed this, although concerns remain about potential off-target effects (Yamanaka, 2009). The ability to derive iPSCs from easily obtainable tissues such as skin and blood raises questions about the scope of donor consent and about the potential for generating embryo-like structures or gametes (Sugarman, 2008), and as iPSC-based therapies move towards clinical application, ensuring their safety and efficacy presents both technical and ethical challenges (Neofytou et al., 2015).

8.3. Chimeras and Organoids

Advanced stem cell applications raise further ethical questions. The creation of human-animal chimeras for research purposes, such as growing human organs in animals, raises concerns about blurring species boundaries (Rashid et al., 2014). As cerebral organoids become more complex, questions arise about their potential for consciousness or sentience (Farahany et al., 2018), and recent advances in creating embryo-like structures from stem cells raise questions about the ethical status of these entities (Aach et al., 2017).

8.4. Equity and Access

The development of stem cell therapies also raises issues of equity and access. Many potential therapies are likely to be expensive, which raises concerns about equitable access (Kimmelman et al., 2016). Ensuring diverse representation in donor pools, particularly for iPSC banks, is key for the equitable development of therapies (Taylor et al., 2012), and the concentration of stem cell research in wealthy countries raises questions of global justice in the development and distribution of these therapies (King & Perrin, 2014).

8.5. Regulatory Frameworks

Different countries have adopted varying regulatory approaches to stem cell research, reflecting their differing ethical and cultural positions. The United Kingdom, Sweden, and Japan have relatively permissive policies that allow research on human embryos under strict regulation (Hyun, 2010), whereas Germany and Italy have more restrictive policies that prohibit or severely limit such research (Gottweis, 2010). The United States has taken a middle position, with federal funding restrictions on hESC research but allowance for research supported by private or state funding (Lo & Parham, 2009).

These ethical considerations continue to evolve as the field advances, necessitating ongoing dialogue between scientists, ethicists, policymakers, and the public.

9. Future Directions in Stem Cell Research

Stem cell research is advancing on several fronts.

9.1. Advanced Gene Editing

CRISPR-Cas9 and other gene editing technologies are advancing stem cell research on several fronts: the precise introduction of disease-associated mutations into stem cells allows more accurate disease models (Hotta & Yamanaka, 2015), the genetic correction of patient-derived cells opens the way to autologous cell therapy (DeWitt et al., 2016), and synthetic biology approaches are engineering stem cells with new functionalities for both research and therapeutic applications (Schukur et al., 2015).

9.2. Single-Cell Technologies

Advances in single-cell analysis are providing striking insight into stem cell biology. Combining genetic lineage tracing with single-cell transcriptomics allows developmental trajectories to be mapped (Wagner et al., 2018), single-cell approaches are clarifying the functional significance of stem cell heterogeneity (Kumar et al., 2014), and

single-cell multi-omics is being used to understand epigenetic regulation in these cells (Kelsey et al., 2017).

9.3. Bioengineering and Biomaterials

The integration of stem cell biology with advanced bioengineering is opening further possibilities. 3D bioprinting allows the creation of complex tissue structures with precise spatial organization of multiple cell types (Murphy & Atala, 2014), smart biomaterials are being developed that can dynamically interact with stem cells to guide their behaviour (Qin et al., 2018), and in situ tissue engineering approaches aim to activate and guide endogenous stem cells for tissue repair.

9.4. Artificial Intelligence and Machine Learning

Artificial intelligence and machine learning are being applied to various aspects of stem cell research: predictive modelling to anticipate stem cell behaviour and optimise culture conditions (Waisman et al., 2019), automated image analysis of stem cell cultures and stem cell-derived tissues (Buggenthin et al., 2017), and AI-driven approaches to identify new compounds that modulate stem cell behaviour (Ekins, 2016).

9.5. In Vivo Reprogramming and Transdifferentiation

Approaches that manipulate cell fate directly in the body are also under development. In vivo reprogramming aims to generate stem cells or specific cell types directly within living tissues (Heinrich et al., 2015), while transdifferentiation converts one cell type directly into another in vivo, bypassing a stem cell state altogether (Srivastava & DeWitt, 2016).

9.6. Stem Cells in Aging Research

The role of stem cells in ageing and age-related disease is a further area of active exploration. Research on stem cell exhaustion seeks to understand how stem cell function declines with age and to develop interventions that maintain stemness (López-Otín et al., 2013), work on rejuvenation investigates the potential of stem cell therapies to reverse aspects of ageing (Ocampo et al., 2016), and studies of senescence explore the interplay between cellular senescence and stem cell function in ageing tissues (Herranz & Gil, 2018).

9.7. Extracellular Vesicles and Stem Cell Secretome

Researchers are also working to harness the therapeutic potential of stem cell-derived factors. The role of stem cell-derived exosomes in intercellular communication and their therapeutic potential is under investigation (Phinney & Pittenger, 2017), and cell-free therapies based on stem cell secreted factors are being developed, potentially avoiding some of the challenges associated with cell transplantation (Vizoso et al., 2017).

9.8. Stem Cells in Personalized Medicine

Finally, stem cell technologies are being integrated into personalised medicine. Patient-derived stem cells are being used to predict individual responses to therapy (Matsa et al., 2016), personalised cancer treatments are being developed on the basis of cancer stem cell models, and regenerative therapies are being tailored to individual patients according to their genetic and epigenetic profiles (Neofytou et al., 2015).

10. Conclusion

The field of stem cell research has made substantial progress in recent decades, from the initial isolation of human embryonic stem cells to the development of induced pluripotent stem cells and advanced organoid models. These advances have opened new avenues for understanding human development, modelling diseases, discovering new drugs, and developing regenerative therapies.

The techniques for isolating and culturing stem cells have become increasingly sophisticated, allowing for greater control over stem cell fate and behaviour. From traditional 2D culture systems, we have progressed to complex 3D organoid cultures that better recapitulate the in vivo environment. Advances in biomaterials and bioengineering have led to the development of tailored culture substrates and microenvironments that can guide stem cell differentiation and organization.

Key achievements in the field include the development of induced pluripotent stem cells by Takahashi and Yamanaka (2006), which substantially changed the field by providing an ethically uncontroversial source of pluripotent stem cells and enabling patient-specific disease modelling and drug screening. The ability to generate organoids, complex self-organizing 3D structures, has provided exceptional models of human organ development and disease (Lancaster & Knoblich, 2014), and the demonstration that one cell type can be directly converted into another

without passing through a pluripotent state has opened new possibilities for regenerative medicine (Vierbuchen et al., 2010). Clinical translation has begun, with the first trials using human embryonic stem cell-derived therapies, such as those for macular degeneration, showing early findings (Schwartz et al., 2015), and the application of single-cell sequencing to stem cell biology has revealed previously unappreciated heterogeneity and developmental trajectories (Tritschler et al., 2017).

Despite these achievements, significant difficulties remain. Many stem cell-derived tissues, particularly in organoid systems, remain developmentally immature, which limits their utility both for modelling adult disease and for cell replacement therapy (Huch et al., 2017). Translating small-scale laboratory protocols to clinically relevant scales while maintaining quality and consistency remains a significant hurdle (Kropp et al., 2017) and ensuring the long-term genetic and epigenetic stability of cultured cells, particularly for clinical applications, is an ongoing challenge (Lund et al., 2012). While iPSCs offer the promise of autologous therapies, concerns remain about their potential immunogenicity (Zhao et al., 2011), and the risk of tumour formation from residual undifferentiated cells in stem cell-derived products remains a concern for clinical use (Lee et al., 2013). New ethical questions arise in step, particularly around the creation of human-animal chimeras, increasingly complex organoids, and synthetic embryo-like structures (Farahany et al., 2018).

Looking to the future, several directions are emerging. The ability to reprogram cells directly in the body could improve regenerative medicine and potentially eliminate the need for cell transplantation in some applications (Heinrich et al., 2015), and developing methods to vascularize organoids will be essential for generating larger, more mature tissues (Wimmer et al., 2019). Creating artificial stem cell niches that precisely control cell behaviour could enhance our ability to expand and differentiate stem cells for clinical use, while machine learning approaches are being applied to predict stem cell behaviour, optimise culture conditions, and analyse large-scale experimental data (Waisman et al., 2019). The combination of iPSC technology with precise gene editing tools such as CRISPR-Cas9 opens new possibilities for treating genetic disorders (DeWitt et al., 2016), and progress in deriving functional gametes from pluripotent stem cells could have substantial implications for reproductive medicine and for our understanding of germ cell development (Hikabe et al., 2016). Insight from stem cell biology is informing strategies to rejuvenate aged tissues, potentially leading to new approaches against age-related disease (Ocampo et al., 2016); despite the ethical challenges, the ability to grow human organs in animal hosts could address the shortage of organs for transplantation (Wu et al., 2017); the generation of embryo-like structures from stem cells is providing new tools for studying early human development (Rivron et al., 2018); and the integration of stem cell technologies with genomics and other omics approaches is supporting the development of truly personalised medicine (Matsa et al., 2016).

As these areas develop, it will be central to continue refining our understanding of the fundamental biology of stem cells. This includes elucidating the molecular mechanisms controlling stem cell fate decisions, understanding the role of the stem cell niche in regulating stem cell behaviour, and unraveling the complexities of cellular reprogramming.

Moreover, as stem cell research moves increasingly towards clinical application, several key areas require attention. Robust, scalable, and GMP-compliant protocols must be developed for generating clinical-grade stem cells and their derivatives (Serra et al., 2012), and full preclinical testing regimens must be established to ensure the safety and efficacy of stem cell-based therapies (Harding & Mirochnitchenko, 2014). Appropriate regulatory frameworks are needed that can keep pace with this fast-moving field while ensuring patient safety (Kimmelman et al., 2016), alongside robust ethical oversight mechanisms capable of addressing the unique challenges posed by advancing technologies (Hyun et al., 2016). Fostering informed public dialogue about the potential and the limitations of stem cell research is likewise important (Robillard et al., 2013), as is ensuring that the benefits of this research are accessible globally, including in low- and middle-income countries (King & Perrin, 2014).

Stem-cell research increasingly intersects with gene editing, bioengineering, and computational methods. These combinations can extend disease modelling and drug discovery, but their translational value depends on reproducibility, product characterisation, safety, and evidence that experimental effects are relevant to human disease.

However, realizing the full promise of stem cell research will require continued efforts to address technical challenges, careful consideration of ethical implications, and the development of appropriate regulatory frameworks. It will also necessitate close collaboration between scientists, clinicians, ethicists, policymakers, and the public to ensure that these powerful technologies are developed and applied in ways that maximize benefit and minimise risk.

Progress in stem-cell research should be matched by attention to safety, reproducibility, ethics, equitable access, and long-term follow-up. With those safeguards in place, the field can continue to clarify human developmental biology and identify regenerative or personalised approaches that merit rigorous clinical testing.

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4.4. MECHANISM OF ACTION IN MS TREATMENT

Cell-based approaches in MS address several proposed mechanisms: immune modulation, support for remyelination, and neuroprotection (Compston & Coles, 2008; Scolding et al., 2017). Most repair-related mechanistic findings come from experimental systems. They need to be interpreted separately from evidence that treatment improves function or disability in patients.

1. Immunomodulation

The immunomodulatory properties of stem cells are central to mitigating the aberrant immune responses characteristic of MS. Various cell products, including haematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and neural stem cells (NSCs), have been investigated for immunomodulatory effects relevant to MS pathogenesis (Burt et al., 2004; Zhang et al., 2005). The interaction between these stem cells and the immune system involves multiple cellular and molecular mechanisms, which collectively work to restore immune homeostasis and potentially halt or reverse the progression of MS.

1.1. Hematopoietic Stem Cell Transplantation (HSCT)

Hematopoietic stem cell transplantation (HSCT) has come to be seen as a promising immunomodulatory strategy for the treatment of multiple sclerosis (MS), particularly in patients with highly active relapsing-remitting or progressive forms of the disease that are unresponsive to conventional therapies. High-dose immunosuppressive therapy followed by autologous haematopoietic stem cell transplantation (AHSCT) has demonstrated the ability to "reset" the immune system, reduce inflammatory activity, and potentially slow disease progression. A recent study by Mariottini et al. (2022) showed that intermediate-intensity AHSCT in aggressive MS patients significantly reduced serum neurofilament light chain (sNfL) levels, a key biomarker of axonal damage, and brain atrophy, supporting the efficacy of AHSCT in limiting neurodegeneration. This approach aims to eradicate the autoreactive immune cells responsible for myelin destruction and replace them with a new, self-tolerant immune system derived from the transplanted HSCs (Burt et al., 1998).

The immunomodulatory effects of HSCT are varied and involve several complex mechanisms:

1.1.1. Depletion of Autoreactive Cells

The conditioning regimen used before transplantation, which typically involves high-dose chemotherapy and sometimes total body irradiation, effectively depletes most autoreactive T cells, B cells, and other immune cells implicated in MS pathogenesis. This "immune reset" provides a clean slate for the reconstitution of a potentially more tolerant immune system (Muraro et al., 2017). The depth of lymphocyte depletion during conditioning may influence long-term outcomes in MS patients undergoing HSCT, which would underline the importance of this initial step in the treatment process.

1.1.2. Immune Reconstitution

Following conditioning, the immune system is rebuilt from the infused haematopoietic progenitors. Innate cells such as neutrophils and NK cells recover within weeks, B cells within several months, and CD4+ T cells more slowly, with renewed thymic output generating a new naïve T-cell compartment whose receptor repertoire differs markedly from the pre-transplant one (Muraro et al., 2005; Massey et al., 2018). This qualitative renewal, rather than lymphodepletion alone, is thought to underlie the durable suppression of inflammatory activity observed in many patients.

1.1.3. Regulatory T Cell Induction

HSCT has been shown to promote the expansion of regulatory T cells (Tregs), which is essential to maintaining immune tolerance and suppressing autoimmune responses. The increased presence of Tregs following HSCT may contribute to the long-term efficacy of this treatment in MS (Abrahamsson et al., 2013). Moreover, recent research has demonstrated that HSCT not only increases the number of Tregs but also enhances their suppressive function, potentially through epigenetic modifications that stabilize the Treg phenotype (Karnell et al., 2017).

1.1.4. Modulation of B Cell Responses

The conditioning regimen depletes circulating B cells, which then repopulate over several months with a predominance of naïve and transitional B cells, while memory B-cell subsets remain reduced for a prolonged period (Karnell et al., 2017; Massey et al., 2018). Because memory B cells act as antigen-presenting cells and sources of pro-inflammatory cytokines in MS, this shift in B-cell composition

may contribute to the immunological effect of transplantation.

1.1.5. Cytokine Profile Alteration

HSCT can lead to significant changes in the cytokine profile of MS patients, with a shift towards a more anti-inflammatory state. This includes reductions in pro-inflammatory cytokines such as IL-17 and IFN-γ and increases in anti-inflammatory cytokines like IL-10 (Darlington et al., 2013). Changes in other regulatory cytokine pathways following HSCT, possibly including IL-35, may also contribute to the restoration of immune tolerance in MS patients.

1.1.6. Restoration of T Cell Receptor Repertoire

HSCT has been shown to profoundly alter the T cell receptor (TCR) repertoire in MS patients. A study by Muraro et al. (2005) demonstrated that HSCT leads to the complete renewal of the T cell pool, with the emergence of a new and diverse TCR repertoire. This renewal may help eliminate autoreactive T cell clones and restore a more balanced immune response.

1.1.7. Modulation of Innate Immunity

While much of the focus on HSCT in MS has been on adaptive immunity, the treatment also affects innate immune cells. Massey et al. (2018) reviewed immune reconstitution after autologous HSCT in MS, including changes in natural killer (NK) cells that may contribute to the overall immunomodulatory effect of the treatment.

1.2. Mesenchymal stem cells (MSCs)

MSCs have demonstrated significant immunomodulatory properties in the context of MS treatment. These multipotent cells, which can be isolated from various tissues including bone marrow, adipose tissue, and umbilical cord, can exert their effects through multiple mechanisms, which are shown together in Figure 4.4.1.

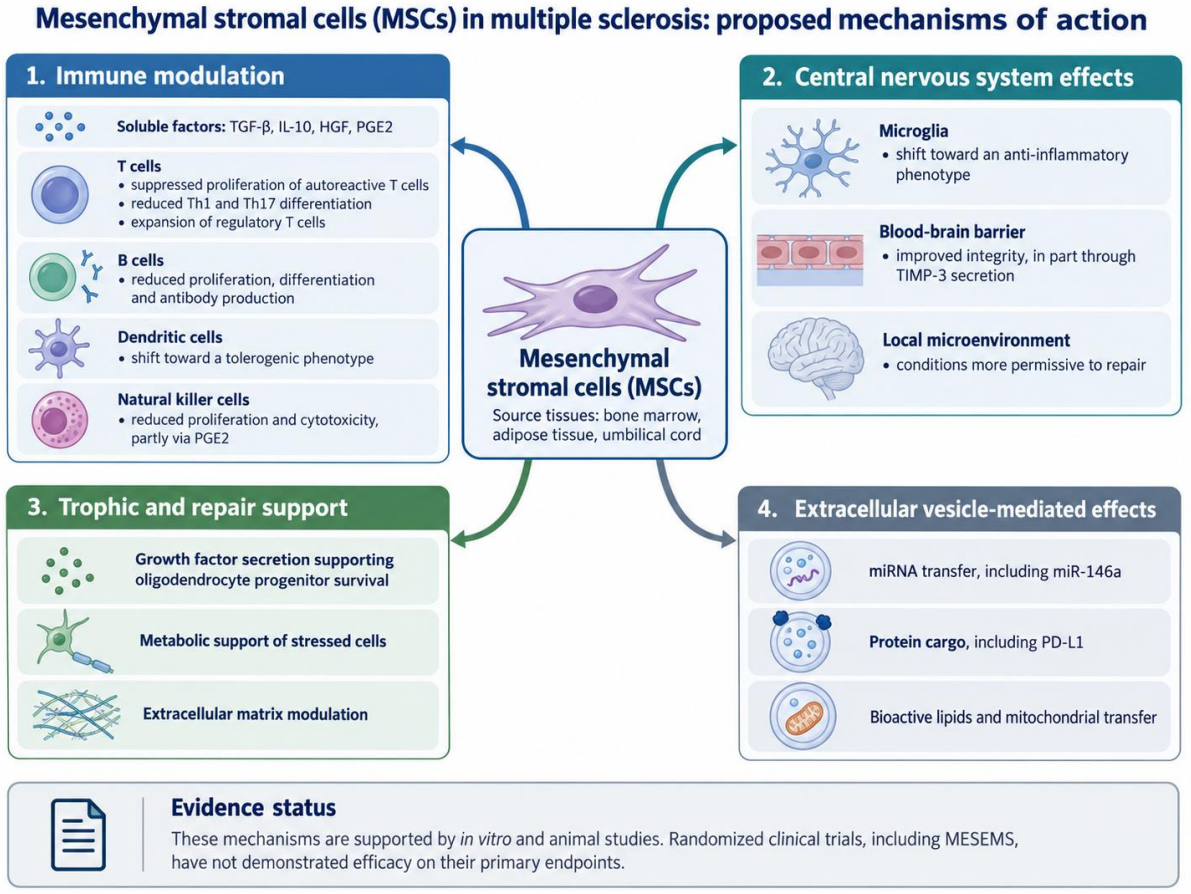


Figure 4.4.1. Proposed mechanisms of action of mesenchymal stromal cells in multiple sclerosis. The pathways shown are derived from in vitro and animal studies; they describe a mechanistic rationale rather than demonstrated clinical efficacy.

1.2.1. Cytokine Secretion

MSCs secrete a wide array of anti-inflammatory cytokines and growth factors, including TGF-β, IL-10, HGF, and PGE2. These factors can suppress the activation and proliferation of autoreactive T cells and promote a more anti-inflammatory environment in the CNS (Uccelli et al., 2008). Recent research has expanded our understanding of the MSC secretome, identifying novel factors such as galectin-1 and semaphorin-3A, which have potent immunomodulatory effects (Gieseke et al., 2010; Lepelletier et al., 2010). See Figure 4.4.2.

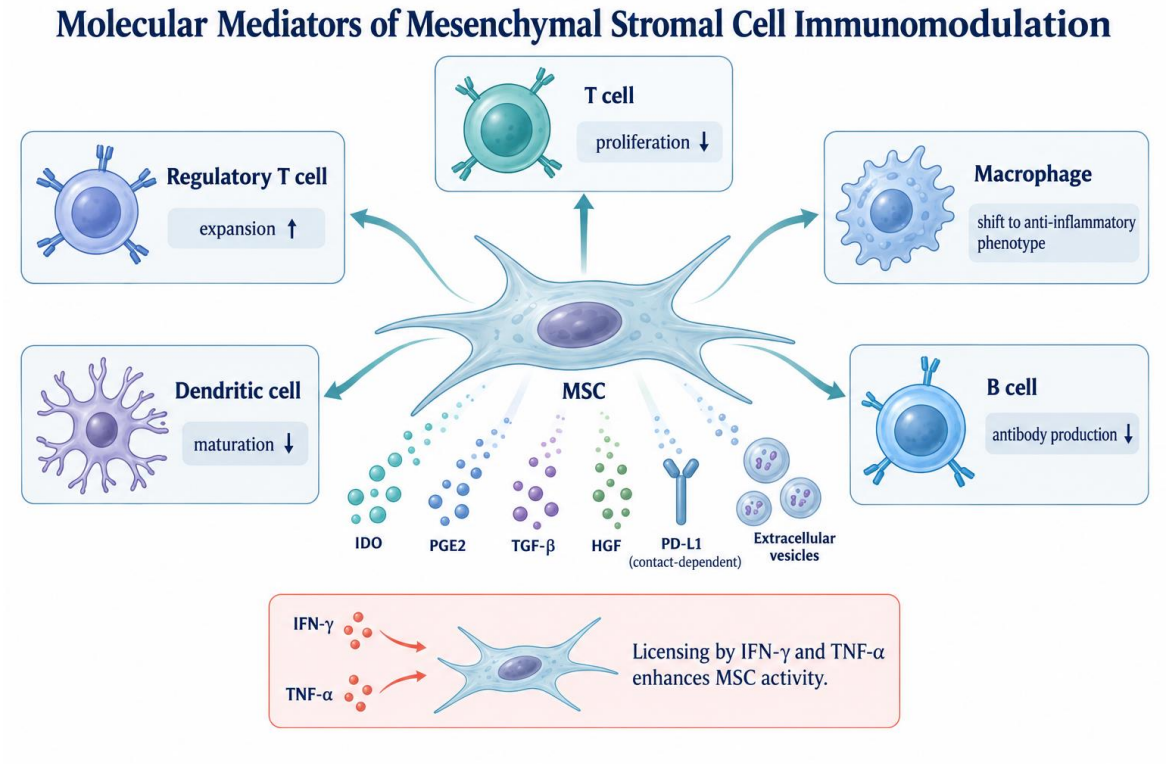


Figure 4.4.2. Molecular mediators of mesenchymal stromal cell immunomodulation and their target cells. Inflammatory licensing by IFN-γ and TNF-α enhances this activity.

1.2.2. T Cell Modulation

MSCs have been shown to suppress the proliferation and activation of autoreactive T cells, inhibit the differentiation of pro-inflammatory Th1 and Th17 cells, and promote the expansion of regulatory T cells. This modulation of T cell responses is important in dampening the autoimmune response in MS (Gerdoni et al., 2007). Recent studies have elucidated additional mechanisms by which MSCs modulate T cell function, including the induction of T cell anergy through the expression of programmed death-ligand 1 (PD-L1) and the modulation of T cell metabolism through the expression of indoleamine 2,3-dioxygenase (IDO) (Davies et al., 2017; Chinnadurai et al., 2015).

1.2.3. B Cell Regulation

MSCs can influence B cell function by inhibiting B cell proliferation, differentiation, and antibody production. This effect on B cells may be particularly relevant in light of the growing recognition of B cells' role in MS pathogenesis (Franquesa et al., 2015). Recent research has shown that MSCs can also induce regulatory B cells (Bregs), which produce anti-inflammatory cytokines and contribute to immune tolerance (Peng et al., 2015).

1.2.4. Dendritic Cell Modulation

MSCs can alter the function of dendritic cells, key players in initiating immune responses. By promoting the development of tolerogenic dendritic cells, MSCs can indirectly suppress T cell activation and promote immune tolerance (Nauta et al., 2006). Recent studies have demonstrated that MSCs can induce a tolerogenic phenotype in dendritic cells through multiple mechanisms, including the secretion of hepatocyte growth factor (HGF) and the modulation of dendritic cell metabolism (Benkhoucha et al., 2010).

1.2.5. Natural Killer Cell Inhibition

MSCs have been shown to inhibit the proliferation and cytotoxic activity of natural killer cells, which may contribute

to their overall immunomodulatory effects in MS (Spaggiari et al., 2008). Recent research has revealed that this inhibition is mediated in part by the secretion of prostaglandin E2 (PGE2) and the expression of HLA-G by MSCs (Sotiropoulou et al., 2006; Rizzo et al., 2008).

1.2.6. Microglial Modulation

In the CNS, MSCs can influence the phenotype and function of microglia, the resident immune cells of the brain and spinal cord. By promoting an anti-inflammatory microglial phenotype, MSCs may help create a more favourable environment for tissue repair and regeneration (Giunti et al., 2014). Recent studies have shown that MSCs can modulate microglial function through the secretion of CX3CL1 (fractalkine) and other factors, promoting a neuroprotective microglial phenotype (Giunti et al., 2012; Zanier et al., 2014).

1.2.7. Modulation of the Blood-Brain Barrier

MSCs have been proposed to influence the integrity and function of the blood-brain barrier (BBB), which is often compromised in MS, although direct evidence in MS models remains limited. Lymphocytes can also enter the CNS through the choroid plexus, where Steffen et al. (1996) found that ICAM-1, VCAM-1, and MAdCAM-1 are expressed on the epithelium rather than the endothelium and mediate lymphocyte binding in vitro.

1.2.8. Exosome-Mediated Effects

Recent research has highlighted the importance of MSC-derived exosomes in mediating many of the immunomodulatory effects of these cells. These nano-sized vesicles contain a variety of bioactive molecules, including microRNAs and proteins, that can modulate immune cell function (Riazifar et al., 2017). Yu et al. (2014) reviewed exosomes derived from MSCs, and experimental studies suggest that such vesicles can suppress T cell activation and promote regulatory T cell expansion, supporting interest in a cell-free therapeutic approach.

1.3. Neural stem cells (NSCs)

NSCs, whether derived from embryonic stem cells, induced pluripotent stem cells, or adult neural tissue, have shown the ability to modulate immune responses in the CNS:

1.3.1. Immunomodulatory Factor Secretion

NSCs can secrete various immunomodulatory factors, such as transforming growth factor-β (TGF-β) and prostaglandin E2 (PGE2), which can suppress the activation and proliferation of autoreactive T cells and promote a more anti-inflammatory environment in the CNS (Pluchino et al., 2005). Recent research has identified additional factors secreted by NSCs, including leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF), which have potent immunomodulatory and neuroprotective effects (Laterza et al., 2013).

1.3.2. Direct Cell-Cell Interactions

NSCs can directly interact with immune cells through cell surface molecules, influencing their activation and function. For example, NSCs expressing the death receptor ligand FasL can induce apoptosis in activated T cells, potentially eliminating autoreactive cells (Knight et al., 2011). Recent studies have also shown that NSCs can modulate T cell function through the expression of programmed death-ligand 1 (PD-L1) and the engagement of the CD47-SIRPα pathway, further contributing to their immunomodulatory effects. These direct interactions between NSCs and immune cells highlight the complexity of stem cell-mediated immunomodulation in the context of MS.

1.3.3. Niche Modulation

Transplanted NSCs can persist in the CNS and modify the local microenvironment, creating immunomodulatory niches that influence the behaviour of resident and infiltrating immune cells (Martino & Pluchino, 2006). Peruzzotti-Jametti et al. (2018) showed in a chronic EAE model that transplanted NSCs sense and take up succinate released by inflammatory macrophages and microglia, thereby reducing chronic neuroinflammation.

1.3.4. Metabolic Regulation

Emerging evidence suggests that NSCs can modulate immune responses through metabolic regulation. Drago et al. (2013) reviewed how the stem cell secretome contributes to brain repair, and later work showed that NSCs respond to metabolites such as succinate released by inflammatory phagocytes (Peruzzotti-Jametti et al., 2018). Such metabolic cross-talk may involve changes in cellular energetics and the production of specific metabolites that influence immune cell function.

1.3.5. Extracellular Vesicle-Mediated Effects

Similar to MSCs, NSCs also release extracellular vesicles (EVs) that can mediate some of their immunomodulatory effects. These EVs contain a variety of bioactive molecules, including microRNAs and proteins, that can influence the function of recipient immune cells. A recent review by Li

et al. (2023) highlighted that extracellular vesicles (EVs) derived from neural stem/progenitor cells (NSCs) could effectively suppress neuroinflammation and promote neuroprotection, suggesting these EVs as a promising cell-free therapeutic approach for neurological diseases, including multiple sclerosis.

1.4. Extracellular Vesicles (EVs) in Stem Cell-Mediated Immunomodulation

The role of extracellular vesicles in mediating the immunomodulatory effects of stem cells has gained significant attention in recent years. EVs, including exosomes and microvesicles, are nano-sized membrane-bound structures that carry a diverse cargo of proteins, lipids, and nucleic acids. These vesicles can function as powerful mediators of intercellular communication, influencing the function of recipient cells in various ways:

1.4.1. miRNA Transfer

Extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) are rich in microRNAs (miRNAs) that can modulate the function of recipient immune cells. Notably, miR-146a, commonly enriched in MSC-derived EVs, has been shown to suppress T cell activation and promote regulatory T cell (Treg) function. For instance, a study demonstrated that exosomal miR-146a from MSCs contributed to enhanced therapeutic efficacy against sepsis by modulating inflammatory responses (Song et al., 2017). Additionally, Riazifar et al. (2019) showed that EVs from interferon-γ-stimulated MSCs reduced disease in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), and characterised the RNA and protein cargo that may contribute to these immunosuppressive effects.

1.4.2. Protein Cargo

EVs can carry immunomodulatory proteins such as PD-L1, which can directly suppress T cell responses upon delivery to recipient cells. Proteomic analyses of stem cell-derived EVs have revealed a complex array of proteins; for instance, Anderson et al. (2016) performed a comprehensive proteomic analysis of MSC-derived exosomes, identifying well over a thousand proteins, and showed that these vesicles modulate angiogenesis via NF-κB signalling.

1.4.3. Lipid Mediators

Some EVs contain bioactive lipids like prostaglandins, which can exert immunomodulatory effects on target cells (Harting et al., 2018). Research has also expanded our understanding of the metabolite composition of stem cell-derived EVs: Showalter et al. (2019) found that primed MSCs package exosomes with metabolites associated with immunomodulation.

1.4.4. Mitochondrial Transfer

Stem cells can transfer mitochondria to recipient cells, altering their metabolic state and function; Islam et al. (2012) showed this for bone marrow-derived stromal cells and lung alveolar epithelium. Court et al. (2020) showed that mitochondrial transfer from MSCs to T cells induces regulatory T cell differentiation and restricts inflammatory responses. The relative contribution of EVs, as opposed to direct cell contact, to such transfer remains under investigation.

1.4.5. Antigen Presentation

Some EVs carry MHC molecules and co-stimulatory factors and could therefore participate in antigen presentation and T cell modulation. Reis et al. (2018) showed that MSC-derived EVs attenuate dendritic cell maturation and function, which may indirectly limit T cell activation.

1.4.6. Complement Regulation

It has been suggested that stem cell-derived EVs may also influence the complement system, a key component of innate immunity, although this has not been examined in MS models. More broadly, Gazdic et al. (2015) reviewed evidence that MSCs can act as either friend or foe in immune-mediated diseases, depending on the inflammatory context.

The interaction between these various immunomodulatory mechanisms contributes to the overall therapeutic effect of stem cell therapy in MS. By targeting multiple aspects of the dysregulated immune response, stem cell-based approaches offer an integrated strategy for modulating the autoimmune processes underlying MS pathogenesis. The continued elucidation of these mechanisms, particularly the emerging role of extracellular vesicles, opens up new avenues for developing more targeted and effective therapies for MS patients.

2. Myelin Regeneration

The capacity of stem cells to promote myelin regeneration represents a critical mechanism in their therapeutic potential for MS treatment. Myelin, the insulating sheath surrounding axons, is essential for proper neuronal signalling and function. In MS, the loss of myelin (demyelination) leads to impaired nerve conduction and contributes to

neurological deficits. Stem cell therapy offers possible routes for enhancing remyelination processes and restoring neuronal function in MS patients (Franklin & Ffrench-Constant, 2008).

2.1. Oligodendrocyte Progenitor Cells (OPCs)

OPCs, which can be derived from various types of stem cell, contribute substantially to myelin regeneration:

2.1.1. Differentiation Potential

OPCs have the capacity to differentiate into mature oligodendrocytes, the primary myelinating cells of the central nervous system (CNS). Studies have shown that transplanted neural stem cells (NSCs) and their derivatives can differentiate into oligodendrocytes and contribute to remyelination in animal models of MS (Martino & Pluchino, 2006). Recent research has further elucidated the molecular mechanisms governing OPC differentiation, identifying key transcription factors such as Olig2, Sox10, and Myrf that regulate the transition from OPCs to myelinating oligodendrocytes (Emery & Lu, 2015).

2.1.2. Migration and Homing

Transplanted OPCs or stem cell-derived OPCs must migrate to the sites of demyelination. This migration is guided by various chemokines and growth factors released in response to CNS injury. Factors such as CXCL12 and its receptor CXCR4 have been implicated in the homing of OPCs to demyelinated lesions (Carbajal et al., 2011). Recent studies have identified additional factors involved in OPC migration, including semaphorin 3A and netrin-1, which can either promote or inhibit OPC migration depending on the specific context (Piaton et al., 2011; Tepavčević et al., 2014).

2.1.3. Proliferation and Survival

Once at the site of injury, OPCs must proliferate and survive in the hostile environment of MS lesions. Stem cell-derived factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF-2), play key roles in promoting OPC proliferation and survival (Muramatsu et al., 2012). Recent research has identified additional factors that support OPC survival in the inflammatory environment of MS lesions, including endothelin-1 and leukemia inhibitory factor (LIF) (Hammond et al., 2014; Barres et al., 1993).

2.1.4. Differentiation and Maturation

The final step in myelin regeneration involves the differentiation of OPCs into mature, myelinating oligodendrocytes. This process is regulated by an interaction of transcription factors, epigenetic modifiers, and environmental cues. Stem cell therapies can influence this process by providing trophic support and modulating the local environment to favor oligodendrocyte differentiation (Rivera et al., 2019). Recent studies have identified novel regulators of OPC differentiation, including the histone deacetylase SIRT1 and the microRNA miR-219, which could serve as potential targets for enhancing remyelination in MS (Rafalski et al., 2013; Dugas et al., 2010).

2.1.5. Integration and Functional Myelination

For successful remyelination, newly differentiated oligodendrocytes must establish contact with demyelinated axons and form functional myelin sheaths. Recent research has begun to map the molecular mechanisms underlying this process, including the role of neuregulin-1 and its receptor ErbB in promoting oligodendrocyte-axon interactions and myelin wrapping (Mei & Nave, 2014). Additionally, studies have identified factors that can enhance the efficiency of myelination, such as the RXR-γ nuclear receptor, which promotes oligodendrocyte differentiation and myelin gene expression (Huang et al., 2011).

2.2. Mesenchymal stem cells (MSCs) and Myelin Regeneration

While MSCs do not typically differentiate into oligodendrocytes themselves, they can enhance myelin regeneration through indirect mechanisms:

2.2.1. Trophic Factor Secretion

MSCs secrete various neurotrophic and growth factors that can promote the survival, proliferation, and differentiation of endogenous OPCs. These factors include PDGF, FGF-2, brain-derived neurotrophic factor (BDNF), and insulin-like growth factor-1 (IGF-1) (Bai et al., 2012). Recent research has identified additional factors secreted by MSCs that contribute to myelin regeneration, including hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) (Bai et al., 2012; Menge et al., 2012).

In a 2026 ancillary analysis of MESEMS, Schiavetti et al. examined peripheral immune-cell profiles in 51 participants. MSC exposure was associated with short-term changes in B-cell and regulatory T-cell populations, with limited longitudinal effects. These exploratory observations document biological activity without overturning the parent trial's negative primary MRI comparison (Schiavetti et al., 2026).

2.2.2. Immunomodulation

By creating a more favourable immune environment, MSCs can reduce the inflammatory factors that inhibit OPC differentiation and remyelination. This immunomodulatory effect can indirectly support the remyelination process (Rivera et al., 2019). Recent studies have demonstrated that MSC-mediated immunomodulation can lead to a shift in the phenotype of CNS-resident microglia and infiltrating macrophages, promoting an anti-inflammatory, pro-regenerative environment that supports remyelination (Giunti et al., 2014).

2.2.3. Extracellular Vesicle (EV) Mediated Effects

MSC-derived EVs can carry bioactive molecules that promote OPC survival, proliferation, and differentiation. These EVs have been shown to enhance remyelination in experimental models of MS (Riazifar et al., 2019). Recent research has identified specific microRNAs contained within MSC-derived EVs, such as miR-146a and miR-181c, which can modulate neuroinflammation and promote a regenerative environment conducive to remyelination (Riazifar et al., 2019).

2.2.4. Metabolic Support

MSCs can provide metabolic support to oligodendrocytes and OPCs, potentially enhancing their survival and function in the challenging environment of MS lesions. Recent studies have shown that MSCs can transfer mitochondria to stressed cells, including oligodendrocytes, through tunneling nanotubes or extracellular vesicles, potentially rescuing these cells from energy failure and supporting their myelinating function (Mahrouf-Yorgov et al., 2017).

2.2.5. Extracellular Matrix Modulation

Mesenchymal stem cells (MSCs) are key contributors to remodeling the extracellular matrix (ECM) within demyelinated lesions, thereby fostering a conducive environment for oligodendrocyte progenitor cell (OPC) migration and differentiation. This remodeling is primarily mediated through the secretion of matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). MMPs are instrumental in degrading various ECM components, facilitating cellular migration and tissue remodeling (Rundhaug, 2003). Concurrently, TIMPs regulate MMP activity, ensuring a balanced ECM turnover and preserving tissue integrity (NAGASE et al., 2006). The orchestrated interplay between MMPs and TIMPs secreted by MSCs is essential for ECM remodeling, which in turn promotes OPC migration and enhances remyelination processes.

2.3. Induced Pluripotent Stem Cells (iPSCs) in Myelin Regeneration

iPSCs offer unique advantages in promoting myelin regeneration:

2.3.1. Directed Differentiation

iPSCs can be differentiated into OPCs or even mature oligodendrocytes in vitro before transplantation. This allows for the generation of a pure population of cells with high remyelination potential (Goldman et al., 2012). Recent advances in differentiation protocols have led to the development of more efficient and scalable methods for generating oligodendrocyte lineage cells from iPSCs, potentially facilitating their clinical application (Ehrlich et al., 2017).

2.3.2. Autologous Transplantation

Patient-specific iPSCs can be generated, potentially allowing for autologous transplantation and reducing the risk of immune rejection (Thiruvalluvan et al., 2016). Recent studies have demonstrated the feasibility of generating and transplanting autologous iPSC-derived OPCs in non-human primate models, supporting the development of future clinical applications in MS.

2.3.3. Genetic Modification

iPSCs can be genetically modified to enhance their remyelination capacity. For example, overexpression of specific transcription factors, such as Sox10 or Olig2, can enhance the oligodendrogenic potential of iPSC-derived neural progenitors (Wang et al., 2013). Advances in gene editing technologies, such as CRISPR-Cas9, have opened up new possibilities for precisely modifying iPSCs to enhance their therapeutic potential in MS (Heman-Ackah et al., 2016).

2.3.4. Disease Modeling

iPSCs derived from MS patients can be used to study disease mechanisms and test potential therapies in vitro, potentially leading to more personalised treatment approaches (Nicaise et al., 2019). Recent studies have used MS patient-derived iPSCs to model oligodendrocyte dysfunction and screen for compounds that can enhance remyelination, identifying several candidates for further investigation (Mei et al., 2014; Lager et al., 2018).

2.4. Enhancing Remyelination Potential

Recent advances have explored ways to enhance the remyelination capacity of transplanted or endogenous stem cells:

2.4.1. Genetic Modification

Overexpression of specific transcription factors or microRNAs in transplanted stem cells can enhance their oligodendrogenic potential and improve remyelination outcomes (Wu et al., 2012). Recent studies have identified novel targets for genetic modification, including transcription factor EB (TFEB). However, rather than enhancing myelination, TFEB overactivation has been shown to repress myelination downstream of the Rag-Ragulator complex, suggesting that precise regulation of TFEB activity is essential when considering therapeutic interventions to promote remyelination (Meireles et al., 2018).

2.4.2. Pharmacological Approaches

Small molecules that promote OPC differentiation or enhance the remyelination capacity of stem cells have been identified. These compounds could be used in combination with stem cell therapies to improve outcomes (Mei et al., 2014). Recent high-throughput screening efforts have identified several promising compounds, including clemastine, miconazole, and clobetasol, which can enhance OPC differentiation and remyelination (Najm et al., 2015; Mei et al., 2014).

2.4.3. Biomaterials and Tissue Engineering

The development of biocompatible scaffolds that can support the survival, differentiation, and integration of transplanted stem cells has shown promise in enhancing the efficiency of cell-based therapies for remyelination. These scaffolds can provide structural support for regenerating axons and oligodendrocytes, as well as serve as delivery vehicles for growth factors and other molecules that promote remyelination (Simitzi et al., 2017). Recent advances in biomaterial design have led to the development of injectable hydrogels that can provide a supportive microenvironment for transplanted stem cells and promote remyelination in situ.

2.4.4. Combination Therapies

Combining stem cell transplantation with other therapeutic approaches, such as immunomodulatory drugs or remyelination-promoting compounds, may synergistically enhance myelin regeneration (Plemel et al., 2017). Recent studies have explored various combinations.

Combining stem cell transplantation with immunomodulatory drugs has been examined directly. Kalincik et al. (2023) compared outcomes after autologous haematopoietic stem cell transplantation (AHSCT) with fingolimod, natalizumab, and ocrelizumab in highly active relapsing-remitting MS. AHSCT was associated with lower relapse activity than fingolimod and natalizumab, while evidence of a difference versus ocrelizumab was less clear. The observational comparative design does not establish general superiority for AHSCT over all high-efficacy therapies; treatment choice also depends on patient selection and transplant-related risk.

Clemastine provides a pharmacological remyelination proof-of-concept rather than evidence for a stem-cell combination. Li et al. (2015) studied clemastine alone after cuprizone-induced demyelination in mice and reported enhanced myelin repair, increased mature oligodendrocytes and myelin basic protein, together with improvement in the behavioural abnormalities assessed in that model. The study did not include stem-cell transplantation, so it should not be cited as evidence that cells and clemastine have been tested together.

Clinical remyelination programmes must distinguish protocols from demonstrated benefit. The CCMR Two protocol evaluates metformin plus clemastine in people with relapsing-remitting MS already receiving disease-modifying therapy, with visual evoked-potential latency as the primary outcome. The protocol itself does not establish efficacy (Riboni-Verri et al., 2025).

Multi-modal strategies have likewise been considered. Zhang et al. (2021) examined how MSC transplantation affects the oligodendrocyte lineage in the brains of rats with EAE, but direct head-to-head comparisons of mesenchymal and neural stem cell strategies remain scarce. Mechanistic explanations remain hypotheses, and animal-model results do not demonstrate a comparative clinical benefit for people with MS.

2.4.5. Exosome-Based Therapies

Recent research has focused on harnessing the regenerative potential of stem cell-derived exosomes for promoting remyelination.

One route is the delivery of microRNAs. Exosomes can be engineered to deliver specific cargo, and Liang et al. (2021) reviewed strategies for engineering exosomes for targeted drug delivery. Vesicles enriched in pro-myelinating microRNAs such as miR-219 are being investigated as a cell-

free approach to promoting OPC differentiation and remyelination.

Stem cell-derived exosomes also carry proteins and other factors that might support repair. Xin et al. (2013) found that systemically administered MSC-derived exosomes promoted functional recovery and neurovascular plasticity after stroke in rats; whether such vesicles deliver growth factors that promote OPC proliferation and differentiation in demyelinated lesions has not been established.

A third route is exosome engineering, which has allowed the creation of "designer" exosomes; for instance, Kojima et al. (2018) showed that exosomes produced by implanted engineered cells could deliver therapeutic cargo into the brain in a Parkinson's disease model. Comparable approaches aimed at oligodendrocytes and remyelination have yet to be established.

2.4.6. Targeting the Microenvironment

Emerging research has focused on modulating the microenvironment of demyelinated lesions to enhance the regenerative potential of endogenous or transplanted stem cells.

One approach is modification of the extracellular matrix. The physical and molecular properties of the extracellular matrix (ECM) can significantly affect remyelination: Segel et al. (2019) showed that age-related stiffening of the niche impairs the function of oligodendrocyte progenitor cells, and inhibitory ECM components such as chondroitin sulfate proteoglycans are also being targeted to enhance repair.

A second targets the microglia and macrophage phenotype. The phenotype of CNS-resident microglia and infiltrating macrophages can significantly influence the success of remyelination. Lloyd et al. (2019) showed that CNS regeneration is driven by necroptosis of pro-inflammatory microglia followed by repopulation with pro-regenerative microglia, and whether transplanted stem cells can promote such a switch is an active question.

Vascular remodelling matters as well: the vasculature helps to create a supportive niche for remyelination; for example, Maki et al. (2018) showed that the secretome of endothelial progenitor cells promoted oligovascular repair in a mouse model of prolonged cerebral hypoperfusion.

2.4.7. Epigenetic Modulation

Recent research has highlighted the importance of epigenetic regulation in oligodendrocyte differentiation and remyelination.

Histone deacetylase (HDAC) activity is required for OPC differentiation, and age-related decline in HDAC recruitment has been linked to impaired remyelination, so epigenetic regulators of this pathway are being explored as targets. Of note, Hait et al. (2014) showed that phosphorylated fingolimod, an approved MS therapy, inhibits histone deacetylases in the brain, although that study examined fear extinction memory rather than remyelination.

Modulating DNA methylation patterns can likewise influence OPC differentiation and remyelination potential. Research by Moyon et al. (2016) demonstrated that changes in DNA methylation are critical for the transition from OPCs to myelinating oligodendrocytes, suggesting potential targets for enhancing remyelination.

Non-coding RNAs add a further layer of control. Long non-coding RNAs (lncRNAs) have become important regulators of oligodendrocyte differentiation and myelination, and lncRNAs expressed in OPCs could be potential targets for promoting remyelination.

2.4.8. Advanced Imaging Techniques

The development of novel imaging techniques has greatly enhanced our ability to track and assess remyelination in vivo.

Positron emission tomography (PET) with myelin-specific tracers has attracted attention as a powerful tool for assessing remyelination. A study by Bodini et al. (2016) demonstrated the utility of [11C]PIB-PET for monitoring remyelination in MS patients, providing a potential biomarker for assessing the efficacy of stem cell therapies.

Advanced magnetic resonance imaging (MRI) techniques, including myelin water imaging (MWI) and magnetization transfer ratio (MTR), have enhanced sensitivity for detecting remyelination in multiple sclerosis (MS). These modalities can identify subtle changes in myelin content, making them valuable tools for assessing the efficacy of remyelination therapies. For instance, studies have demonstrated that MTR correlates with histological measures of myelin, providing a non-invasive biomarker for myelin integrity. Similarly, MWI has been utilised to detect demyelination and remyelination processes, offering insights into the dynamics of myelin repair. These imaging techniques are instrumental in evaluating the outcomes of stem cell therapies aimed at promoting remyelination in MS (Laule et al., 2007).

Advances in intravital microscopy have allowed real-time visualisation of remyelination in living animals. Orthmann-Murphy et al. (2020) used longitudinal in vivo

two-photon imaging in a cuprizone model to show that remyelination alters the pattern of myelin in the cerebral cortex; similar approaches could be used to follow the fate of transplanted cells.

These advanced approaches and emerging technologies are pushing the boundaries of stem cell-mediated myelin regeneration in MS. By combining multiple strategies and leveraging our growing understanding of the complex biology of remyelination, researchers are supporting the development of more effective and targeted therapies for MS patients. The field continues to expand, with new discoveries constantly refining our approaches to promoting myelin repair and functional recovery in MS.

3. Neuroprotection

Neuroprotection is a proposed mechanism of investigational cell-based approaches in MS. Neuronal and axonal loss contribute to disability, and experimental studies suggest that trophic and immunomodulatory actions of some cell products may limit tissue injury (Scolding et al., 2017). Such findings should not be interpreted as established slowing or reversal of neurodegeneration in treated MS patients.

3.1. Neurotrophic Factor Secretion

One of the primary ways stem cells exert neuroprotective effects is through the secretion of neurotrophic factors.

Among the most important of these is brain-derived neurotrophic factor. BDNF supports neuronal survival, promotes axonal growth, and enhances synaptic plasticity. Mesenchymal stem cells (MSCs) and neural stem cells (NSCs) have been shown to secrete BDNF, which can protect neurons from inflammatory damage and promote neuronal repair (Barhum et al., 2010). Recent studies have demonstrated that genetically modified stem cells overexpressing BDNF show enhanced neuroprotective effects in experimental models of MS (Makar et al., 2009).

Nerve growth factor (NGF) supports the survival and differentiation of neurons, particularly in the peripheral nervous system. Stem cell-derived NGF can promote axonal regeneration and protect neurons from oxidative stress (Uccelli et al., 2011). Razavi et al. (2017) reported improved remyelination after transplantation of neurotrophic factor-secreting cells in a rat model of spinal cord injury; comparable evidence in MS models is lacking.

Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for various neuronal populations. Stem cells secreting GDNF can protect motor neurons and dopaminergic neurons, which may be relevant in MS-related motor dysfunction (Drago et al., 2013). Research has explored the use of GDNF-secreting stem cells in combination with other neuroprotective strategies to enhance their therapeutic efficacy in MS models (X. Gao et al., 2016).

Ciliary neurotrophic factor (CNTF) has been shown to promote the survival of oligodendrocytes and neurons. Stem cells engineered to overexpress CNTF have demonstrated enhanced neuroprotective effects in experimental models of MS.

Fifth is insulin-like growth factor-1. Insulin-like growth factor-1 (IGF-1) exhibits significant neuroprotective and neurotrophic properties. Stem cell-derived IGF-1 has been shown to promote neuronal survival and enhance remyelination. Recent evidence suggests that IGF-1 signalling plays a central immunomodulatory role in the central nervous system (CNS). Specifically, IGF-1 receptor (IGF-1R) activity in CNS-resident macrophages regulates their morphology and gene expression, thereby influencing the severity of autoimmune inflammation. In a recent study, genetic deletion of IGF-1R in these macrophages resulted in exacerbated disease severity in an EAE model, underlining the protective role of IGF-1R signalling in neuroinflammation (Ivan et al., 2023). Additionally, a systematic review and meta-analysis revealed no significant difference in serum IGF-1 levels between MS patients and healthy controls, though the therapeutic potential of IGF-1, especially in neurorepair and remyelination, remains a possible route for future research (Yaghoobpoor & Ghayyem, 2024).

3.2. Anti-Apoptotic Effects

Stem cells, especially MSCs, have demonstrated potent anti-apoptotic properties in the context of MS.

The first concerns the modulation of apoptotic pathways. Recent research demonstrated that conditioned medium derived from human amniotic epithelial stem cells (hAEC-CM), in combination with ponesimod, can effectively modulate apoptotic pathways in oligodendrocytes. This combination treatment reduced the expression of pro-apoptotic proteins such as caspase-3, caspase-8, and Bax, while increasing the expression of the anti-apoptotic protein Bcl-2, ultimately elevating the Bcl-2/Bax ratio. This suggests that stem cell-derived factors, in conjunction with pharmacological agents, could provide an effective strategy to prevent programmed cell death in oligodendrocytes, which is a key pathological feature of MS lesions (Safaeinejad et al., 2021).

Stem cells and their products may also inhibit the activation of caspases, key enzymes in the apoptotic cascade, and MSC-derived exosomes have shown anti-inflammatory effects in other injury models; for example, Li et al. (2016) found that exosomes from human umbilical cord MSCs attenuated burn-induced excessive inflammation through miR-181c. Direct evidence that such exosomes inhibit neuronal caspase activation in MS models is lacking.

Stem cells also appear to stabilise mitochondria in stressed neurons, preventing the release of pro-apoptotic factors. Recent studies indicate that mesenchymal stem cells (MSCs) can transfer functional mitochondria to damaged neurons, rescuing them from apoptosis. For instance, Hayakawa et al. (2016) demonstrated that astrocytes transfer mitochondria to neurons after stroke, promoting neuronal survival and recovery. Additionally, Islam et al. (2012) reported that MSCs can donate mitochondria to pulmonary alveoli, protecting against acute lung injury. On this evidence, mitochondrial transfer via stem cells is a candidate therapeutic strategy for neurodegenerative diseases characterised by mitochondrial dysfunction.

3.3. Oxidative Stress Modulation

Stem cells contribute to neuroprotection by modulating oxidative stress, a key contributor to neuronal damage in MS.

One mechanism is the upregulation of antioxidant enzymes. Stem cells, especially MSCs, have been shown to upregulate antioxidant enzymes such as superoxide dismutase (SOD) and catalase in the CNS, thereby mitigating oxidative stress-induced neuronal injury (Witherick et al., 2011). Recent studies have explored the use of genetically modified stem cells overexpressing these antioxidant enzymes to enhance their neuroprotective effects in MS models (Wang et al., 2014).

Stem cells and their secreted factors may also help to reduce oxidative damage to neurons and other CNS cells. Shalaby et al. (2016) showed that adipose-derived MSCs modulated the immune response in a chronic EAE model, but whether MSCs or their vesicles directly scavenge free radicals in the CNS has not been established.

Recent research has shown that stem cells can activate the Nrf2 pathway, a master regulator of antioxidant responses, in CNS cells. Activation of this pathway leads to the expression of numerous antioxidant and detoxifying enzymes, providing broad-spectrum protection against oxidative stress (Mohammadzadeh et al., 2014).

3.4. Mitochondrial Function and Energy Metabolism

Recent studies have highlighted the role of stem cells in promoting mitochondrial function and energy metabolism in neurons affected by MS.

One mechanism is mitochondrial transfer. Cells can transfer healthy mitochondria to stressed neighbouring cells through tunnelling nanotubes or extracellular vesicles, as shown for astrocytes and neurons after stroke (Hayakawa et al., 2016) and for bone marrow-derived stromal cells and lung epithelium (Islam et al., 2012). Whether transplanted stem cells rescue neurons in this way in MS, and whether such transfer can occur over long distances in the CNS, remains to be demonstrated.

Stem cell-derived factors, particularly those secreted by mesenchymal stem cells (MSCs), can also reprogram the metabolic state of neural and glial cells. One of the key mechanisms underlying this effect involves the activation of the AMP-activated protein kinase (AMPK) pathway, a central regulator of cellular energy homeostasis. By activating AMPK, MSCs reduce the production of pro-inflammatory mediators and oxidative stress markers in microglial cells, which are often implicated in neuroinflammatory and neurodegenerative processes. This shift not only suppresses neuroinflammation but also promotes neuronal survival and metabolic adaptation under conditions of cellular stress. Such metabolic reprogramming, especially through AMPK signalling, offers a promising therapeutic target for neurological diseases characterised by chronic inflammation and metabolic dysfunction (Cao et al., 2019).

Stem cells can also promote the generation of new mitochondria in neurons through the activation of key transcription factors such as PGC-1α. This increase in mitochondrial mass can enhance neuronal energy production and resilience.

3.5. Immunomodulation-Mediated Neuroprotection

The immunomodulatory properties of stem cells also contribute indirectly to their neuroprotective effects.

One route is modulation of the microglial phenotype. By dampening the inflammatory response and creating a more favourable immune environment in the CNS, stem cells can reduce the neurotoxic effects of prolonged inflammation. This includes the modulation of microglial activation states, shifting them from a pro-inflammatory to a more neuroprotective phenotype (Barati et al., 2019). Moreover,

mesenchymal stem cells directly influence microglial function through the secretion of transforming growth factor-beta (TGF-β), making clear their potential therapeutic value in neuroinflammatory conditions (Noh et al., 2016).

Stem cell-derived exosomes have been shown to modulate T cell responses by transferring specific microRNAs, leading to the induction of regulatory T cells (Tregs) and suppression of autoreactive T cell activity. For instance, mesenchymal stem cell (MSC)-derived exosomes can deliver immunomodulatory microRNAs that influence T cell differentiation and function, thereby providing a novel mechanism for their immunomodulatory and neuroprotective effects. These findings make stem cell-derived exosomes worth pursuing as a therapeutic strategy in autoimmune diseases such as multiple sclerosis (Ma et al., 2020).

Stem cells can also help maintain the integrity of the BBB, reducing the infiltration of inflammatory cells and molecules into the CNS. Recent research has shown that stem cell-derived factors, such as tissue inhibitor of metalloproteinase-3 (TIMP-3), can enhance BBB function and provide neuroprotection in MS models (Menge et al., 2012).

3.6. Extracellular Vesicle-Mediated Neuroprotection

Stem cell-derived extracellular vesicles (EVs) have been investigated as potent mediators of neuroprotection in MS.

EVs can deliver microRNAs that promote neuronal survival; for example, Li et al. (2019) found that increased miR-124-3p in microglial exosomes conferred neuroprotection by targeting FIP200-mediated neuronal autophagy after traumatic brain injury. Neuroprotective miRNA cargo in MSC-derived EVs has not yet been characterised in MS models.

Their protein cargo matters as well: stem cell-derived EVs contain various proteins with neuroprotective properties. Recent proteomic analyses have identified novel EV-associated proteins, such as galectin-1 and peroxiredoxins, that contribute to their neuroprotective effects (Keerthikumar et al., 2016).

EVs can also carry bioactive lipids, including specialized pro-resolving mediators (SPMs), which have been proposed to promote the resolution of neuroinflammation and to provide neuroprotection; this possibility has not yet been tested directly in MS models.

3.7. Promoting Neural Circuit Repair and Plasticity

Stem cells can enhance neural circuit repair and plasticity, contributing to their neuroprotective effects.

One aspect concerns axonal sprouting. While stem cell-derived factors generally promote axonal regeneration, certain molecules such as Semaphorin 3A (Sema3A) have been found to negatively impact axonal growth and dendritic organization when overexpressed. Elevated levels of Sema3A may lead to axonal retraction and aberrant dendritic arborization, thereby impairing neural circuit formation and connectivity (Ferretti et al., 2022).

Second is synapse formation. Stem cells can promote the formation of new synapses and the strengthening of existing ones, enhancing neural circuit function.

By secreting factors that promote neuroplasticity, such as brain-derived neurotrophic factor (BDNF), stem cells can enhance the brain's ability to compensate for damage and maintain function. Recent research has shown that stem cell-derived exosomes containing high levels of BDNF can promote neuroplasticity and cognitive function in MS models.

The neuroprotective effects of stem cell therapy in MS are heterogeneous and involve complex interactions between transplanted cells, endogenous neural cells, and the CNS microenvironment. By leveraging these various mechanisms, stem cell-based approaches offer a holistic strategy for protecting neurons from damage, promoting repair, and potentially slowing or halting the progression of neurodegeneration in MS.

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4.5. PRECLINICAL STUDIES: ANIMAL MODELS AND IN VITRO STUDIES

Multiple Sclerosis (MS) stands as one of the most prevalent chronic inflammatory diseases of the central nervous system (CNS), characterised by demyelination, neurodegeneration, and an interaction between the immune system and neural cells (Mao et al., 2022; Procaccini et al., 2015). This debilitating condition affects millions worldwide, presenting with a wide array of neurological symptoms and progressive disability. The aetiology of MS remains complex, involving genetic predisposition, environmental factors, and autoimmune processes that lead to the destruction of myelin and subsequent neuronal damage in the brain, spinal cord, and optic nerves. Figure 4.5.1 compares the models used in this field and the question each can answer.

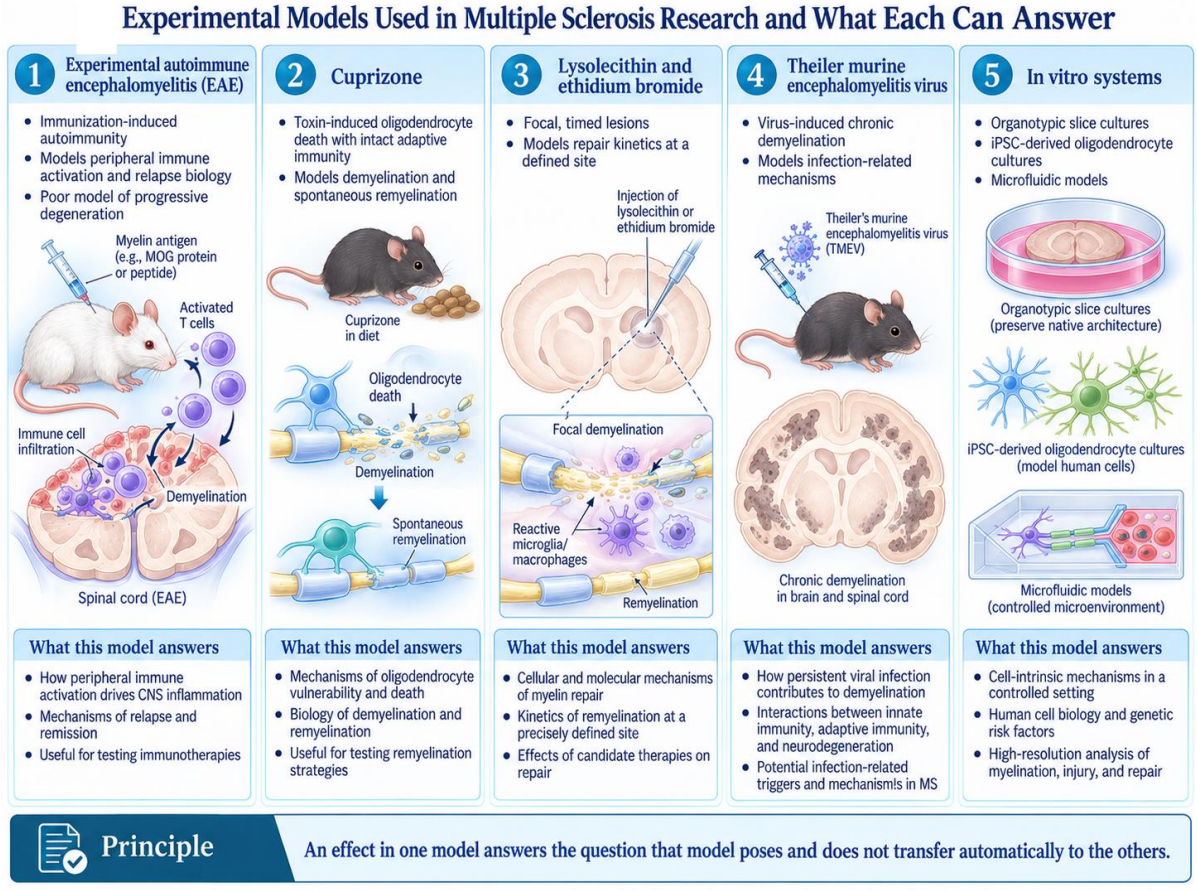


Figure 4.5.1. Experimental models used in multiple sclerosis research and the question each model can answer. An effect obtained in one model does not transfer automatically to the others.

Preclinical studies are central to investigating MS pathophysiology and evaluating candidate interventions. Animal models, in vitro systems, and molecular analyses address different questions and should be interpreted according to their specific strengths and limitations. See Figure 4.5.2.

Animal models, cellular systems, and molecular experiments answer different questions about MS. Their value depends on whether the model captures the process being studied and whether the reported endpoint is relevant to the proposed clinical use.

1. Animal Models in MS Research

Animal models have been instrumental in advancing our understanding of MS pathogenesis and in the preclinical evaluation of potential therapeutic interventions. These models aim to recapitulate various aspects of MS pathology, including inflammation, demyelination, and neurodegeneration. While no single model perfectly mimics the complexity of human MS, each offers unique insights into specific disease mechanisms and treatment strategies.

1.1. Experimental Autoimmune Encephalomyelitis (EAE)

Experimental Autoimmune Encephalomyelitis (EAE) remains the most widely used and well-established animal model for studying MS. This model, typically induced in rodents, mimics key aspects of MS pathology, including immune-mediated demyelination and neurodegeneration in the CNS (Zhang et al., 2013). The induction of EAE often involves immunization with myelin components, such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), or proteolipid protein (PLP), combined with adjuvants to stimulate an autoimmune response against myelin (Shahzadeh Fazeli et al., 2010).

EAE has been instrumental in elucidating various facets of MS pathogenesis and in the preclinical evaluation of potential therapeutic interventions. For instance, studies utilising the EAE model have provided insights along several lines.

One line of work concerns cellular mechanisms. Research by Mc Guire et al. (2010) demonstrated that oligodendrocyte-specific deletion of the Fas-associated death domain (FADD) protein protects mice from autoimmune-mediated demyelination in EAE. This finding highlights the critical role of oligodendrocyte apoptosis in the pathogenesis of demyelinating diseases like MS.

The model has also served immunomodulation research: the regulatory role of natural killer cells in MS has been explored using the EAE model. Takahashi et al. (2004) showed that these cells play an important part in modulating the autoimmune response targeting myelin components, suggesting potential therapeutic strategies focusing on natural killer cell manipulation.

Studies investigating neuroprotective agents have likewise used the EAE model to assess their efficacy. Candidate agents are often characterised first in other settings; for example, Xue et al. (2010) reported neuroprotective effects of minocycline in cell culture and in a mouse model of intracerebral haemorrhage, findings that cannot be transferred directly to EAE or MS.

The EAE model has also been important in studying remyelination processes and potential therapies to enhance myelin repair. Aharoni et al. (2008) used EAE to investigate the effects of glatiramer acetate on promoting remyelination and arresting demyelination, providing insights into potential regenerative therapies for MS.

It has been instrumental, too, in testing innovative treatment strategies. For example, Mohammadzadeh et al. (2016) evaluated the use of adipose-derived mesenchymal stem cells (AD-MSCs) as a vehicle for interferon-β delivery in EAE, demonstrating early findings in ameliorating disease progression.

Recent advancements in EAE models have focused on developing more targeted approaches to induce disease. For instance, Krishnamoorthy et al. (2006) developed a spontaneous EAE model in transgenic mice expressing T cell and B cell receptors specific to MOG, providing insights into the role of B cells in MS pathogenesis. This model has been particularly valuable in studying the interplay between T cells and B cells in driving autoimmune responses in CNS.

Moreover, the use of humanized EAE models has gained traction in recent years. These models involve the expression of human MS-associated genes in mice or the engraftment of human immune cells into immunodeficient mice. For example, Madsen et al. (1999) developed a humanized EAE model expressing the human HLA-DR15 allele, a major genetic risk factor for MS. This model has provided valuable insights into the role of specific genetic factors in MS susceptibility and has been used to evaluate targeted therapies.

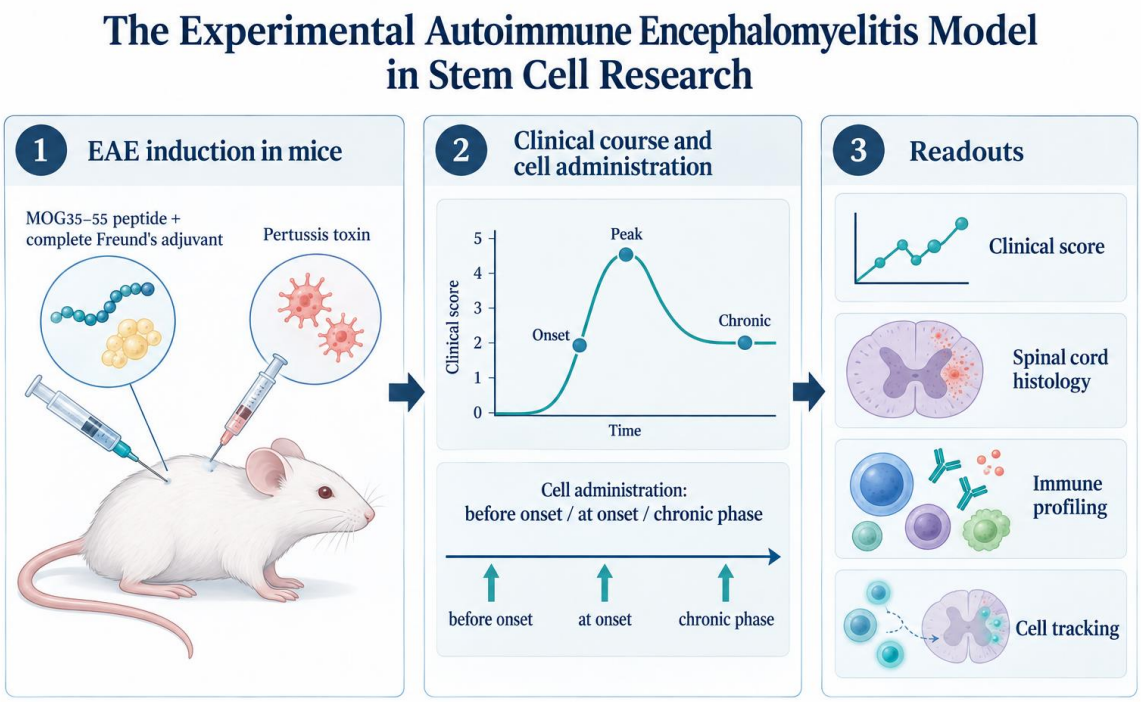


Figure 4.5.2. The experimental autoimmune encephalomyelitis (EAE) model as used in stem cell research: induction, clinical course, timing of cell administration and principal readouts.

1.2. Cuprizone-Induced Demyelination Model

While EAE primarily models the inflammatory aspects of MS, the cuprizone-induced demyelination model focuses on the demyelination and remyelination processes without significant T cell involvement. This model involves feeding mice with the copper chelator cuprizone, which results in oligodendrocyte death and subsequent demyelination, particularly in the corpus callosum (Praet et al., 2014).

The cuprizone model has been valuable in several areas of study.

The first is remyelination dynamics. The cuprizone-induced demyelination model has played a critical role in understanding remyelination and related neurobehavioral outcomes in multiple sclerosis (MS). Lei et al. (2023) employed this model to evaluate the therapeutic effects of bone marrow-derived mesenchymal stem cells (BM-MSCs) on myelin repair and emotional disturbances triggered by demyelination. Their results demonstrated that BM-MSC transplantation not only facilitated remyelination but also alleviated anxiety- and depression-like behaviours in mice, possibly through modulation of monoaminergic neurotransmitter systems such as norepinephrine and serotonin. These findings support the potential of BM-MSCs as a multimodal therapeutic strategy addressing both neurological damage and mood-related symptoms in MS.

Zarini et al. (2022) explored the glial response to intranasal mesenchymal stem cell delivery in an intermittent cuprizone model, providing insights into non-invasive treatment approaches and their impact on glial cells and myelin recovery.

The third is combination therapy. Recent studies have explored the synergistic effects of combining mesenchymal stem cell (MSC) transplantation with transcranial direct current stimulation (tDCS) to enhance remyelination in the cuprizone-induced demyelination model. This combination therapy has been shown to significantly improve motor coordination and balance, increase oligodendrocyte populations, and reduce apoptosis, supporting further investigation for myelin repair strategies in multiple sclerosis (MS) (Madadi et al., 2022).

Advancements in the cuprizone model have also focused on developing chronic demyelination paradigms to better mimic the progressive nature of MS. Bai et al. (2016) described a cuprizone-based mouse model designed for testing remyelinating therapies.

Furthermore, the combination of cuprizone-induced demyelination with subsequent experimental autoimmune encephalomyelitis (EAE) induction has developed into a

powerful tool for studying the interplay between demyelination and inflammation in MS. Rüther et al. (2017) demonstrated that this combined model results in enhanced inflammation and exacerbated clinical symptoms, offering insights into the mechanisms underlying disease progression in MS.

1.3. Viral Models of MS

Viral models of MS have gained increasing attention in recent years, given the growing evidence supporting a potential role of viral infections in MS aetiology. These models aim to recapitulate the viral-induced inflammation and demyelination observed in some MS patients.

The first is the Theiler's murine encephalomyelitis virus model. The TMEV model has been extensively used to study viral-induced demyelination and chronic progressive neurological disease. Olson et al. (2001) demonstrated that TMEV infection in susceptible mouse strains leads to persistent CNS infection, chronic inflammation, and progressive demyelination, mirroring aspects of progressive MS.

The mouse hepatitis virus (MHV) model, particularly the neurotropic strain MHV-A59, has been valuable in studying virus-induced demyelination and remyelination processes. Bender and Weiss (2010) showed that MHV infection leads to acute encephalitis followed by chronic demyelination, providing insights into the mechanisms of viral-induced myelin damage and repair.

Recent work on viral models has also considered human-specific viruses. Virtanen and Jacobson (2012) reviewed the evidence linking viruses, including human herpesvirus 6 (HHV-6), to MS pathogenesis. Experimental systems that reproduce infection with such human-specific viruses could help clarify their potential role in MS-like pathology.

1.4. Toxin-Induced Demyelination Models

In addition to cuprizone, other toxin-induced models have been developed to study specific aspects of demyelination and remyelination.

The first is the lysolecithin model. The focal injection of lysolecithin into white matter tracts induces rapid demyelination followed by spontaneous remyelination. Jeffery and Blakemore (1995) demonstrated the utility of this model in studying the cellular dynamics of remyelination, particularly the roles of oligodendrocyte precursor cells and astrocytes in myelin repair.

Similar to lysolecithin, focal injection of ethidium bromide induces demyelination, but it also kills astrocytes within the lesion. BLAKEMORE (1982) characterised ethidium bromide-induced demyelination in the cat spinal cord, and the model has since been used to study remyelination in lesions depleted of astrocytes.

Recent developments in toxin-induced models have combined these approaches with genetic tools to study the cellular sources of remyelination. For example, Zawadzka et al. (2010) used genetic fate mapping in mice to show that CNS-resident glial progenitor cells generate remyelinating Schwann cells as well as oligodendrocytes after CNS demyelination, challenging long-held assumptions about the cellular sources of myelin repair in the CNS.

1.5. Transgenic Models

Genetically modified mice expressing specific myelin antigens or altered immune components have provided insights into the genetic factors contributing to MS susceptibility and progression.

In the 2D2 TCR transgenic model, Bettelli et al. (2003) developed mice expressing a T cell receptor specific for MOG, providing a valuable tool for studying the activation and regulation of myelin-specific T cells in MS.

A second comprises humanized HLA transgenic models. Madsen et al. (1999) created mice expressing human HLA-DR15, a major genetic risk factor for MS, offering insights into how specific HLA alleles contribute to disease susceptibility.

A third comprises conditional knockout models, in which myelin-related genes can be inactivated in specific cells at defined times, allowing myelin maintenance and repair to be studied in a temporally controlled manner.

Recent advancements in transgenic models have leveraged CRISPR-Cas9 technology to create more precise genetic modifications, which may allow MS-associated risk variants to be introduced into mice so that their functional consequences can be studied.

1.6. Primate Models

While rodent models have been the mainstay of MS research, primate models offer unique advantages due to their closer evolutionary relationship to humans.

The first is the marmoset EAE model. EAE in common marmosets reproduces several features of MS immunopathology, including cortical lesions and a contribution of B cells; 't 't Hart et al. (2011) discussed the strengths and limitations of this and other EAE models.

In rhesus monkeys, EAE is typically acute and often fatal, which limits its value for modelling chronic disease; Haanstra et al. (2015) used this model to show that

selective blockade of CD28-mediated T cell costimulation protected against acute fatal EAE.

These primate models offer a valuable platform for preclinical evaluation of advanced therapeutic approaches, particularly those targeting human-specific pathways or involving complex drug delivery systems.

The diverse array of animal models available for MS research provides a broad toolkit for investigating various aspects of disease pathology and evaluating potential therapeutic interventions. While each model has its limitations, the complementary use of multiple models, along with the continuous refinement and development of new models, offers a robust approach to advancing our understanding of MS and developing more effective treatments.

2. In Vitro Studies and Cellular Mechanisms

Complementing animal models, in vitro studies have been key in elucidating cellular and molecular mechanisms underlying MS pathology. These investigations provide controlled environments to study specific cell types, signalling pathways, and potential therapeutic targets. The advent of advanced cell culture techniques, including 3D cultures and organoids, has further enhanced the relevance and complexity of in vitro MS models.

2.1. Oligodendrocyte Biology and Myelination

Oligodendrocytes, the myelin-producing cells of the CNS, are central to MS pathology. In vitro studies have significantly advanced our understanding of oligodendrocyte biology and myelination processes.

One focus is the oligodendrocyte precursor cell. Research by Goldman and Kuypers (2015) has characterised the behaviour and potential of OPCs in remyelination, using in vitro cultures to study factors influencing their differentiation and myelinating capacity. They demonstrated that OPCs retain considerable plasticity and can be directed towards enhanced remyelination through manipulation of specific signalling pathways.

Simons and Nave (2015) used advanced in vitro techniques to work out the molecular mechanisms of myelin formation and maintenance. Their work revealed the interaction between axons and oligodendrocytes in establishing and maintaining myelin sheaths, providing insights into potential targets for promoting remyelination in MS.

Building on the work of Fang & Bai (2023), further in vitro studies have investigated the cellular mechanisms leading to oligodendrocyte death in multiple sclerosis. Blagov et al. (2022) identified mitochondrial dysfunction as a key contributor to oligodendrocyte vulnerability. Their findings highlight how mitochondrial DNA damage, oxidative stress, and impaired ATP production compromise the survival of oligodendrocytes under MS-like conditions. These insights offer possible routes for developing neuroprotective strategies aimed at preserving myelin-producing cells in the CNS.

Recent advancements in oligodendrocyte research have leveraged induced pluripotent stem cell (iPSC) technology to generate patient-specific oligodendrocytes. Mozafari et al. (2020) developed a protocol for generating mature oligodendrocytes from MS patient-derived iPSCs, providing a powerful tool for studying disease mechanisms and screening potential therapies in a patient-specific context.

2.2. Immune Cell Interactions

In vitro models have been instrumental in studying the complex interactions between immune cells and CNS components in MS.

One focus is T cell activation. In vitro systems have been used extensively to investigate the activation and regulation of autoreactive T cells, and Baecher-Allan et al. (2018) reviewed how these mechanisms inform immunotherapy for MS.

In vitro models of microglial activation have likewise helped elucidate the role of these cells in neuroinflammation and potential strategies for modulating their activity in MS (Voet et al., 2019).

In vitro BBB models have been developed to study the mechanisms of immune cell infiltration into the CNS and potential strategies to maintain BBB integrity in MS; Lopes Pinheiro et al. (2016) reviewed immune cell trafficking across the barriers of the CNS in MS and stroke.

Recent advances in immune cell research have focused on the role of B cells in MS pathogenesis. Jelcic et al. (2018) used in vitro co-culture systems to show that memory B cells from MS patients can activate brain-homing, autoreactive CD4+ T cells, which supports the rationale for B cell-targeted therapies in MS. Li et al. (2018) reassessed the contributions of B cells to MS, including antibody-independent functions such as antigen presentation and cytokine production.

The role of astrocytes in MS pathology has gained increasing attention in recent years. Ponath et al. (2018) reviewed the role of astrocytes in MS, including their interactions with immune cells and their capacity to adopt neurotoxic phenotypes in response to inflammatory stimuli. This

work has been complemented by Wheeler et al. (2020), who combined single-cell transcriptomics with experimental studies to identify MAFG-driven astrocyte populations that promote CNS inflammation in EAE and MS.

Extracellular vesicle-mediated communication is another. The role of extracellular vesicles (EVs) in cell-to-cell communication in MS has come to be seen as an active area of research. Sáenz-Cuesta et al. (2014) reviewed early evidence on EVs in MS, including altered EV profiles in patients. Lombardi et al. (2019) showed in experimental systems that EVs released by microglia can exert both detrimental and protective actions on myelin lesions, with astrocytes contributing to remyelination failure.

2.3. Molecular Pathways and Therapeutic Targets

In vitro studies have been essential in identifying and validating molecular pathways and potential therapeutic targets in MS.

One such pathway is PI3K/Akt/mTOR. In vitro studies have been instrumental in elucidating molecular pathways and identifying potential therapeutic targets in multiple sclerosis (MS), and the PI3K/Akt/mTOR cascade has emerged as a candidate target in oligodendrocyte biology and neuroinflammation. Mammana et al. (2018) reviewed the role of macrophages in the neuroinflammatory and neurodegenerative pathways shared by Alzheimer's disease, amyotrophic lateral sclerosis, and MS.

Building upon this foundation, Shi et al. (2025) demonstrated that gastrodin, a compound derived from traditional Chinese medicine, promotes oligodendrocyte maturation and remyelination in both in vitro and in vivo models. Their study revealed that gastrodin activates the PI3K/Akt/mTOR signalling pathway, enhancing myelin sheath formation and offering a possible route for therapeutic intervention in demyelinating conditions such as MS.

Turning to microRNA regulation, Aljawadi et al. (2016) investigated the expression levels of specific microRNAs, miR-20a, miR-146a, miR-155, and miR-145, in the peripheral blood leukocytes of patients with multiple sclerosis (MS). Their study revealed a significant downregulation of miR-20a and an upregulation of miR-155 in MS patients compared to healthy controls, suggesting these microRNAs play important roles in the dysregulation of gene expression associated with MS pathogenesis. No significant changes were observed for miR-146a and miR-145, indicating a more nuanced role for these microRNAs in MS. These findings contribute to the growing body of evidence that microRNAs are integral to the molecular mechanisms underlying MS and may serve as potential biomarkers or therapeutic targets.

On mitochondrial dysfunction, Zambonin et al. (2011) reported increased mitochondrial content in remyelinated axons, with implications for understanding metabolic alterations in MS progression. Witte et al. (2013) found that reduced expression of PGC-1α partly underlies mitochondrial changes and correlates with neuronal loss in the MS cortex; in vitro systems are now being used to test compounds that might rescue such mitochondrial dysfunction.

Fourth concerns neuroprotective compounds. Candidate neuroprotective agents such as minocycline have been evaluated in cell culture (Xue et al., 2010), and fingolimod entered clinical development after early human studies in renal transplantation (Budde et al., 2002). Faissner et al. (2019) reviewed the pathophysiology of progressive MS and therapeutic strategies, emphasizing the need for more physiologically relevant multicellular systems for screening neuroprotective compounds.

2.4. Advanced In Vitro Models

Recent years have seen the development of increasingly sophisticated in vitro models that better recapitulate the complexity of the CNS environment.

Lancaster and Knoblich (2014) reviewed the development of organoid technologies, including brain organoids, which have since been adapted for MS research. Madhavan et al. (2018) induced myelinating oligodendrocytes in human cortical spheroids derived from pluripotent stem cells, providing a 3D platform in which myelination, and potentially MS-relevant processes, can be studied in a human-specific environment.

MS-specific human models have advanced further in 2026. Patient-derived cerebral and spinal-cord organoids showed impaired oligodendrocyte maturation and myelination, particularly in primary progressive MS, with p21/PAK1/E2F1 dysregulation implicated in the phenotype (Sadiq et al., 2026). Separately, a human iPSC-derived spheroid model containing myelinating oligodendrocytes and reactive microglia reproduced myelin injury, debris clearance, oligodendrocyte generation, and remyelination after lysolecithin exposure (Ramesan et al., 2026). These systems improve mechanistic and drug-screening relevance but remain experimental models rather than evidence of therapeutic efficacy in patients.

Park et al. (2019) developed a microfluidic BBB model using induced pluripotent stem cell-derived human brain microvascular endothelium. This model mimicked in vivo BBB characteristics and allowed for reliable in vitro drug permeability studies under recirculating perfusion. A recent study (Berjaoui et al., 2024) introduced a "Neuroinflammation-on-a-Chip" model to study MS. This model integrates multiple cell types and allows for real-time monitoring of neuroinflammation and demyelination processes, providing insights into MS development and potential therapeutic approaches.

Bioengineered axon tracts combine human iPSC-derived neurons and oligodendrocytes to model myelinated tracts. Such systems allow myelination and demyelination to be studied in a controlled environment and could provide tools for screening remyelinating therapies.

2.5. Single-Cell Technologies in MS Research

The advent of single-cell technologies has substantially changed our ability to study cellular heterogeneity in MS.

Jäkel et al. (2019) used single-nucleus RNA sequencing of post-mortem white matter from MS patients and controls to reveal altered heterogeneity of oligodendrocyte subpopulations in MS. Schirmer et al. (2019) combined single-nucleus RNA sequencing with in situ validation in MS lesions, revealing selective vulnerability of upper-layer cortical neurons and lineage-specific changes in glial cells, including oligodendrocytes.

Single-cell proteomic approaches such as mass cytometry are increasingly used to characterize immune and glial cell populations relevant to MS. Combining single-cell proteomics with functional assays may help map changes in immune cell phenotypes during MS progression and in response to therapy.

Single-cell epigenomic technologies are transforming our understanding of immune dysregulation in multiple sclerosis (MS). In a landmark study, de la Fuente et al. (2020) applied single-cell ATAC-seq to map chromatin accessibility in peripheral immune cells from MS patients. Their analysis revealed distinct epigenetic states and transcription factor activity patterns in monocytes and T cells, many of which were enriched in genomic loci associated with MS susceptibility. These findings provide critical insight into how genetic risk variants may shape cell type–specific regulatory programs in MS. Subsequent studies are now expanding upon this work by integrating single-cell chromatin accessibility with transcriptomic and proteomic data to build a full atlas of MS-related immune alterations at single-cell resolution.

3. Molecular and Genetic Investigations

Molecular and genetic studies have significantly contributed to our understanding of MS pathogenesis, identifying potential biomarkers and genetic factors associated with disease susceptibility and progression. The advent of high-throughput sequencing technologies and advanced bioinformatics approaches has substantially changed this field, allowing for detailed analyses of the genetics and molecular biology of MS.

3.1. Genetic Factors in MS

The complex genetics of MS has been a subject of intensive research.

The complex genetic architecture of multiple sclerosis (MS) has been extensively investigated through genome-wide association studies (GWAS). A major study conducted by the International Multiple Sclerosis Genetics Consortium identified 233 genetic variants significantly associated with MS susceptibility, implicating various immune pathways, including those involving peripheral immune cells and microglia, in the pathogenesis of the disease (International Multiple Sclerosis Genetics Consortium, 2019).

The strong association between specific HLA alleles and MS risk has been well established. Moutsianas et al. (2015) showed that class II HLA interactions modulate genetic risk for MS, further refining our understanding of these associations. Menegatti et al. (2021) reported that HLA-DRB1*15:01 can act as a co-receptor for Epstein–Barr virus, linking genetic and environmental risk factors for MS.

Research by Koch et al. (2013) has explored epigenetic modifications in MS, highlighting the role of DNA methylation and histone modifications in disease pathogenesis and potential therapeutic targets. Recent advances in this field include the work of Kular et al. (2018), who performed an integrated analysis of genetic and epigenetic factors in MS, revealing complex interactions between genetic variants and epigenetic marks that influence disease risk and progression.

The role of non-coding RNAs, particularly long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), in MS pathogenesis has gained increasing attention. Recent studies have identified several lncRNAs associated with disease activity and progression. For instance, Ding et al. (2021) constructed a lncRNA-associated competing

endogenous RNA (ceRNA) network in MS, identifying hub lncRNAs that may play critical roles in disease mechanisms. Additionally, research has characterised specific circRNAs involved in oligodendrocyte differentiation and myelination, revealing potential new targets for promoting remyelination in MS.

3.2. Biomarker Discovery

The identification of reliable biomarkers for MS diagnosis, prognosis, and treatment response has been a focus of preclinical research.

Cerebrospinal fluid biomarkers are one group. Teunissen et al. (2015) reviewed body fluid biomarkers for MS, including neurofilament light chain (NfL) and chitinase-3-like protein 1 (CHI3L1), which may aid in diagnosis and monitoring of disease progression, and Varhaug et al. (2019) reviewed the evidence for NfL as a biomarker in MS.

Among serum biomarkers, Disanto et al. (2017) showed that serum NfL levels reflect neuronal damage and are associated with disease activity and treatment in MS. Khalil et al. (2018) reviewed neurofilaments as biomarkers across neurological disorders, and combinations of NfL with other markers are being explored to improve the prediction of disease progression.

Building on the work of Aljawadi et al. (2016), further studies have explored microRNA profiles as potential biomarkers for MS diagnosis and prognosis. Keller et al. (2009) found that microRNA expression profiles in blood accurately differentiated patients with relapsing-remitting MS from healthy controls.

Metabolomic biomarkers complete this picture. Metabolomics has become an active approach for biomarker discovery in multiple sclerosis (MS). Bhargava and Calabresi (2016) reviewed metabolomic studies in MS, which have reported alterations in amino acid metabolism, energy pathways, and lipid profiles that may yield biomarkers for disease monitoring and therapeutic targeting.

3.3. Molecular Mechanisms of Disease Progression

Preclinical studies have elucidated various molecular mechanisms contributing to MS progression.

Research by Haider et al. (2011) has highlighted the role of oxidative stress in MS pathology, particularly in driving neurodegeneration and mitochondrial dysfunction. Recent studies by Lassmann and van Horssen (2016) have further characterised the interaction between oxidative stress, mitochondrial dysfunction, and neurodegeneration in MS, identifying potential targets for neuroprotective interventions.

Sorbara et al. (2014) revealed pervasive axonal transport deficits in MS models, providing insights into the mechanisms of axonal degeneration and potential neuroprotective strategies. Sleigh et al. (2019) reviewed the role of axonal transport defects across neurological diseases and the tools available to study them.

Kotter et al. (2011) reviewed factors that inhibit remyelination in MS lesions and strategies to enhance repair. Keough et al. (2016) targeted one such factor, showing that an inhibitor of chondroitin sulfate proteoglycan synthesis promoted CNS remyelination in mice.

The role of chronic neuroinflammation and glial activation in driving MS progression has been a focus of recent research. Luo et al. (2017) reviewed the role of microglia in MS, including how chronic microglial activation may contribute to ongoing neurodegeneration. Complementing this work, Wheeler et al. (2020) used single-cell transcriptomics and experimental models to identify disease-promoting astrocyte states in EAE and MS, revealing potential targets for modulating glial responses.

The contribution of blood-brain barrier (BBB) dysfunction to MS pathogenesis has gained increasing attention, and Ortiz et al. (2014) reviewed the molecular players in BBB dysfunction in MS that could serve as therapeutic targets.

These molecular and genetic studies have significantly advanced our understanding of MS pathogenesis, offering new insights into disease mechanisms and potential therapeutic targets. The integration of multi-omics data, coupled with advanced bioinformatics approaches, is providing a broader view of the molecular biology of MS, supporting the development of personalised medicine approaches in MS treatment.

4. Emerging Therapeutic Approaches

Preclinical studies have provided a basis for numerous innovative therapeutic approaches in MS treatment, targeting various aspects of disease pathology from immune modulation to neuroprotection and remyelination. These emerging therapies offer hope for more effective and targeted treatments for MS patients.

4.1. Stem Cell Therapies

Stem cell-based therapies have shown promise in preclinical MS models.

Mesenchymal stem cells have been studied most extensively. Recent studies have confirmed the efficacy of MSCs in modulating immune responses and promoting remyelination in experimental autoimmune encephalomyelitis (EAE) models, which closely mimic key aspects of multiple sclerosis (MS). Yang et al. (2020) performed a systematic review and meta-analysis showing that MSC therapy significantly reduced clinical scores and CNS inflammation in EAE, with observable histological improvements and neuroprotective effects.

To enhance the therapeutic potential of MSCs, Liao et al. (2016) engineered MSCs to express both selectin ligands and interleukin-10 (IL-10), improving their homing to inflamed CNS tissues and increasing their immunomodulatory efficiency. These genetically modified MSCs demonstrated superior suppression of disease severity in murine EAE models compared to unmodified MSCs. This argues for optimising MSC-based therapy through targeted engineering in preclinical MS research.

Neural stem cells have been examined in the same way: Pluchino et al. (2003) showed that injection of adult neurospheres induced recovery in a chronic model of MS. Peruzzotti-Jametti et al. (2018) later reported that neural stem cells sense succinate released by inflammatory macrophages and use this signal to suppress chronic neuroinflammation.

For induced pluripotent stem cells, Thiruvalluvan et al. (2016) examined the survival and functionality of human iPSC-derived oligodendrocytes after transplantation in a nonhuman primate model of MS. Khazaei et al. (2017) reviewed the use of iPSCs for traumatic spinal cord injury, work that may inform cell-based remyelination strategies in MS.

4.2. Nanoparticle-Based Therapies

Nanoparticle-based approaches offer promising strategies for targeted drug delivery in MS.

One approach uses myelin antigen-coupled particles. Getts et al. (2012) showed that microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate EAE. Building on this work, Clemente-Casares et al. (2016) developed nanoparticles coated with disease-relevant peptide–MHC class II complexes, showing therapeutic efficacy across several autoimmune disease models, including EAE.

Dendrimer nanoparticles can target activated microglia and deliver anti-inflammatory agents to the CNS; Kannan et al. (2012) demonstrated this approach in a rabbit model of neuroinflammation and cerebral palsy, and its application to MS models remains to be established.

Moving beyond drug delivery, recent research has focused on engineering nanoparticles that mimic molecular cues required for oligodendrocyte differentiation and myelination. For instance, Zhao and Jacob (2023) discussed various strategies for enhancing remyelination in MS, including the use of nanoparticles to deliver therapeutic agents directly to the central nervous system. These approaches have shown promise in promoting remyelination in both in vitro and in vivo models of MS, offering a new paradigm for nanomedicine in MS treatment.

4.3. Gene Therapy Approaches

Preclinical studies have investigated various gene therapy strategies for MS treatment.

Building on the work of Doudna and Charpentier (2014) on CRISPR-Cas9 technology, studies have begun to explore its potential in MS, for example by editing T cells to reduce their pathogenic potential. This approach could open new avenues for personalised cell therapy in MS, but it remains experimental.

Early work by Furlan et al. (2003) explored the use of viral vectors for delivering therapeutic genes, specifically cytokines, into the central nervous system (CNS) via intrathecal injection in animal models of MS. These studies demonstrated the feasibility and immunomodulatory potential of gene transfer approaches in experimental autoimmune encephalomyelitis (EAE), a widely used MS model.

Recent advances have focused on adeno-associated virus (AAV)-mediated gene delivery for targeting neuroprotective factors to the CNS. A 2025 study (Tong et al., 2025) using AAV1.NT-3 gene therapy showed that targeted delivery of the neurotrophin-3 (NT-3) gene led to improved motor function, enhanced remyelination, and reduced disease severity in chronic EAE mice. This highlights the therapeutic potential of AAV vectors in progressive MS.

A third avenue is non-viral gene delivery. To address the safety concerns associated with viral vectors, non-viral systems based on biodegradable polymeric nanoparticles are being developed; the size and shape of such particles influence the immune responses they elicit (Kumar et al., 2015), which must be considered in any gene therapy application in MS.

4.4. Combination Therapies

Preclinical research has increasingly focused on combination therapies to address multiple aspects of MS pathology.

One approach combines immunomodulation with neuroprotection. Experimental studies have combined cell-based or immunomodulatory approaches with candidate neuroprotective agents. Reported changes in EAE or other preclinical models (Frid et al., 2024) should be described as activity observed under those experimental conditions, not as demonstrated clinical synergy or superior efficacy in MS patients.

A second targets multiple pathways at once. Experimental MSC effects include immunomodulatory and trophic signalling. Whether these mechanisms produce remyelination or disability improvement in patients requires direct clinical evidence (Wu et al., 2024).

Research by Madadi et al. (2022) has investigated the combined effects of astrocyte ablation and MSC transplantation in promoting remyelination and functional recovery in cuprizone-induced demyelination models, demonstrating the potential benefits of combining cellular therapies with targeted cell ablation approaches.

AHSCT is primarily an immune-reconstitution approach. Neural cell transplantation has a distinct experimental rationale involving support or replacement of neural lineages. Their evidence and risks should be assessed separately (Wu et al., 2024).

Mechanistic diversity supports testing defined products and hypotheses. It does not establish therapeutic efficacy or justify combining interventions without appropriate controlled evaluation (Wu et al., 2024).

While specific studies detailing a novel approach of sequential combination therapy involving initial aggressive immunosuppression followed by neuroprotective and remyelination-promoting agents are limited, the rationale for such strategies has been discussed. For instance, Costello et al. (2007) highlighted the scientific rationale for combination therapy in MS, suggesting that combining approved and experimental drugs may positively impact clinical disease activity and neurorepair.

4.5. Precision Medicine Approaches

The advent of high-throughput technologies and advanced data analytics has provided a basis for precision medicine approaches in MS treatment.

One such direction is pharmacogenomics, with studies examining genetic factors that influence treatment response in MS. Patient-level clinical factors also matter: Kowalec et al. (2017) found in a prospective study that comorbidity increases the risk of relapse in MS, underscoring the need to account for such factors when personalizing treatment.

For biomarker-guided therapy, Manouchehrinia et al. (2020) found that plasma neurofilament light levels are associated with the risk of disability in MS, and multi-modal panels combining fluid and imaging markers are being explored to support more personalised treatment strategies.

Gurevich et al. (2025) used machine learning to predict disease progression in primary progressive MS; extending such models to recommend individual treatment strategies will require prospective validation.

4.6. Regenerative Medicine Approaches

Beyond traditional pharmacological interventions, regenerative medicine approaches aim to restore lost function in MS.

Biomaterial scaffolds are one such approach, designed to provide a supportive environment for remyelination in the CNS. Their design is informed by findings such as those of Segel et al. (2019), who showed that increasing niche stiffness underlies the age-related decline in the function of CNS oligodendrocyte progenitor cells.

Osorio-Querejeta et al. (2020) showed that extracellular vesicles enriched in miR-219a-5p promoted oligodendrocyte progenitor cell differentiation and improved EAE, and engineered vesicles from other sources, including neural stem cells, are being explored as carriers of remyelination-promoting factors.

Recent regenerative-medicine work has also explored the potential of in situ reprogramming to generate oligodendrocytes from resident glial cells within demyelinated lesions. In a central study, Mokhtarzadeh Khanghahi et al. (2018) demonstrated that overexpression of the transcription factor Sox10 can reprogram astrocytes into oligodendrocyte-like cells in vivo, promoting remyelination in a demyelinated mouse model. This approach offers a possible route for treating progressive forms of MS by harnessing the plasticity of resident glial cells to restore myelin integrity.

5. Challenges in Translating Preclinical Findings

Despite the significant advances in preclinical MS research, translating these findings into effective clinical treatments remains a significant challenge. Several factors contribute to this translational gap. Wong (2025) describes the same pattern for MSC-based therapy in particular: animal studies report lower clinical scores, reduced lesion burden and

histological evidence of remyelination with some regularity, while the clinical studies that follow are smaller, differ in protocol and yield results that do not align.

While animal models of MS have been invaluable in understanding disease mechanisms and testing potential therapies, they do not fully recapitulate the complexity of human MS. Procaccini et al. (2015) highlighted the limitations of current EAE models in mimicking the chronic progressive phase of MS, which is particularly relevant for developing therapies for progressive MS forms.

The immune system and CNS biology differ substantially between rodents and humans, which limits how far preclinical findings can be carried over. Baker and Amor (2014) discussed the challenges in translating immunological findings from animal models to human MS, emphasizing the need for more human-relevant research tools.

Timing is a further problem. Many preclinical studies test prevention or very early intervention, whereas patients usually present with established disease. Mahad et al. (2015) emphasized the importance of developing and testing therapies in models of chronic, established MS to better mimic the clinical reality.

The outcome measures used in preclinical studies often do not directly correlate with clinically relevant endpoints in human MS trials. Vesterinen et al. (2010) conducted a systematic review highlighting the discrepancies between preclinical and clinical outcome measures in MS research, calling for more aligned and translatable endpoints.

The tendency to publish only positive results can lead to an overestimation of treatment effects in preclinical studies; Sena et al. (2010) demonstrated this in animal stroke studies, where publication bias led to a major overstatement of efficacy, and similar concerns apply to preclinical MS research (Vesterinen et al., 2010).

6. Future Directions and Emerging Technologies

To address these challenges and advance MS research, several promising directions and technologies are emerging.

The use of human induced pluripotent stem cells (iPSCs) to generate CNS cell types and organoids offers a more human-relevant system for studying MS pathology and testing potential therapies. Madhavan et al. (2018) generated oligodendrocyte-containing cortical spheroids from human pluripotent stem cells, and similar platforms derived from MS patients could support drug screening and personalised medicine approaches.

High-resolution imaging techniques allow more detailed study of MS pathology in both preclinical models and patients. Absinta et al. (2016) reviewed advanced MRI methods, including ultra-high-field imaging of the central vein sign, for staging MS lesions and potentially improving diagnostic accuracy.

Single-cell technologies offer unusual resolution in studying cellular heterogeneity in MS. Schirmer et al. (2019) used single-nucleus RNA sequencing to characterize cell-type-specific changes in MS lesions, including stress responses in oligodendrocytes, which may reveal new targets for promoting remyelination.

Fourth is artificial intelligence and machine learning. AI and machine learning approaches are increasingly being applied to analyse complex MS datasets. Eshaghi et al. (2021) developed a machine learning algorithm to predict MS progression from MRI data, demonstrating the potential of AI in improving prognostic accuracy and treatment planning.

Experimental autoimmune encephalomyelitis (EAE) serves as a key model for understanding multiple sclerosis (MS) pathophysiology. Traditional EAE models have significantly contributed to our knowledge of MS. However, they often fall short in fully replicating the human disease's complexity. Recent advancements have led to the development of humanized EAE models, which incorporate human immune components into mice, thereby providing a more accurate representation of MS. These models have been instrumental in elucidating the roles of human-specific antibodies and immune responses in MS progression. For instance, studies have demonstrated that antibodies derived from MS patients can exacerbate EAE in humanized mice, highlighting the pathogenic potential of these antibodies (Khare et al., 2018). Such work argues for humanized models in MS research and their potential in developing targeted therapies.

Microfluidic organ-on-a-chip systems allow for the study of complex cellular interactions in a controlled, human-relevant environment. Park et al. (2019) developed a hypoxia-enhanced blood-brain barrier chip using iPSC-derived brain microvascular endothelium that recapitulates human barrier function and the shuttling of drugs and antibodies; adapting such chips to study immune cell infiltration in MS is a logical next step.

The application of CRISPR technology for large-scale genetic screens in MS-relevant immune cell types offers new opportunities for identifying disease mechanisms and

therapeutic targets. For example, Shifrut et al. (2018) conducted a genome-wide CRISPR-Cas9 screen in primary human T cells, identifying key regulators of immune function. These findings highlight the power of CRISPR-based functional genomics in uncovering molecular players that could be targeted to modulate autoimmune responses in MS.

While significant questions remain open in translating preclinical findings to effective MS treatments, the field is moving quickly, with new technologies and approaches that promise to bridge the translational gap. By leveraging these advanced tools and adopting more human-relevant research paradigms, the future of MS research has investigational potentials for developing more effective, targeted, and personalised treatments for this complex and devastating disease.

New technologies are reshaping preclinical MS research. These advancements hold the promise of bridging the gap between laboratory findings and clinical applications, potentially leading to more effective treatments for MS patients.

6.1. Advanced Imaging Techniques

The development of sophisticated imaging technologies has substantially changed our ability to visualise and quantify MS pathology in both preclinical models and human patients.

Building on the work of Absinta et al. (2016), recent studies by Kilsdonk et al. (2016) have utilised 7 Tesla MRI to provide marked detail of MS lesion morphology and evolution. This high-resolution imaging has revealed subtle changes in lesion characteristics that correlate with clinical outcomes, offering new biomarkers for disease progression and treatment response.

Recent studies have developed novel PET tracers to visualise neuroinflammatory processes in MS models. For instance, Kuo et al. (2024) introduced [^18F]OP-801, a radiolabeled hydroxyl dendrimer that crosses the blood-brain barrier and is taken up by activated microglia and macrophages. In experimental autoimmune encephalomyelitis (EAE) models, this tracer enabled real-time tracking of microglial activation and lymphocyte infiltration, providing a powerful tool for assessing therapeutic efficacy in vivo.

Integrating multiple imaging modalities could provide a more complete picture of MS pathology. For example, Magon et al. (2020) showed in a longitudinal MRI study that volume loss in the deep grey matter and thalamic subnuclei is associated with disability progression in MS, and combining such structural measures with functional and molecular imaging, such as PET, is an active area of research.

6.2. Artificial Intelligence and Machine Learning

The application of AI and machine learning in MS research has expanded rapidly, offering new ways to analyse complex datasets and generate predictive models.

One such application is AI-driven image analysis. The application of artificial intelligence (AI) and machine learning (ML) in multiple sclerosis (MS) research has grown significantly, enabling more effective analysis of high-dimensional clinical and imaging datasets. Eshaghi et al. (2021) developed a machine learning framework using MRI data from thousands of MS patients to model individualized brain atrophy and lesion patterns. This approach enabled the creation of a data-driven subtyping system for MS, outperforming conventional classification based on clinical criteria alone. Such AI-driven tools can enhance diagnostic precision, predict disease progression, and optimise patient stratification for clinical trials, supporting progress toward personalised treatment strategies.

6.3. Single-Cell and Spatial Omics Technologies

Advancements in single-cell and spatial omics technologies have provided striking insights into the cellular and molecular complexity of MS.

Schirmer et al. (2019) employed single-nucleus RNA sequencing to analyse MS lesions, revealing selective vulnerability and loss of excitatory neurons in upper cortical layers, as well as stress responses in oligodendrocytes. Their study highlighted the diverse cellular responses within MS lesions, contributing to a deeper understanding of disease pathogenesis and identifying potential therapeutic targets.

In spatial biology, high-resolution transcriptomics can map gene expression patterns within tissue sections, an approach applicable to MS lesions. Applied to MS tissue, such methods have begun to map the spatial organization of immune and glial cells within lesions, offering new insights into lesion evolution and repair processes.

Pushing spatial resolution further, Vickovic et al. (2019) developed high-definition spatial transcriptomics for in situ tissue profiling at near-cellular resolution. Applying such methods to MS tissue could reveal localised gene expression changes associated with early myelin damage.

6.4. Organoid and Microphysiological Systems

The development of more complex, human-relevant in vitro models is providing new platforms for studying MS pathology and testing potential therapies.

Building on the foundational work of Madhavan et al. (2018), recent studies have advanced the development of brain organoids that incorporate multiple CNS cell types and recapitulate key features of MS pathology. Notably, Daviaud et al. (2023) derived cerebral organoids from induced pluripotent stem cells (iPSCs) of patients with various MS subtypes, including primary progressive MS (PPMS), secondary progressive MS (SPMS), and relapsing-remitting MS (RRMS). These patient-derived organoids offer a system for studying disease mechanisms and, potentially, for screening remyelinating therapies. The study highlighted a decrease in proliferation markers and oligodendrocyte differentiation, particularly in PPMS organoids, suggesting intrinsic cellular dysregulation linked to the disease's progression.

Building on organ-on-a-chip technology, microfluidic devices that expose brain endothelial barriers to inflammatory stimuli and immune cells could allow immune cell trafficking and barrier dysfunction to be monitored in real time (Park et al., 2019; Vatine et al., 2019). MS-specific versions of such chips would provide valuable tools for studying MS pathogenesis and testing BBB-targeted therapies.

6.5. Gene Editing and Cell Therapy Advancements

Recent developments in gene editing and cell therapy approaches are being investigated for additional roles for treating MS.

Building on advances in CRISPR–Cas9 technology, researchers have explored methods to enhance the function and specificity of regulatory T cells (Tregs). For instance, CRISPR–Cas9 has been utilised to edit genes within T cells to improve their adaptability to specific microenvironments, thereby enhancing their therapeutic potential in autoimmune diseases like MS (Chen et al., 2024). These CRISPR-modified Tregs demonstrated enhanced efficacy in modulating immune responses, suggesting a promising cell therapy approach for MS.

In vivo reprogramming for remyelination converts resident glial cells into oligodendrocyte-lineage cells (see Section 4.6); whether this can produce meaningful remyelination and functional recovery in chronic EAE models, and thus offer a strategy for progressive MS, remains to be established.

CRISPR-based transcriptional and epigenetic editing tools could, in principle, be used to selectively activate neuroprotective genes in CNS cells. Whether targeted epigenetic activation of specific genes can reduce neuroinflammation and promote repair in MS models remains to be tested.

6.6. Systems Biology and Network Medicine

The application of systems biology approaches to MS research is providing a more holistic understanding of disease mechanisms and potential therapeutic strategies.

The integration of systems biology approaches into multiple sclerosis (MS) research enabling a more detailed and network-level understanding of disease mechanisms. Cervantes-Gracia and Husi (2018) applied a multi-omics strategy, combining transcriptomic, proteomic, and genomic data to construct molecular interaction networks relevant to MS. Their analysis identified disease-specific modules and pathways, revealing key regulatory hubs involved in immune activation, neuroinflammation, and myelin degradation. This system-level framework not only supports the development of novel therapeutic hypotheses but also provides a platform for the identification of potential biomarkers and drug targets, ultimately contributing to more personalised and precise interventions in MS.

Drawing on large-scale genomic and proteomic datasets, Liu et al. (2024) used network analysis techniques to identify novel drug targets and repurpose opportunities for multiple sclerosis (MS). Their approach uncovered unexpected connections between MS and other diseases, leading to the identification of several FDA-approved drugs with potential efficacy in MS.

Advancing precision medicine in multiple sclerosis (MS) involves integrating individual patient data into large-scale molecular interaction networks to develop personalised therapeutic strategies. Recent research has emphasized the importance of incorporating patient-reported outcome measures (PROMs) into artificial intelligence (AI)-driven healthcare solutions for MS. Helme et al. (2025) discuss the challenges associated with utilising PROMs at scale, arguing for effective integration of patient input to enhance care quality and accelerate research and innovation.

The reliability and trustworthiness of AI models are critical in the context of MS research and care. Werthen-Brabants and Dhaene (2025) explore key aspects of trustworthy machine learning, such as out-of-distribution generalization, explainability, uncertainty quantification, and calibration, emphasizing their significance for healthcare applications.

Digital remote monitoring technologies have attracted attention as valuable tools for continuously assessing motor and non-motor symptoms in MS patients. Dini et al. (2025) conducted a narrative review focusing on the application of AI and big data in digital remote monitoring, utilising data from wearable sensors, smartphones, and other connected devices to facilitate personalised and timely interventions.

Furthermore, the combination of biomarkers from different modalities has been investigated to predict early disability accumulation in MS. Fleischer et al. (2025) examined the prognostic value of single and multimodal biomarker combinations, finding that integrating various biomarkers enhances the prediction of four-year disability progression in MS patients.

Collectively, these approaches contribute to the development of patient-specific disease networks and the prediction of optimal treatment combinations, thereby providing a framework for truly personalised MS therapy.

These emerging technologies and approaches represent the forefront of MS research, offering new tools and strategies for understanding and treating this complex disease. While many of these advancements are still in the early stages of development, they hold tremendous promises for advancing MS research and treatment in the coming years.

Further progress will depend on linking advanced imaging, computational analysis, single-cell methods, and human-relevant experimental models to questions that can be tested clinically. The value of these technologies lies in whether they improve mechanistic inference, patient selection, endpoint sensitivity, or treatment development.

Moreover, the increasing focus on personalised and precision medicine approaches in MS research reflects a growing recognition of the heterogeneity of the disease and the need for tailored treatment strategies. By leveraging advanced technologies and integrative approaches, researchers are moving closer to the goal of developing targeted, personalised therapies that address the specific underlying mechanisms driving MS in individual patients.

However, as we embrace these new technologies and approaches, it is central to maintain a balanced perspective and rigorous scientific methodology. The complexity of MS and the challenges of translating preclinical findings to clinical applications necessitate careful validation and replication of results across multiple models and systems.

Furthermore, ethical considerations must remain at the forefront as we explore new areas of MS research, particularly in areas such as gene editing and the use of human-derived tissues and organoids. Ensuring that these advanced research tools are developed and applied in an ethically responsible manner will be important for maintaining public trust and realizing their full potential to benefit MS patients.

Preclinical MS research increasingly uses human-derived models, single-cell methods, advanced imaging, and computational analysis. Their value will depend on reproducibility, biological validity, and whether findings can be linked prospectively to mechanisms or outcomes in people with MS.

7. Future Prospects and Potential Impacts

As we look towards the future of Multiple Sclerosis (MS) research, it is clear that the convergence of emerging technologies and innovative approaches holds potential to transform our understanding and treatment of this complex disease. The integration of advanced imaging, artificial intelligence, single-cell technologies, and sophisticated in vitro models is creating a powerful ecosystem for MS research that promises to bridge the gap between preclinical studies and clinical applications.

7.1. Precision Medicine and Personalized Therapies

The future of MS treatment is likely to be increasingly personalised, tailored to the specific pathophysiological mechanisms driving disease progression in individual patients.

7.2. Next-Generation Neuroprotection and Remyelination Strategies

Recent MS research has increasingly focused on neuroprotection and remyelination in addition to control of inflammatory activity. Several approaches studied during the past five years remain experimental and should be interpreted according to their stage of evidence.

Nanoparticles designed to cross the blood-brain barrier are emerging as a promising method for delivering therapeutics directly to damaged neurons and oligodendrocytes. Robinson et al. (2020) demonstrated that nanocrystalline gold (CNM-Au8) significantly enhanced remyelination in animal models of MS by promoting oligodendrocyte maturation and myelin repair.

Reprogramming endogenous cells into oligodendrocyte progenitors represents a novel therapeutic strategy for MS. Reprogramming reactive astrocytes into oligodendrocyte

lineage cells has been explored as a regenerative strategy in spinal cord injury models.

Exosomes engineered to deliver therapeutic molecules, represent an innovative approach for enhancing remyelination. Osorio-Querejeta et al. (2020) showed that miR-219a-5p-enriched extracellular vesicles significantly promoted oligodendrocyte progenitor cell differentiation, resulting in improved outcomes in experimental autoimmune encephalomyelitis (EAE) compared to traditional nanoparticles and liposomes.

7.3. Advanced Disease Modeling and Drug Discovery

The integration of advanced in vitro models with AI-driven analytics is poised to advance MS drug discovery.

AI and machine learning are increasingly used to accelerate the identification and design of new therapeutic molecules, including peptide-based drugs (Zhai et al., 2025), and approaches such as computational drug repositioning and virtual screening could help identify candidates targeting neuroinflammation in MS.

Organ-on-a-chip systems offer powerful platforms to replicate complex CNS interactions. Vatine et al. (2019) developed human iPSC-derived blood-brain barrier chips for disease modelling and personalised medicine applications; adapting such systems to reproduce MS-relevant features, such as immune cell infiltration and BBB disruption, could facilitate analyses of disease mechanisms and therapeutic responses.

Computational modelling enables virtual simulations of clinical trials and could accelerate drug evaluation. In silico MS models that integrate patient-specific data are being developed to predict individual responses to therapies, which may in future streamline drug evaluation and reduce reliance on animal models, although their predictive accuracy still requires validation.

7.4. Non-Invasive Neuromodulation and Cognitive Enhancement

Future MS therapies may increasingly incorporate non-invasive neuromodulation techniques to address both physical and cognitive symptoms.

Non-invasive brain stimulation methods, such as transcranial magnetic stimulation (TMS), have shown promise in improving cognitive and motor function in MS. Personalised TMS protocols guided by neuroimaging have been proposed to enhance neuroplasticity and symptom management in MS, but supporting evidence remains limited.

Neurofeedback systems using real-time neuroimaging may have the potential to improve cognitive outcomes in MS, but controlled evidence is still limited. Machine learning applied to neuroimaging is also being used to characterize subtle brain changes; for example, Buyukturkoglu et al. (2022) used machine learning to investigate superficial white matter integrity in early MS.

Virtual reality-based cognitive rehabilitation provides immersive, adaptive environments for cognitive training in MS patients. A systematic review by Maggio et al. (2019) indicated that VR interventions significantly enhance cognitive functions, including memory, attention, and executive skills, by dynamically adjusting to patient-specific performance and brain activation patterns.

7.5. Microbiome-Based Interventions

The growing understanding of the gut-brain axis in MS is likely to lead to novel microbiome-based therapeutic approaches.

Building on current microbiome research, future MS treatments may involve targeted probiotic formulations designed to modulate specific aspects of immune function. The iMSMS Consortium (2022) characterised the gut microbiome of people with MS and their paired household healthy controls, identifying microbial associations with disease risk and course that could inform such personalised approaches.

Beyond single-strain probiotics, future microbiome-based therapies may employ engineered microbial communities designed to produce specific immunomodulatory metabolites.

The identification of microbiome-derived metabolites that influence MS pathogenesis may lead to novel small molecule therapies.

7.6. Ethical Considerations and Societal Impact

As these advanced technologies and therapies move closer to clinical application, it is important to consider their ethical implications and potential societal impacts.

The increasing use of genetic profiling in MS management raises concerns about genetic privacy and potential discrimination.

The development of highly personalised and potentially costly MS therapies raises questions about equitable access to advanced treatments.

Emerging technologies and new experimental approaches widen the range of questions that can be asked about MS, and in some areas the range of treatments that can be

tested. However, realizing this potential will require not only scientific and technological advancements but also careful consideration of the ethical, social, and economic implications of these new approaches. Addressing these questions alongside the science is what will determine whether individualized treatment becomes broadly available rather than confined to a few centres.

The coming decade could change how MS is diagnosed, treated and followed. Computational analysis and improved in vitro models should shorten the interval between a hypothesis and a testable result, though neither halts progression on its own. Whether that happens depends on sustained funding, collaboration across disciplines, and a willingness to carry findings through to the point where patients notice a difference.

As MS research becomes more varied and more technically demanding, training has to keep pace. Interdisciplinary programmes that combine neuroscience, immunology, bioinformatics and data science would give future researchers the range of skills the work now requires.

MS research should prioritise outcomes that matter to people living with the disease, including function, symptoms, participation, and quality of life. Patient involvement in study design can help align experimental endpoints with these clinical priorities.

The road ahead in MS research holds both promise and difficulty. Sustained collaboration, methodological rigour and attention to ethics are the conditions under which the field is most likely to convert mechanistic insight into treatments that change how patients live.

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4.6. CLINICAL TRIALS AND OUTCOMES OF STEM CELL THERAPY IN MULTIPLE SCLEROSIS:

1. Introduction

Multiple Sclerosis (MS) is a chronic immune-mediated neurological disorder characterised by chronic inflammation, demyelination, and neurodegeneration within the central nervous system. Despite the availability of disease-modifying therapies (DMTs) aimed at reducing relapse frequency and decelerating disease progression, a substantial unmet need persists for interventions that can effectively repair the extensive neural damage and restore lost neurological function, particularly in patients with progressive forms of MS. See Figure 4.6.1.

Clinical Trial Landscape of Stem Cell Therapies in Multiple Sclerosis

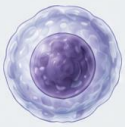
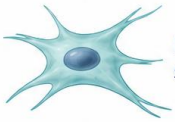
Cell type	Key trials	Phase	Main finding status
 Autologous hematopoietic stem cell transplantation	HALT-MS, MIST; ongoing BEAT-MS, RAM-MS, STAR-MS	Phase II–III	Strong suppression of inflammatory activity
 Mesenchymal stromal cells	MESEMS	Phase I–II	No effect on primary MRI endpoint in MESEMS
 MSC-derived neural progenitors (MSC-NP)	Harris et al. 2024 randomized phase II	Phase I–II	Primary EDSS-Plus endpoint not met

Figure 4.6.1. Representative stem-cell studies in MS by cell product and study design. MSC-NP denotes MSC-derived neural progenitors and should not be conflated with bona fide neural stem/progenitor cell products. The randomised phase II MSC-NP study did not meet its primary EDSS-Plus endpoint; this overview does not quantify effect size or probability of benefit and is not a general safety guarantee (Harris et al., 2024).

MSC and extracellular-vesicle (EV) approaches have therefore attracted interest as investigational candidates. MSCs have immunomodulatory and anti-inflammatory properties, while MSC-derived EVs carry bioactive molecules that may influence intercellular communication. These mechanisms are hypotheses requiring controlled clinical validation.

The evidence base is heterogeneous. MSC studies include early clinical trials, whereas exosome/EV studies in MS are predominantly preclinical, biomarker, mechanistic or laboratory investigations, and these evidence levels should not be treated as equivalent. An updated 2024 systematic review and meta-analysis of MSC intervention studies found no significant reduction in annualised relapse rate and reported route-dependent signals for EDSS, while emphasizing substantial heterogeneity and uncertainty (Vaheb et al., 2024). Reviews published in 2025 similarly concluded that short-term safety is generally reassuring but efficacy remains unresolved because cohorts are small and cell source, dose, route and outcome definitions vary (Nahayati et al., 2025; Sheikhi et al., 2025). A September 2026 systematic review of 16 distinct clinical trials reached the same broad conclusion at the biomarker level: randomised studies showed divergent clinical efficacy and candidate biomarker changes were not sufficiently consistent to establish a validated response signature (Najafi et al., 2026).

The 2026 literature has clarified this position rather than reversed it. The randomised SMART-MS trial did not

meet its primary neurophysiological endpoint, and a 2026 meta-analysis restricted to randomised progressive-MS studies found no significant pooled EDSS benefit, while pooled short-term adverse-event comparisons did not show an excess; this does not exclude the spinal safety signals observed in SMART-MS (Kvistad et al., 2026; Guo et al., 2026b). Recent overviews of human cell-therapy studies likewise place AHSCT on a different evidentiary footing from MSC and neural-cell products: AHSCT has the most consistent evidence for selected highly active relapsing disease, whereas regenerative cell products still require larger controlled trials with standardised manufacturing and clinically meaningful endpoints (Mohamed Fathy Kamal et al., 2026; Michel, 2026).

Additional recent syntheses reinforce the same caution. A randomised-trial meta-analysis and a network meta-analysis published in 2024 combined heterogeneous stem-cell products and study populations, while broader reviews published from 2024 to 2026 emphasized continuing variation in cell source, manufacturing, route, dose, disease phenotype, and outcome selection (Nawar et al., 2024; Fan et al., 2024; Christodoulou et al., 2024; Nahayati et al., 2025; Sheikhi et al., 2025; Yildirim & Avci, 2026). Taken together, these publications support continued clinical investigation but do not establish a class-wide efficacy effect or routine use of MSC-based therapy in MS.

2. Clinical Trials and Results

Table 4.6.1 summarizes the principal studies before each is described in detail.

Table 4.6.1. Summary of principal clinical studies of stem cell–based therapies in multiple sclerosis. Sample sizes and endpoints are as reported in the cited publications; uncontrolled studies are identified as such and do not support efficacy conclusions.

Study	Design / phase	n	Product and route	Primary endpoint	Reported outcome
Mohyeddin Bonab et al. (2007)	Uncontrolled pilot	10	Autologous BM-MSC, intrathecal	None pre-specified	Feasibility and short-term tolerability only; mixed clinical course
Karussis et al. (2010)	Uncontrolled pilot	15	Autologous BM-MSC, intrathecal and intravenous	Safety / immunological	Tolerated; immunological changes exploratory
Yamout et al. (2010)	Uncontrolled pilot	10	Autologous BM-MSC, intrathecal	None pre-specified	Clinical and MRI findings diverged; exploratory
Connick et al. (2012)	Uncontrolled, open-label	10	Autologous MSC, intravenous	Safety	Tolerated; visual/neurophysiological changes exploratory
Llufriu et al. (2014)	Randomized, placebo-controlled crossover	9	Autologous BM-MSC, intravenous	Gd-enhancing lesions	No significant treatment effect on the reported MRI endpoint
Lublin et al. (2014)	Randomized, placebo-controlled	16	Placental-derived MSC, intravenous	Safety	Tolerated; efficacy not established
Cohen et al. (2018)	Open-label phase I	24	Autologous BM-MSC, intravenous	Feasibility / safety	Feasible; no efficacy inference
Harris et al. (2018, 2021)	Uncontrolled phase I with 2-year follow-up	20	Autologous MSC-NP, intrathecal	Safety	No treatment-related serious adverse events; functional changes exploratory

Study	Design / phase	n	Product and route	Primary endpoint	Reported outcome
Petrou et al. (2020)	Randomized, double-blind, sham-controlled phase II	48	Autologous MSC, intrathecal / intravenous	Treatment failure rate	Signals favouring intrathecal arm; single-centre, requires confirmation
Uccelli et al. (2021) MESEMS	Multicentre randomised, double-blind crossover phase II	144	Autologous BM-MSC, intravenous	Gd-enhancing lesion count	Negative: rate ratio 0.94 (95% CI 0.58–1.50; p=0.78)
Harris et al. (2024)	Single-centre randomised, double-blind phase II	54	Autologous MSC-NP, intrathecal (6 doses)	EDSS-Plus	Primary endpoint not met; ambulatory and bladder findings exploratory
Burt et al. (2019) MIST	Randomized controlled trial	110	Non-myeloablative AHSCT vs DMT	Disease progression	Progression in 3/55 (AHSCT) vs 34/55 (DMT); four centres (most patients at one site), relapsing MS
Kvistad et al. (2026), SMART-MS	Randomised placebo-controlled crossover; phase II	18	Autologous bone-marrow MSCs; intrathecal	Combined evoked-potential latency at 6 months	Primary endpoint not met; spinal MRI abnormalities in 7 participants, arachnoiditis in 1

The individual studies are described in detail in the sections that follow.

2.1. Phase I Clinical Trials

The primary objective of Phase I clinical trials is to evaluate the safety and tolerability of novel treatments in a small cohort of patients. In the context of Multiple Sclerosis (MS), several pioneering studies have been conducted to explore the potential of mesenchymal stem cells (MSCs) and related therapies.

Mohyeddin Bonab et al. (2007) reported an uncontrolled pilot study in ten patients with progressive MS who received intrathecal autologous bone-marrow-derived MSCs. Clinical outcomes varied, including improvement, stability and worsening. The report provides early feasibility observations; it cannot establish efficacy or a general safety profile.

Karussis et al. (2010) extended this line of investigation by treating 15 MS patients, with an average age of 35.3 years, using intrathecal injections of 63.2 × 10^6 MSCs. After six months, 66.6% of the participants exhibited a decline in their EDSS scores, suggesting a potential therapeutic effect, albeit the small sample size limited the statistical power of the study.

Yamout et al. (2010) conducted a ten-patient intrathecal BM-MSC pilot study. Clinical and MRI findings diverged, and outcome-specific denominators were smaller than the enrolled cohort. The evaluable results and limitations are summarized in Chapter 7.

The study by Odinak et al. (2011) involved eight MS patients aged 24 to 47 years with a disease duration of 4 to 14 years. The patients received intravenous MSCs at a dose of 2.0 × 10^6 cells/kg, resulting in 75% of them showing improvements after 12 months, a result that supports further study of intravenous MSC administration in MS.

Mohyeddin Bonab et al. (2012) treated 25 patients with progressive MS in an open-label study. Mean age was 34.7 years, eligibility EDSS was 4.0–6.5, and the mean intrathecal MSC dose was 29.5 million cells. Three patients withdrew. Among the 22 followed clinically, EDSS improved in four, worsened in six and was unchanged in twelve; mean EDSS rose from 6.1 to 6.3. MRI was unchanged in fifteen patients, while six had new T2 or gadolinium-enhancing lesions and one lacked MRI follow-up. Reported short-term adverse effects included fever, nausea or vomiting, lower-limb weakness and headache. No major delayed adverse effect was reported during the study. The uncontrolled design, incomplete follow-up and mixed clinical

course preclude attribution of improvement or stability to treatment.

Connick et al. (2012) evaluated ten patients with MS who received autologous MSCs in an early, uncontrolled study. Reported changes in neurological and visual outcomes were exploratory signals; the small sample size and absence of a concurrent control group preclude attribution of clinical benefit to the intervention.

In a first-in-human Phase I clinical trial, Harris et al. (2018) evaluated the safety, feasibility, and potential clinical effects of intrathecal administration of mesenchymal stem cell-derived neural progenitors (MSC-NPs) in patients with progressive multiple sclerosis (MS). A total of 20 patients with either primary or secondary progressive MS received up to three intrathecal injections of autologous MSC-NPs, administered three months apart.

No treatment-related serious adverse events were reported in this small phase I cohort. Observed functional changes were exploratory and require a controlled comparison before a therapeutic effect can be attributed to MSC-NPs.

This pioneering study provided early evidence supporting the safety of MSC-NP therapy in progressive MS and suggested potential therapeutic benefits that warrant further investigation in larger, controlled trials.

2.2. Phase I/II Clinical Trials

Dahbour et al. (2017) conducted a phase I/IIa clinical study assessing the safety and efficacy of mesenchymal stem cells (MSCs) and MSC-conditioned media (MSC-CM) in multiple sclerosis (MS) patients. Ten patients, eight with secondary progressive MS and two with relapsing-remitting MS, were treated with autologous bone marrow-derived MSCs (93-168×10^6 cells) administered intrathecally, followed one month later by intrathecal injection of 13-20 mL of MSC-CM. The primary outcome was safety, with no serious adverse events reported, though patients experienced minor side effects such as headaches and site pain. Secondary outcomes included clinical measures such as the Expanded Disability Status Scale (EDSS), Timed 25-Foot Walk (T25FW), and 9-Hole Peg Test (9HPT), as well as MRI and ophthalmological assessments. The EDSS showed a mean reduction of -0.1 points, with two patients experiencing marked improvement (4 and 3.5 points, respectively). MRI scans showed increased lesion volumes in most patients, though some displayed a reduction in enhancing lesions. Inflammatory biomarkers, including IL-6, IL-8, and VEGF, were highly secreted in MSC-CM, and higher concentrations correlated with fewer baseline white matter lesions. The study concluded that both MSC and MSC-CM treatments are safe and may provide clinical stabilization or improvement in some MS patients. However, further large-scale trials are required to confirm efficacy.

In this phase I/IIa randomised controlled trial, Nabavi et al. (2023) evaluated both safety and potential efficacy outcomes following intravenous infusion of autologous bone marrow-derived mesenchymal stromal cells (MSCs) in patients with multiple sclerosis (MS). A total of 21 patients were enrolled and followed over an 18-month period.

Safety observations showed no severe immediate or delayed adverse events after the intervention within the study follow-up, and the procedure was well tolerated across all patients, with no acute or delayed infusion-related toxicities observed; a cohort of 21 patients, however, cannot reliably estimate uncommon adverse events. In terms of clinical efficacy, there was no statistically significant difference in the number of clinical relapses between the treatment and control groups during the 18-month follow-up, and no significant between-group difference in EDSS was demonstrated, stability in both groups cannot establish that MSC treatment caused clinical stabilization , while neurocognitive assessments did not show any statistically significant differences between the baseline and post-treatment periods in either group. On MRI, scans revealed no significant differences between groups in terms of new or enlarging T2 lesions or gadolinium-enhancing lesions after treatment, and the study did not demonstrate a significant treatment effect on the reported MRI findings. The cerebrospinal fluid analysis likewise did not demonstrate a significant treatment-related difference in the assessed biomarkers, and a nonsignificant biomarker result neither proves biological inactivity nor establishes neuroprotection.

Interpretation of Findings

Nabavi et al. (2023) provided limited safety and feasibility observations, without demonstrated clinical, MRI or CSF-biomarker efficacy. Alternative cell sources, routes or dosing schedules remain research questions; the negative result itself does not validate any alternative regimen.

Riordan et al. (2018) conducted a Phase I/II clinical trial involving 20 MS patients with a mean age of 41.1 years and a disease duration of 7.7 years. Participants received intravenous umbilical cord-derived MSCs at a dose of 20.0 × 10^6 cells/day. The study reported a mean decrease in EDSS scores after 12 months, suggesting a potential

therapeutic effect, although larger studies are needed to confirm these findings.

Meng et al. (2018) reported clinical observations following umbilical cord MSC transplantation in MS. Such exploration reports should be interpreted according to their study design and evaluated alongside controlled evidence. They do not, by themselves, establish treatment efficacy or long-term safety.

The MESEMS protocol (Uccelli et al., 2019) and completed phase 2 trial (Uccelli et al., 2021) should be distinguished. The published randomised result is summarized below under multicentre controlled evidence; its primary efficacy endpoint was not met.

Harris et al. (2021) conducted a two-year follow-up of a phase I clinical trial evaluating the safety and potential therapeutic effects of intrathecally administered mesenchymal stem cell-derived neural progenitors (MSC-NPs) in patients with progressive multiple sclerosis (MS). Twenty patients with primary or secondary progressive MS received three intrathecal injections of autologous MSC-NPs at three-month intervals.

The study found that the treatment was safe and well tolerated, with no serious adverse events attributed to the therapy. Some participants exhibited sustained clinical improvements, including enhanced motor function and bladder control. However, the extent and durability of the clinical responses varied among individuals. The findings suggest that MSC-NP therapy may offer a potential therapeutic benefit in progressive MS and support further investigation in controlled trials.

2.3. Controlled Studies and Related Exploratory Reports

Phase II trials are designed to assess the efficacy of a treatment while continuing to monitor its safety, typically involving a larger cohort of patients. Several studies at this stage have sought to evaluate MSC-based therapies in MS.

Llufriu et al. (2014) conducted a randomised, placebo-controlled trial involving nine patients with relapsing-remitting MS (RRMS), predominantly female, with a mean age of 36.8 years and a disease duration of 8.1 years. The participants received intravenous bone marrow-derived MSCs at a dose of 1.87 × 10^6 cells/kg. While no significant changes in EDSS scores were observed, the treatment was deemed safe, showing the necessity of larger trials to more thoroughly explore efficacy.

A randomised controlled trial by Lublin et al. (2014) involved 16 MS patients aged 18 to 65 years, treated with intravenous placental-derived MSCs at doses of 150.0 × 10^6 or 600.0 × 10^6 cells. The results were mixed, with no consistent improvements in EDSS observed after 12 months, suggesting that further research is necessary to ascertain the optimal dosage and patient selection criteria for MSC therapy in MS.

Cohen et al. (2018) reported an open-label phase I feasibility study: 24 patients received intravenous autologous BM-MSCs, including 10 with RRMS and 14 with SPMS; mean age was 46.5 years. The study supported further investigation of feasibility and tolerability but could not establish efficacy without a control group.

A Phase I/II clinical trial by Fernandez et al. (2018) included 30 SPMS patients, predominantly female, with a mean age of 46.3 years. They were treated with intravenous adipose-derived MSCs at doses of 1.0 × 10^6 or 4.0 × 10^6 cells/kg. No significant changes in EDSS scores were observed after 12 months, reinforcing the need for further research to refine MSC-based therapies for SPMS.

Baldassari et al. (2019) conducted a phase 1/2 clinical trial to assess the effect of autologous mesenchymal stem cell (MSC) transplantation on serum neurofilament light chain (sNfL-c) levels in multiple sclerosis (MS) patients. sNfL-c, a known biomarker of axonal injury, was measured alongside clinical assessments and MRI results. The study involved 24 patients, with serum samples taken at baseline and post-transplantation. The results revealed significant correlations between sNfL-c levels and MRI markers of disease activity, such as gadolinium-enhancing lesions and T2 lesion volume. However, MSC transplantation did not result in a statistically significant decrease in sNfL-c levels post-treatment. sNfL-c levels remained stable in patients without active disease. This study suggests that sNfL-c is a promising biomarker for disease monitoring in MS, though MSC therapy's direct impact on axonal injury requires further investigation.

Harris et al. (2024) randomised 54 patients with progressive MS, 27 per group, in a single-centre, double-blind phase II trial. Participants received six intrathecal injections of autologous MSC-NPs or saline at two-month intervals.

The primary EDSS-Plus outcome did not differ between groups: improvement occurred in 33% with MSC-NPs and 37% with saline. Exploratory analyses suggested

ambulatory benefit in participants with baseline EDSS 6.0–6.5, alongside bladder-function and imaging signals.

These exploratory findings require confirmation. The negative primary comparison precludes describing this trial as demonstrating overall efficacy or reversal of progressive MS disability.

In a 2022 study, Tremblay et al. conducted a Phase II randomised controlled trial to assess the safety and potential benefits of autologous mesenchymal stem cell (aMSC) infusion in patients with multiple sclerosis (MS). The study enrolled 20 adults with confirmed MS, randomly assigning them to receive either aMSC infusion (n = 9) or a placebo (n = 11) at Week 0. At Week 24, the placebo group also received the aMSC infusion. Blind assessments were conducted at Weeks 0, 24, and 48, utilising transcranial magnetic stimulation (TMS) to measure corticomotor excitability and motor conduction, alongside evaluations of motor impairments and disability.

Post-infusion assessments revealed no significant changes in corticomotor excitability or in measures of intra- or interhemispheric inhibition. The latency of motor evoked potentials and central motor conduction time were notably prolonged following aMSC infusion, and these delays in motor conduction were correlated with declines in hand dexterity post-infusion.

Conclusion:

The study concluded that, in this cohort of MS patients, aMSC therapy did not lead to improvements in clinical or neurophysiological measures. While stem cell therapy remains worth pursuing, its efficacy in altering disease activity in MS patients remains uncertain.

Petrou et al. (2021) conducted a prospective open-label study assessing the long-term safety and clinical efficacy of repeated mesenchymal stem cell (MSC) injections in patients with progressive multiple sclerosis (MS). Twenty-four patients with secondary progressive MS (SPMS) and primary progressive MS (PPMS) were treated with MSCs. They received up to eight intrathecal and intravenous MSC treatments over a 4-year period. The primary aim was to evaluate safety, while secondary outcomes included clinical and immunological effects. At baseline, the mean Expanded Disability Status Scale (EDSS) score was 6.75 (±0.68). The study found no serious treatment-related adverse effects. Forty-one mild or moderate side effects were recorded, including headaches, low-grade fever, and back pain. Clinically, 22 out of 24 patients showed either stability or improvement in their EDSS scores by the last follow-up. Patients who received more than two treatments experienced statistically significant improvements, with the mean EDSS decreasing to 6.42 (±0.84) at the final visit. The improvements were especially evident in patients who had multiple relapses or MRI activity prior to treatment. Immunologically, MSC therapy induced a transient increase in CD4+CD25high+FoxP3+ regulatory T cells and a reduction in the proliferation of lymphocytes, suggesting immunomodulatory effects. However, these effects diminished over time. These are uncontrolled follow-up observations, not proof that repeated MSC treatment produces clinical benefit or establishes general safety. The study leaves MSCs worth pursuing as a treatment for progressive MS, though larger controlled studies are necessary to confirm these results and optimise treatment protocols.

Cohen et al. (2023) evaluated intrathecal neurotrophic factor-secreting MSCs in an open-label progressive MS study. Two participants developed back and leg pain consistent with arachnoiditis. These events warrant attention to procedure- and product-related safety; uncontrolled clinical observations cannot establish efficacy.

2.4. Additional Randomized Phase II Evidence

Petrou et al. (2020) studied 48 patients with active or worsening progressive MS in a randomised, double-blind, sham-controlled phase II trial. Autologous bone marrow MSCs were administered intrathecally or intravenously at 1 × 10^6 cells/kg. Treatment failure was less frequent with either MSC route than with sham treatment. These short-term controlled findings warrant replication and do not establish durable regeneration.

2.5. Multicenter Randomized Evidence

The study below is a multicentre phase 2 trial. Trial phase, randomisation, comparator, prespecified endpoint, and follow-up should be considered separately when assessing evidence maturity.

Uccelli et al. (2021) reported the randomised, double-blind, placebo-controlled crossover MESEMS phase 2 trial of intravenous autologous bone marrow MSCs in active MS.

The trial enrolled 144 adults with active relapsing-remitting or progressive MS, aged 18 to 50 years, with EDSS scores of 2.5 to 6.5, across 15 centres in nine countries. Participants were randomised to an early-MSC group (n = 69), which received MSCs at baseline and then placebo at week 24, or to a delayed-MSC group (n = 75), which received placebo at baseline and then MSCs at week 24. Patients,

treating physicians, and radiologists were blinded to treatment order. The primary efficacy endpoint was the cumulative gadolinium-enhancing lesion count at weeks 4, 12, and 24, while the primary safety endpoint was the number and severity of adverse events, with safety monitoring continuing throughout follow-up.

The prespecified primary comparison for gadolinium-enhancing lesions was negative, with a rate ratio of 0.94 (95% CI 0.58-1.50; p = 0.78). On safety and tolerability, there were 93 adverse events in the early-MSC group and 120 in the delayed-MSC group; infections and infestations accounted for 54 of 213 adverse events, which is an event-based proportion, and no treatment-related serious adverse events or deaths were reported. Two interpretive limits apply to these results: tolerability within a trial does not establish universal or long-term safety, and a nonsignificant comparison is not proof of equivalence.

Feasibility does not establish efficacy, and this regime did not demonstrate the intended anti-inflammatory MRI benefit. Several implications for future research follow. Future trials may consider longer follow-up, different biomarkers such as neurofilament light chain, or functional outcomes such as relapse rate and disability progression. The exploration of repeated dosing, intrathecal delivery, or allogeneic MSC sources may also be warranted. Finally, future studies should prespecify populations and test effect modification rather than assume greater benefit in an unvalidated subgroup.

2.6. Small Studies and Supporting Experimental Literature

In addition to large-scale clinical trials, several case reports and small-scale studies have offered insights into the potential benefits and challenges of MSC-based therapies in MS treatment.

The ten-patient pilot study by Mohyeddin Bonab et al. (2007), introduced above, reported mixed clinical outcomes after intrathecal MSC administration. The sample and uncontrolled design are insufficient to establish treatment efficacy or the frequency of uncommon complications.

The study by Odinak et al. (2011) assessed the safety and tolerability of autologous multipotent mesenchymal stem cell (MSC) therapy in multiple sclerosis (MS) patients. Eight patients received intravenous MSC infusions at a dose of 2.0 × 10^6 cells/kg of body mass every 30 days over 4 to 8 months. All patients tolerated the treatment well, with no significant side effects. Improvement in the Expanded Disability Status Scale (EDSS) was observed in 5 of 8 patients after 4 months, and in 6 of 8 patients after 12 months. One patient experienced disease progression, and another showed stabilization. The observed changes in this eight-patient uncontrolled series cannot establish efficacy; a concurrent comparator and adequate follow-up are required.

Sahraian et al. (2014) conducted a five-year follow-up study on six patients with progressive multiple sclerosis (MS) and neuromyelitis optica (NMO) who received intrathecal autologous mesenchymal stem cell (MSC) injections. The patients had non-responsive progressive disease and were treated with MSCs after discontinuing other immunomodulatory therapies. The study found that two patients experienced no change in their Expanded Disability Status Scale (EDSS) scores, while three had an increase of 1-2 points. One patient, initially diagnosed with Devic's disease, improved by 1 point in EDSS but experienced four relapses. No serious adverse reactions, infections, or malignancies were reported during the five-year follow-up. The small mixed-diagnosis cohort provides limited follow-up observations. It cannot establish long-term safety or efficacy in MS, and NMO outcomes should not be pooled with MS outcomes.

Sahraian et al. (2019) conducted a pilot study investigating the safety and efficacy of intrathecal mesenchymal stem cell (MSC) therapy in patients with multiple sclerosis (MS). The study involved four patients (three with secondary progressive MS, one with relapsing-remitting MS) who received 50 to 80 million MSCs derived from bone marrow via intrathecal injection. Two patients received a booster injection after one year. No significant adverse effects were noted, and clinical stabilization was observed in three secondary progressive MS patients. MRI scans showed no new or enhanced plaques. The study suggested that MSC therapy, combined with booster injections, may help stabilize MS, particularly in secondary progressive cases.

Jafarzadeh Bejargafshe et al. (2019) conducted a review to assess the safety and efficacy of stem cell therapy for treating neural damage in patients with multiple sclerosis (MS). The article highlights the promising potential of mesenchymal stem cells (MSCs) due to their immunomodulatory and neuroregenerative properties in repairing MS-related neural damage. The authors analysed data from clinical trials, suggesting that these therapies may improve clinical outcomes, though further research is needed to confirm their efficacy and long-term effects. They also emphasized the importance of closely monitoring potential

adverse effects given the innovative nature of stem cell treatments. This study provides a valuable foundation for understanding the future role of stem cell therapies in MS treatment.

Gerdoni et al. (2007) investigated MSC-mediated immunomodulation in experimental autoimmune encephalomyelitis. This animal study informs mechanism research; it is not a human trial and does not establish a clinical dose, route or treatment benefit in MS.

Li et al. (2014) conducted a clinical study investigating the potential therapeutic benefits of human umbilical cord-derived mesenchymal stem cell (hUC-MSC) in patients with multiple sclerosis (MS). The study enrolled 23 patients, randomly assigning 13 to receive hUC-MSC therapy combined with anti-inflammatory treatment, while 10 patients constituted the control group receiving only anti-inflammatory therapy. The researchers aimed to evaluate the efficacy, safety, and immunological effects of hUC-MSC treatment.

Clinical assessments revealed significant improvements in overall symptoms among patients treated with hUC-MSC compared to the control group. Patients receiving stem cell therapy exhibited notably lower Expanded Disability Status Scale (EDSS) scores and reduced relapse rates. Immunologically, the treatment appeared to shift cytokine profiles from a pro-inflammatory Th1 pattern toward an anti-inflammatory Th2 profile, suggesting modulation of the immune response beneficial to MS treatment.

The authors concluded that umbilical cord-derived MSC therapy is a promising and safe approach for treating MS, showing positive clinical outcomes and immunological modulation. They recommended further larger-scale studies to validate and expand upon these findings.

Lu et al. (2020) conducted a 10-year follow-up study to assess the long-term safety and feasibility of combined intravenous (IV) and intrathecal (IT) infusions of human umbilical cord mesenchymal stem cells (hUC-MSCs) in patients with relapsing-remitting multiple sclerosis (RRMS) and neuromyelitis optica (NMO). The study included five patients with RRMS and five with NMO. Patients received four low-dose IV infusions (4×10^7 cells) and three IT infusions (2×10^7 cells) over 21 days. The results showed no severe adverse effects, such as tumour formation or organ dysfunction, during the 10-year follow-up. Nine of the 10 patients demonstrated a reduced relapse rate, and the treatment was well tolerated. This small uncontrolled series provides follow-up observations in two distinct diseases; it cannot establish long-term safety or efficacy in either condition. However, further large-scale clinical trials are necessary to confirm these findings.

3. Discussion and Future Directions

The reports discussed here illustrate heterogeneous outcomes and differences in cell product, route, comparator and follow-up. Protocols, secondary analyses, reviews and repeated descriptions of the same cohort should not be counted as independent clinical trials. The strongest causal conclusions depend on controlled comparisons with prespecified outcomes.

3.1. Variability in Outcomes and Factors Influencing Efficacy

Patient outcomes varied considerably across the reviewed studies. While some individuals experienced significant improvements in their Expanded Disability Status Scale (EDSS) scores, others showed minimal or no benefit. This variability is likely attributable to several interrelated factors, including the source of MSCs, the route of administration, the stage and subtype of MS, and the dosages utilised.

The reviewed studies used MSCs derived from various sources, including bone marrow, adipose tissue, and umbilical cords. Each source may possess distinct biological properties that influence the therapeutic potential of the cells. For instance, umbilical cord-derived MSCs are believed to have a higher proliferative capacity and more potent immunomodulatory effects, which might account for their relative success in some studies. However, the consistency of these effects across larger and more diverse populations remains to be conclusively determined.

The studies also employed different routes for MSC administration, such as intrathecal (directly into the cerebrospinal fluid), intravenous, or a combination of both. Intrathecal administration, which allows MSCs to reach the central nervous system more directly, may explain the more favourable outcomes observed in some studies. However, this method also carries higher procedural risks, which must be carefully weighed against the potential therapeutic benefits.

Patient selection matters as much: MS is a heterogeneous disease with multiple forms (e.g., relapsing-remitting MS, secondary progressive MS, primary progressive MS), and patients at different stages of the disease may exhibit varied responses to MSC therapy. For instance, progressive forms of MS, characterised by more extensive neural damage,

may be less responsive to treatment, as the repair mechanisms required are more complex. Tailoring MSC treatment to specific MS subtypes and disease stages could enhance therapeutic efficacy.

MSC doses and treatment schedules varied across studies. Reported dosing was heterogeneous and was not directly comparable because some studies used total-cell doses whereas others used weight-based dosing. Dose, frequency, and treatment duration therefore require product- and protocol-specific interpretation rather than a single pooled range.

3.2. Safety and Long-Term Effects

Most studies reported that MSC therapies are generally safe, with side effects such as fever or mild immunological reactions being relatively manageable. However, the long-term safety profile of MSC therapy remains largely unexplored. Only a few studies provided long-term follow-up data, and even these were limited to observation periods of a few years. Given the chronic nature of MS, long-term studies are essential for assessing the durability of therapeutic effects and the potential for delayed adverse events.

The potential for immunological side effects, particularly in the context of allogeneic (donor-derived) MSCs, also warrants further investigation. Although MSCs are renowned for their immunomodulatory properties, the risk of immune rejection or the development of adverse immune responses over time cannot be entirely discounted. Meticulous patient monitoring is therefore required and possibly the development of biomarkers to predict and manage such risks.

3.3. Potential Mechanisms of Action

A clearer understanding of the mechanisms by which MSCs exert their therapeutic effects is critical for optimising their clinical application in MS. Several mechanisms have been proposed, including:

The first is immunomodulation. MSCs are believed to modulate the immune system by attenuating the inflammatory processes that drive MS progression. This is supported by studies demonstrating reductions in pro-inflammatory cytokines and subsequent clinical improvements.

MSCs may also contribute to neural repair and protection by secreting growth factors and other bioactive molecules that support neuronal survival and function.

The third is remyelination. Some evidence suggests that MSCs might play a role in remyelinating damaged neurons, thereby potentially reversing some of the neurological damage inflicted by MS. However, the extent to which MSCs can promote remyelination in human patients remains an area of active research.

Further studies are necessary to elucidate these mechanisms fully and to determine how they can be harnessed to improve therapeutic outcomes. For instance, the combination of MSCs with other neuroprotective or regenerative therapies could enhance their efficacy.

3.4. Challenges and Future Research Directions

The current body of research has established a foundational understanding of the potential of MSC therapies in MS, yet significant problems remain. Addressing these challenges will be key for the future development and clinical translation of MSC therapies.

The first is standardisation and protocol optimisation. One of the most pressing challenges is the lack of standardised protocols across studies. Future research should focus on establishing a consensus regarding the optimal source of MSCs, dosage, route of administration, and treatment schedule. Standardisation would not only improve the consistency of outcomes but also facilitate the comparison of results across different studies, thereby accelerating the development of MSC therapies.

Given the variability in patient responses, future studies should prioritise the identification of biomarkers that can predict which patients are most likely to benefit from MSC therapy. This could involve genetic, immunological, or imaging-based markers that correlate with treatment outcomes. A personalised medicine approach, where therapies are tailored to individual patient profiles, could significantly enhance the effectiveness of MSC treatments.

Long-term studies are indispensable for determining the sustainability of the benefits observed in short-term trials and for monitoring the occurrence of delayed adverse effects. Such studies should include larger patient populations and extended follow-up periods to capture the full spectrum of potential outcomes.

Exploring the synergistic potential of MSCs in combination with other treatments, such as disease-modifying therapies (DMTs), neuroprotective agents, or even gene therapies, could provide a more comprehensive approach to MS treatment. For instance, combining MSCs with agents that promote remyelination or protect against neurodegeneration could address multiple aspects of MS pathology concurrently.

The cost and accessibility of MSC therapies are central considerations for their widespread clinical adoption. Future research should explore strategies to reduce the costs associated with MSC production and administration, thereby making these therapies more accessible to a broader patient population. This may include the development of more efficient manufacturing processes or the utilization of alternative sources of MSCs.

4. Meta-Analyses and Reviews

In their systematic review and meta-analysis, Mesa Bedoya et al. (2024) aimed to evaluate the safety profile of mesenchymal stem cell (MSC) therapy when administered via intrathecal injection for various neurological disorders. Among the 540 patients included across 9 randomised controlled trials (RCTs), a significant proportion were diagnosed with Multiple Sclerosis (MS). This makes the study particularly relevant to ongoing discussions about the utility of MSCs in the treatment of MS, a chronic, immune-mediated neurodegenerative disease characterised by demyelination, neuroinflammation, and progressive disability.

Rationale and Context for MS Inclusion

MS represents a logical and promising target for cell-based therapies, especially due to the broad immunomodulatory, neurotrophic, and tissue-repair potential of MSCs. Intrathecal delivery is seen as particularly advantageous in neurodegenerative conditions like MS due to its direct access to the cerebrospinal fluid (CSF) and central nervous system (CNS), allowing for potentially greater therapeutic reach.

Several of the RCTs included in this meta-analysis incorporated MS patients either as the primary study population or as part of a broader neurological cohort. These trials administered bone marrow-derived or umbilical cord-derived MSCs, most commonly in single or repeated doses ranging from 1 to 100 million cells, delivered intrathecally via lumbar puncture.

Safety Findings Relevant to MS Patients

The pooled safety findings require distinction between serious and severe adverse events and between intervention-related and all-cause events. Limited sample sizes and follow-up do not exclude uncommon harm. Procedure-related headache or back pain should still be included in an assessment of the intervention as delivered (Mesa Bedoya et al., 2024).

The pooled results should not be interpreted as proof that intrathecal MSCs cannot trigger disease activity or neurological complications in MS. Diagnosis-specific outcomes and event attribution require review at the individual trial level.

The pooled analysis used CTCAE version 5.0 categories and found an increased risk of musculoskeletal and connective-tissue adverse events with intrathecal MSCs (RR 1.61, 95% CI 1.19–2.19). This safety signal should not be dismissed as clinically unimportant; its relevance depends on event type and severity (Mesa Bedoya et al., 2024).

Therapeutic Implications for MS

Safety-focused pooled evidence cannot establish an adjunctive therapeutic role in progressive MS. That question requires MS-specific controlled trials with clinically meaningful efficacy outcomes and adequate monitoring.

The authors call for larger, MS-focused randomised controlled trials with longer follow-up to confirm these findings, and recommend that future work address optimal dosing strategies, repeated versus single administration protocols, longitudinal monitoring of MRI and CSF biomarkers, and the evaluation of functional outcomes such as EDSS, MSFC, and quality-of-life scores.

Conclusion

Mesa Bedoya et al. (2024) pooled nine randomised trials across neurological disorders, involving 540 participants. The mixed-diagnosis evidence does not provide a standalone estimate of MS-specific efficacy or uncommon long-term harms. Product, route and event definitions matter when interpreting the pooled safety findings.

In their 2024 article, Faile and Gallicchio explore the potential of stem cell therapy as a regenerative treatment for Multiple Sclerosis (MS), a chronic autoimmune disease affecting the central nervous system (CNS). The authors review various stem cell types, their mechanisms of action, and clinical trial outcomes to assess the efficacy and safety of these therapies in MS patients.

Introduction to Multiple Sclerosis and Rationale for Stem Cell Therapy

MS is characterised by demyelination, neuroinflammation, and neurodegeneration, leading to symptoms such as fatigue, pain, and motor dysfunction. Traditional disease-modifying therapies (DMTs) have limited success in halting disease progression, especially in progressive forms of MS. Stem cell therapy has been investigated as a promising

alternative due to its potential for immunomodulation and neuroregeneration.

Types of Stem Cells Investigated

The authors discuss several stem cell types explored in MS treatment. Mesenchymal stem cells, derived from bone marrow, adipose tissue, and umbilical cord, exhibit anti-inflammatory properties and promote remyelination. Haematopoietic stem cells are used in autologous haematopoietic stem cell transplantation to reset the immune system and reduce autoreactive lymphocytes, while neural stem cells are capable of differentiating into neural lineages and thus offer potential for repairing CNS damage. Induced pluripotent stem cells and human embryonic stem cells are pluripotent cells that can differentiate into various cell types, including neural cells, but pose ethical and safety concerns. Adipose-derived stem cells are known for their anti-inflammatory nature, which arises from their cytokine and growth factor production, and human amniotic epithelial cells exhibit immune-regulatory effects and regenerative properties with decreased antigenicity. MSC-derived exosomes, finally, are considered potential cell-free therapeutic alternatives because of their role in cell communication and regeneration.

Mechanisms of Action

Stem cells may benefit MS patients through three broad routes: immunomodulation, by suppressing autoreactive T and B cells, reducing inflammation, and promoting regulatory T cell development; neuroprotection, by releasing neurotrophic factors that support neuron survival and inhibit apoptosis; and neuroregeneration, by differentiating into neural cells to replace damaged tissue and by promoting remyelination.

Clinical Trials and Outcomes

The article reviews several clinical trials.

Riordan et al. (2018) reported an uncontrolled study of umbilical-cord-derived MSCs in 20 patients. Changes in disability and symptoms were exploratory; MRI results depend on the number with available follow-up and do not establish causal efficacy.

Bonab et al. (2012) reported an open-label progressive-MS cohort, discussed earlier in this chapter. Repeated summaries of that cohort do not provide independent evidence.

Secondary biomarker analyses from the Petrou MSC program should be interpreted as analyses of the parent trial, with attention to multiplicity, missing data and prespecification. A biomarker change does not by itself establish disability benefit.

Harris et al. (2021) reported two-year follow-up of the earlier phase I MSC-derived neural progenitor study. Sustained changes in some participants were uncontrolled observations and require confirmation in a randomised comparison.

The MIST trial randomised 110 patients with highly active RRMS. Progression occurred in three HSCT recipients and 34 DMT recipients during trial follow-up; these are not one-year event counts (Burt et al., 2019).

Pooled transplant outcomes require study-specific definitions of relapse freedom, progression, MRI activity and survival. Estimates from heterogeneous cohorts or follow-up periods should not be presented as interchangeable probabilities or as direct comparisons with contemporary DMTs.

Safety and Adverse Effects

Safety must be assessed separately for AHSCT, MSCs, neural cell products and EVs. AHSCT involves conditioning-related infection, organ toxicity, infertility and mortality risks. Small MSC studies cannot exclude uncommon procedural or product-related harms. A low event frequency in selected cohorts should not be generalized across all cell therapies.

Future Directions and Challenges

The authors highlight several needs: standardised treatment protocols and patient selection criteria, long-term safety and efficacy studies, attention to ethical concerns, particularly regarding the use of hESCs, and the separation of AHSCT conditioning and immune reconstitution from investigational combinations involving MSCs, neural cells, or extracellular vesicles.

MSC-based therapies remain investigational in MS. Priorities include standardised product characterisation, reproducible treatment protocols, appropriate patient selection, controlled efficacy testing, and sufficiently long follow-up to detect delayed adverse events.

The controlled trials set limits on efficacy claims. MESEMS and the MSC-NP trial did not meet their primary efficacy endpoints; favourable observations in small open-label cohorts cannot resolve those findings. Future trials need a defined product, a specified patient population, and durable effects on clinically meaningful outcomes.

Shokati et al. (2025) reported a phase I study of intravenous placenta-derived MSCs in five participants with SPMS. No serious complications were reported during the primary six-month safety assessment; two patients had transient headache. Clinical, imaging, and immune

measures were exploratory. The small uncontrolled design was intended to assess feasibility and tolerability, not establish efficacy.

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4.7. ADMINISTRATION ROUTES AND METHODS

1. Introduction

The administration route affects exposure, procedural risk, and the interaction of a cell product with host tissues. MS phenotype and disease activity also influence how a treatment result should be interpreted (Compston & Coles, 2008; Lublin et al., 2014; Mesa Bedoya et al., 2024). Figure 4.7.1 compares the routes and their limitations.

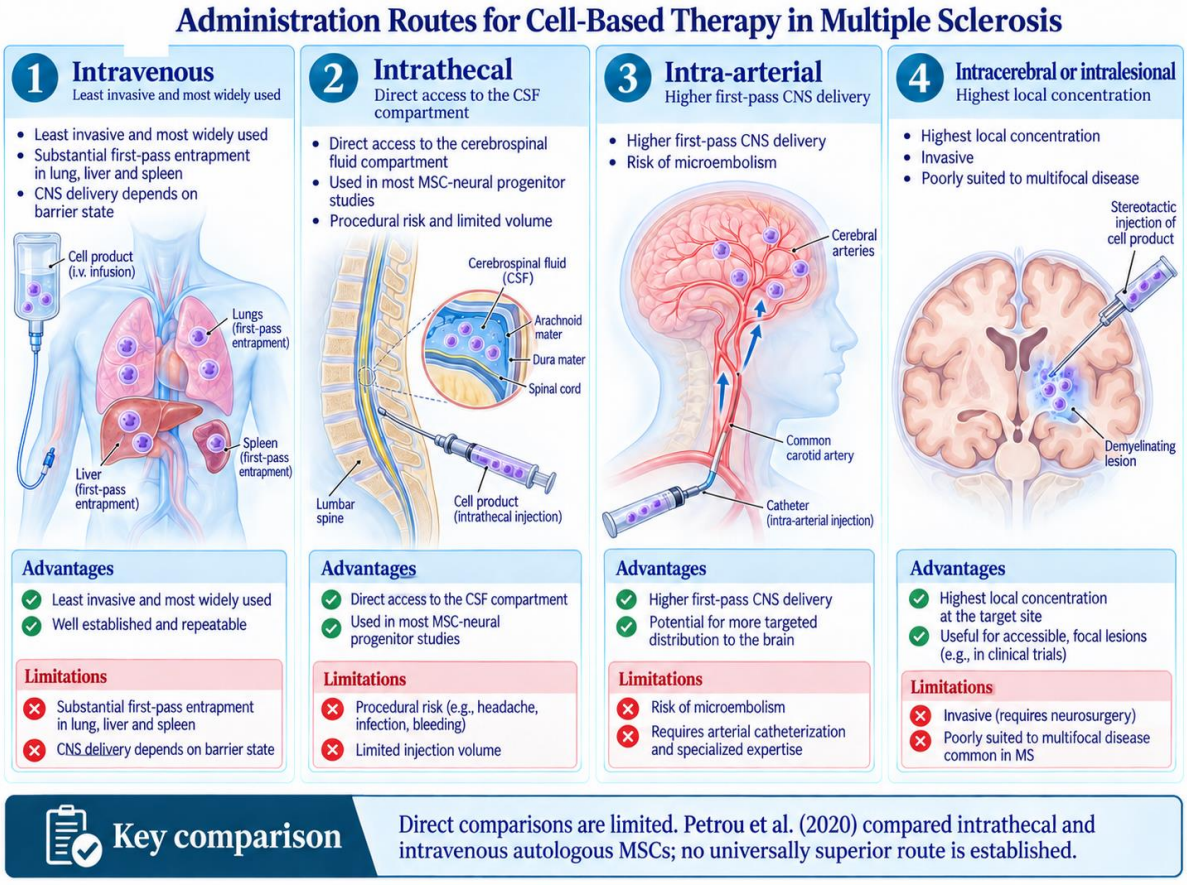


Figure 4.7.1. Administration routes for cell-based therapy in multiple sclerosis. Direct comparisons are limited. Petrou et al. (2020) compared intrathecal and intravenous administration of autologous MSCs; a universally superior route has not been established.

The potential of stem cell therapy in MS lies in its heterogeneous mechanisms of action, which include immunomodulation, neuroprotection, and regeneration (Uccelli et al., 2019). Different types of stem cell, including mesenchymal stem cells (MSCs), neural stem cells (NSCs), and haematopoietic stem cells (HSCs), have been investigated for their therapeutic potential in MS, each offering unique advantages and challenges (Scolding et al., 2017).

Delivery can influence cell biodistribution, survival, and interaction with host tissue (Yousefi et al., 2019). The following comparison covers intravenous, intrathecal, and local administration, without assuming that greater anatomical proximity to the CNS produces greater clinical benefit.

The following sections compare the principal administration routes with respect to biological rationale, delivery constraints, safety, and available evidence. They also examine whether route selection, repeated administration, or combination with other treatments has been tested directly, rather than assuming that a theoretical delivery advantage will translate into clinical benefit.

2. Intravenous Administration

2.1. Overview and Mechanisms of Action

Intravenous (IV) administration is one of the most extensively studied routes for delivering stem cell therapy in MS, owing to its relative simplicity, minimal invasiveness, and potential for systemic distribution of therapeutic cells. This method involves injecting stem cells directly into the bloodstream, allowing for circulation throughout the body, including the CNS. The rationale behind this

approach is predicated on the ability of stem cells to potentially cross the blood-brain barrier (BBB) and home to areas of inflammation and damage within the CNS, thereby exerting their therapeutic effects through various mechanisms.

The mechanisms by which intravenously administered stem cells exert their therapeutic effects in MS are complex and continue to be an area of active research. While initial hypotheses focused on the potential of stem cells to directly replace damaged CNS cells, current evidence suggests that the primary mechanisms of action may be more related to immunomodulation and neuroprotection (Uccelli et al., 2019).

2.1.1. Immunomodulation

Intravenously administered MSCs have been shown to modulate the immune response by suppressing pro-inflammatory T cells and promoting the expansion of regulatory T cells, thereby potentially attenuating the autoimmune attack on myelin. Extracellular vesicles derived from MSCs have shown related anti-inflammatory effects in a progressive MS model, including fewer inflammatory infiltrates in the spinal cord (Laso-García et al., 2018). This immunomodulatory effect is mediated through both cell-cell interactions and the secretion of soluble factors, such as TGF-β, IL-10, and prostaglandin E2 (PGE2).

Recent research has elucidated the complex roles of B cells in multiple sclerosis (MS) pathogenesis, revealing their involvement in both pro-inflammatory and regulatory processes. Kumar and Axtell (2023) discuss how B cells contribute to MS by producing autoantibodies, presenting antigens, and secreting cytokines that influence T cell activity. Conversely, regulatory B cells (Bregs) have been identified as important regulators capable of suppressing autoimmune responses through the production of anti-inflammatory cytokines such as IL-10. This dual role argues for therapeutic strategies that restore the balance between pathogenic and regulatory B cell functions.

In this context, mesenchymal stem cell (MSC) therapy has come to be seen as a possible approach in MS treatment due to its immunomodulatory and neuroprotective properties. Sheikhi et al. (2025) provide an in-depth analysis of MSC mechanisms of action, including immune system regulation, promotion of remyelination, and neuroregeneration. They highlight that MSCs can modulate B cell responses, potentially leading to a reduction in pathogenic antibody production and an increase in Bregs. This modulation contributes to dampening autoimmune responses and supports the regeneration of neural tissues, thereby addressing multiple aspects of MS pathology.

According to Wang et al. (2014), mesenchymal stem cells (MSCs) possess considerable plasticity in immunomodulation, enabling them to either suppress or enhance immune responses depending on the inflammatory signals they encounter. Their ability to communicate with the inflammatory microenvironment is important for their therapeutic role, particularly in diseases characterised by immune dysregulation such as multiple sclerosis (MS). Exogenously administered MSCs home to damaged tissues, where they exert their effects through direct cell-to-cell interactions and secretion of immunomodulatory factors (Wang et al., 2014).

2.1.2. Neuroprotection and Trophic Support

Beyond their immunomodulatory effects, intravenously administered mesenchymal stem cells (MSCs) have been shown to provide neuroprotection through the secretion of various neurotrophic factors. Proteomic analyses have identified a diverse array of neuroprotective molecules in the MSC secretome, including brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF). These factors can promote neuronal survival, stimulate endogenous repair mechanisms, and potentially support remyelination processes. Additionally, MSC-derived factors have been demonstrated to significantly promote oligodendrogenesis from cultured primary adult neural stem cells, suggesting a role in fostering the differentiation and maturation of oligodendroglial lineage cells (Samper Agrelo et al., 2020; Teixeira et al., 2016).

2.1.3. Extracellular Vesicles and Paracrine Effects

Recent studies have shown the significance of the mesenchymal stem cell (MSC) secretome, particularly extracellular vesicles (EVs), in mediating therapeutic effects in multiple sclerosis (MS). These EVs, including exosomes, are membrane-coated particles that transport various proteins and nucleic acids, serving as paracrine mediators. They have been shown to influence the fate of neural stem cells by enhancing oligodendrogenesis and neurogenesis, potentially leading to increased remyelination in MS. Additionally, MSC-derived EVs have demonstrated potent immunomodulatory and remyelinating properties in rodent models, suggesting their potential as a cell-free therapeutic modality to prevent disease progression and promote

remyelination in MS (Gavasso et al., 2024; Kråkenes et al., 2024).

2.2. Clinical Evidence and Efficacy

The efficacy and safety of intravenous stem cell administration in MS patients have been investigated in several clinical studies. An early open-label study by Connick et al. (2012) found intravenous infusion of autologous mesenchymal stem cells (MSCs) to be safe and well tolerated in patients with secondary progressive MS and reported improvements in visual function and an increase in optic nerve area; as an uncontrolled study of ten patients, these findings require confirmation.

A systematic review and meta-analysis summarized heterogeneous MSC studies in MS and reported proportions of participants whose measured status improved, remained stable, or worsened (Islam et al., 2023). Such pooled descriptive proportions depend on study design, outcome definitions, and follow-up. They should not be interpreted as the treatment effect relative to a control group or as proof that MSC therapy is clinically effective.

The MESEMS results should be read together with the detailed controlled-evidence summary in Section 4.6. Small-study improvement signals cannot override a negative prespecified randomised comparison (Uccelli et al., 2021).

2.3. Challenges and Limitations

Despite the early findings observed in clinical trials, several obstacles persist in optimizing the intravenous delivery of stem cells for MS treatment. One primary concern is the limited engraftment of stem cells in the CNS following IV administration. Studies have shown that a significant proportion of intravenously administered stem cells are trapped in the lungs and other organs, potentially reducing the number of cells reaching the target sites in the brain and spinal cord (Fischer et al., 2009). This phenomenon, known as the "pulmonary first-pass effect," has prompted researchers to explore various strategies to enhance the homing and engraftment of stem cells to the CNS following IV administration.

An important study by Zhao et al. (2011) demonstrated that, unlike ESCs, iPSC derivatives can trigger T-cell mediated immune responses even in syngeneic recipients due to abnormal gene expression, which means iPSC-based therapies require careful immunological assessment before clinical application (Zhao et al., 2011).

2.4. Innovative Approaches and Future Directions

2.4.1. Cell Preconditioning

One approach to overcome the limitations of IV delivery involves pre-conditioning stem cells before infusion to enhance their migratory capacity and survival. Hemeda et al. (2010) showed that pre-treating MSCs with interferon-γ (IFN-γ) or tumour necrosis factor-α (TNF-α) differentially affects their cytokine expression and migration properties, although whether such pre-conditioning improves CNS homing and therapeutic efficacy in MS models requires further study. Delivery parameters also matter: Cui et al. (2015) showed in rats that cerebral embolism after intra-arterial delivery of allogeneic bone marrow MSCs is related to cell dose and infusion velocity.

Building on this concept, Rosová et al. (2008) explored the use of hypoxic preconditioning to enhance the therapeutic potential of IV-administered MSCs. By exposing the cells to low oxygen conditions prior to infusion, the researchers observed increased motility and improved therapeutic potential. This strategy may enhance the efficacy of MSC-based therapies in conditions such as multiple sclerosis (MS).

2.4.2. Genetic Engineering and Cell Modification

Genome editing provides tools for investigating immune-cell trafficking in experimental models. Kendirli et al. (2023) used a genome-wide in vivo CRISPR screen in EAE to identify regulators of T-cell migration into the CNS, including ITGA4, CXCR3 and G-protein-coupled receptor kinase 2 (GRK2). GRK2 is a kinase, not a G-protein-coupled receptor. This study investigated pathogenic T cells rather than an engineered MSC treatment; translation to MSC homing or clinical therapy in MS remains a research hypothesis.

The adhesion molecule ITGA4, targeted by the monoclonal antibody natalizumab, was among the most critical factors regulating T cell migration into the CNS, and its deletion significantly reduced T cell infiltration in preclinical models. Additionally, chemokine receptor CXCR3 is essential to guiding immune cells to sites of inflammation, with studies demonstrating that its overexpression enhances stem cell migration efficiency. The involvement of the GRK2 kinase in controlling T cell trafficking via the S1PR1 pathway highlights another potential target for

genetic modification strategies aimed at improving stem cell-based treatments.

Further research has also identified transcription factors such as ETS1 that act as critical regulators of T cell responsiveness and limit their entry into the CNS. Engineering MSCs to modulate these pathways could enhance their therapeutic potential by improving their ability to suppress autoimmune responses and promote tissue repair in MS patients.

Despite the experimental signals reported to date, translating genetically modified stem cell therapies into clinical practice presents challenges, including concerns about long-term stability, potential off-target effects, and regulatory compliance. Preclinical studies are essential to address these issues and ensure the safety and efficacy of such approaches before clinical applications can proceed.

Genetic engineering, including CRISPR-Cas9 methods, could be used experimentally to alter targeting or functional properties of cell products, although this adds substantial safety and regulatory requirements. Ongoing research efforts should focus on optimising genetic modifications to maximize therapeutic outcomes while ensuring patient safety.

2.4.3. Nanoparticle-Based Approaches

Recent advancements in nanotechnology have significantly enhanced the potential for improving the delivery and efficacy of intravenously administered stem cells in multiple sclerosis (MS). Nanoparticle-based strategies, such as magnetic targeting and cell surface modifications, have been explored to optimise the direct migration of stem cells to the central nervous system (CNS). These approaches aim to overcome the challenges posed by the blood-brain barrier (BBB), which acts as a major obstacle to the effective delivery of therapeutic agents.

A thorough review by Ghosh et al. (2023) highlighted the potential of nano-engineered stem cells to promote remyelination and neuroprotection in MS. They discussed various nanotechnology techniques, including nano-script, nano-exosome hybrids, and nano-topography, which enhance the homing, engraftment, and survival of stem cells. The review emphasized that these nano-engineered stem cells exhibited improved immunomodulatory effects and more precise targeting of MS-affected regions.

A study by Rittchen et al. (2015) showed that nanoparticles encapsulating leukemia inhibitory factor (LIF) and targeted to oligodendrocyte precursor cells (OPCs) through antibodies against NG2 chondroitin sulfate proteoglycan increased myelin repair in vivo in a mouse model of demyelination. The study supports the potential of nanotechnology-based approaches to neurorepair, although their relevance to MS remains to be tested.

In a study by Li et al. (2020a,b), magnetically targeted mesenchymal stem cells (MSCs) labeled with Fe_3O_4 nanoparticles were used to enhance targeted delivery to injured tissues. Their findings indicated that magnetic nanoparticles improved the homing capabilities of MSCs and promoted anti-inflammatory cytokine secretion, resulting in enhanced angiogenesis and tissue repair (Li et al., 2020a,b).

Additionally, Toader et al. (2024) reviewed recent advancements in nanoparticle strategies for treating central nervous system disorders, emphasizing the role of receptor-mediated transport and stimuli-responsive systems in overcoming BBB challenges. Their review discussed how nanoparticles could facilitate the precise delivery of therapeutic agents, improving treatment outcomes in neurodegenerative diseases (Toader et al., 2024).

These studies make a case for combining nanotechnology with stem cell therapies to improve targeted delivery and therapeutic efficacy in MS. However, challenges such as potential toxicity, immune responses, and regulatory approval remain hurdles that need to be addressed before widespread clinical application.

2.4.4. Optimizing Administration Protocols

The timing and frequency of intravenous stem cell administration have become critical factors influencing treatment efficacy in multiple sclerosis (MS). While single infusions have shown benefits in some studies, growing evidence suggests that repeated administrations may lead to more sustained therapeutic effects.

Repeated administration remains a protocol question. The MESEMS publication (Uccelli et al., 2021) does not establish three-year benefit from repeated dosing. Dose, interval, and cumulative exposure require direct prospective comparison with adequate follow-up.

Administration strategies therefore need to be tailored to the individual in achieving the best possible therapeutic outcomes in MS treatment.

2.5. Future Perspectives

Several key areas warrant further investigation. Work is needed to elucidate the optimal cell dose, timing of administration, and frequency of infusions in order to maximize therapeutic benefits while minimising potential adverse effects, and to develop standardised protocols for cell

preparation, characterisation, and administration so as to ensure reproducibility and facilitate larger-scale clinical trials. Integrating advanced imaging techniques, such as real-time cell tracking, could provide valuable insight into the biodistribution and fate of intravenously administered stem cells. Combination therapies that leverage the systemic effects of IV administration alongside more targeted approaches, such as intrathecal or localised delivery methods, also merit exploration, as does the potential of cell-free therapies based on the stem cell secretome, including extracellular vesicles and engineered nanoparticles carrying therapeutic cargo. Finally, personalised treatment approaches that take account of individual patient characteristics, disease subtypes, and biomarkers may help optimise the efficacy of intravenous stem cell therapy.

Intravenous administration of stem cells is a promising and fast-developing approach for MS treatment, offering a minimally invasive method with the potential for systemic therapeutic effects. While difficulties remain, ongoing research into cell preconditioning, genetic engineering, and nanotechnology-based approaches may allow enhancing the efficacy of this delivery route. As the mechanisms underlying stem cell therapy in MS continues to grow, intravenous administration is likely to play an increasingly important role in the management of this complex neurological disorder, potentially offering new hope for patients suffering from MS.

3. Intrathecal Administration

3.1. Overview and Rationale

Intrathecal administration, involving the direct injection of stem cells into the cerebrospinal fluid (CSF) within the spinal canal, has attracted attention as a promising alternative to intravenous delivery for stem cell therapy in Multiple Sclerosis (MS). Delivery into the CSF places the cells closer to the affected tissue and avoids the pulmonary first pass and the blood-brain barrier, although the assumption that proximity translates into engraftment has not held up uniformly: an early intrathecal study in progressive MS found that cells delivered this way did not reach the lesions in the numbers expected, which shifted attention from the choice of route to what happens to the cells after they arrive (Pluchino & Mozafari, 2026). This route offers a more targeted approach to delivering stem cells to the central nervous system (CNS), potentially overcoming some of the limitations associated with intravenous administration, such as the blood-brain barrier (BBB) and systemic distribution (Kim et al., 2020). The rationale behind intrathecal delivery is rooted in the premise that it allows for a higher concentration of stem cells to reach the CNS, thereby enhancing their therapeutic potential in addressing the pathological hallmarks of MS, including inflammation, demyelination, and neurodegeneration.

3.2. Mechanisms of Action

The mechanisms by which intrathecally administered stem cells exert their therapeutic effects in MS are diverse and continue to be an area of active research. Recent studies have begun to describe the interplay between transplanted stem cells and the host CNS environment, revealing several key mechanisms of action:

3.2.1. Immunomodulation

Ruzicka et al. (2017) compared three different types of stem cells for the treatment of spinal cord injury in rats; whether intrathecally administered MSCs modulate the inflammatory microenvironment within the CNS of people with MS has not been established. Such immunomodulation is thought to be a key mechanism by which stem cells might attenuate the autoimmune attack on myelin and promote a more favourable environment for repair and regeneration.

Recent studies have demonstrated that mesenchymal stem cells (MSCs) can modulate microglial activation states, promoting a shift from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype. This phenotypic transition is associated with increased production of neurotrophic factors and enhanced remyelination in experimental autoimmune encephalomyelitis (EAE) models of multiple sclerosis (MS). For instance, research indicates that MSC-derived exosomes can significantly reduce central nervous system inflammation and demyelination, leading to improved clinical outcomes in EAE (Kråkenes et al., 2024). Additionally, studies have shown that M2-polarized microglia/macrophages are essential for efficient remyelination, as they drive oligodendrocyte differentiation through the secretion of factors like activin-A. MSC-based therapies may therefore modulate neuroinflammation and promote repair mechanisms in MS (Gavasso et al., 2024).

3.2.2. Direct Remyelination and Neuroprotection

The potential of intrathecally administered stem cells to directly contribute to remyelination and neuroprotection has been a subject of growing interest. Advanced imaging techniques can be used to track the fate of intrathecally delivered neural stem cells (NSCs) in experimental models of

MS, and experimental studies have reported that a subset of such cells can differentiate into oligodendrocytes. Direct evidence that intrathecally delivered cells remyelinate axons in patients is, however, lacking.

Building on these findings, Villoslada and Steinman (2020) provided critical insights into the molecular mechanisms underlying remyelination and neuroprotection in MS. Their research emphasized the importance of oligodendrocytes and their precursor cells in facilitating the repair process. They highlighted the necessity of targeting key pathways involved in oligodendrocyte differentiation and myelination, such as the Wnt/β-catenin and Notch signalling pathways, which play essential roles in regulating remyelination dynamics.

The study also discussed the potential of combining immunotherapies with regenerative approaches to achieve more effective long-term outcomes in MS patients. They pointed out that while current immunomodulatory therapies can reduce relapse rates, they do not prevent chronic neurodegeneration and progressive disability, reinforcing the importance of remyelination-focused interventions. Moreover, they suggested that improving drug delivery to the central nervous system (CNS), identifying predictive biomarkers, and optimising clinical trials through visual pathway assessments could accelerate the translation of these therapies into clinical practice.

The integration of stem cell therapy with targeted neuroprotective and remyelination strategies offers a possible route for MS treatment. Future research should focus on refining these approaches to enhance oligodendrocyte precursor cell differentiation and improve the overall efficacy of intrathecal stem cell therapies.

3.2.3. Neurotrophic Factor Secretion

Stem cells, and MSCs in particular, secrete a variety of neurotrophic factors that may promote neuroprotection and stimulate endogenous repair mechanisms. Key factors include brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF), all of which have been implicated in neuroprotection and repair processes in MS; whether intrathecally delivered cells raise the CSF levels of these factors in patients has not been established.

Recent research by Brown et al. (2021) explored the neurotrophic effects of neural stem cells (NSCs) derived from primitive mesenchymal stem cells (MSCs) in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS. Their findings indicated that transplanted NSCs significantly upregulated the expression of key neuroprotective genes such as BDNF, TrkB, and PI3K/Akt signalling pathways, which play key roles in neuronal survival and repair. Additionally, NSC transplantation led to an increase in the secretion of fibroblast growth factor (FGF), a critical modulator of neural progenitor cell proliferation and differentiation.

Furthermore, Razavi et al. (2015) reviewed neurotrophic factors and their effects in MS treatment, including trophic factors secreted by MSCs that may reduce inflammation and support remyelination by promoting oligodendrocyte differentiation.

These findings indicate the therapeutic potential of intrathecally administered stem cells in MS, emphasizing their ability to modulate the CNS microenvironment by secreting neurotrophic factors that support neuroprotection and repair.

3.3. Clinical Evidence and Efficacy

The feasibility and efficacy of intrathecal stem cell administration in multiple sclerosis (MS) have been explored in several pioneering studies. A seminal pilot study by Karussis et al. **(2010)** investigated the safety and immunological effects of both intrathecal and intravenous administration of autologous mesenchymal stem cells (MSCs) in patients with MS and amyotrophic lateral sclerosis (ALS). This notable research demonstrated the safety of intrathecal MSC administration and provided evidence of cell migration to key areas of the central nervous system (CNS), including the occipital horns of the ventricles, subarachnoid space, and spinal cord.

These studies provide preliminary information on feasibility and tolerability of intrathecal stem cell administration in MS. They do not establish a safe and effective treatment option; larger controlled trials and longer follow-up are needed to determine efficacy and uncommon adverse effects.

That expectation has since been tested directly. The SMART-MS trial randomised 18 patients with progressive MS to a single intrathecal dose of autologous bone-marrow-derived mesenchymal stem cells or placebo in a crossover design, with change in combined evoked-potential latency at six months as the primary endpoint. No significant between-group difference was observed for the primary endpoint (β = -0.31, 95% CI -1.84 to 1.22; p = 0.668). At six months, secondary analyses showed less cerebral atrophy (p = 0.044) and numerically lower serum GFAP, without statistical significance (p = 0.054); neither signal

was sustained at 12 months. Seven participants developed spinal MRI abnormalities with fluid loculations and nerve-root clumping; one developed chronic coccygeal pain attributed to arachnoiditis, and one serious adverse event was judged probably treatment-related. The trial therefore did not demonstrate a neuroregenerative effect and supports caution with intrathecal MSC administration (Kvistad et al., 2026). This result also argues against assuming that intrathecal delivery is intrinsically more effective than systemic administration merely because cells are introduced closer to the central nervous system.

3.4. Challenges and Limitations

Despite the early findings observed in clinical trials, intrathecal administration is not without its challenges. One potential concern is the risk of complications associated with the lumbar puncture procedure required for intrathecal injection. While serious adverse events are rare, minor complications such as post-procedure headache and transient back pain have been reported in some patients (Smith et al., 2021).

Another challenge lies in optimising the distribution of intrathecally administered stem cells within the central nervous system (CNS). The flow dynamics of cerebrospinal fluid (CSF) can significantly influence the dispersion of transplanted cells, potentially limiting their reach to certain regions of the brain and spinal cord. Studies have shown that CSF flow patterns contribute substantially to the transport of intrathecally delivered therapeutics, suggesting that understanding these dynamics is essential for improving delivery strategies (Naseri Kouzehgarani et al., 2021). Additionally, research indicates that CSF flow dynamics may be altered in multiple sclerosis (MS) patients, which could further impact the distribution and efficacy of intrathecally administered stem cells (Gorucu et al., 2011). These insights have led researchers to explore various strategies to enhance the widespread distribution of intrathecally delivered stem cells, such as harnessing CSF circulation for more effective drug delivery to brain tissues (Naseri Kouzehgarani et al., 2021).

3.5. Innovative Approaches and Future Directions

3.5.1. Advanced Delivery Techniques

To overcome the limitations associated with traditional lumbar puncture-based intrathecal delivery, researchers have been exploring alternative methods aimed at minimising invasiveness while maximizing therapeutic efficacy. Malysz-Cymborska et al. (2018) developed an innovative MRI-guided technique for the intrathecal transplantation of hydrogel-embedded glial progenitors in large animal models. This approach enables precise targeting of specific regions within the central nervous system (CNS), potentially enhancing the therapeutic efficacy of stem cell treatments while reducing procedural risks.

Building upon this concept, recent advancements have introduced minimally invasive intrathecal delivery systems utilising flexible, steerable microcatheters. These systems, guided by real-time imaging techniques such as MRI, allow for precise navigation through the subarachnoid space, facilitating targeted delivery of stem cells to specific regions of the brain and spinal cord affected by multiple sclerosis (MS). Preclinical studies have demonstrated early findings, including improved cell distribution and enhanced functional outcomes compared to traditional intrathecal injection methods (Lamanna et al., 2017).

These advanced delivery techniques represent significant progress in the field of regenerative medicine for MS, offering the potential to improve the precision and efficacy of stem cell therapies. Further research and clinical trials are necessary to validate these findings and to establish standardised protocols for their implementation in clinical practice.

3.5.2. Biomaterial-Enhanced Delivery

Biomaterials and tissue-engineering approaches are being investigated as ways to support cell survival and local retention after delivery. Jafarisavari et al. (2024), for example, evaluated a polycaprolactone-chitosan-hyaluronic-acid nanofibrous conduit in a rat model and reported effects on neural regeneration and functional recovery. Such data are preclinical and do not establish benefit for intrathecal cell delivery in MS.

Building on this concept, Yun et al. (2024) developed collagen protein-chitosan nerve conduits enriched with neuroepithelial stem cells to promote peripheral nerve regeneration. Their findings indicated significant improvements in axonal growth and functional outcomes compared to traditional cell suspension delivery methods. These studies suggest that the integration of bioactive scaffolds with stem cell therapy could create a supportive microenvironment, mimicking key aspects of the native CNS niche.

While these biomaterial-based approaches offer promising advancements, challenges such as long-term biocompatibility, potential immune responses, and regulatory

approval remain key considerations before clinical translation.

3.5.3. Combination Therapies

Combining intrathecal mesenchymal stem cell (MSC) delivery with other therapeutic modalities has been proposed as a way to address the complex pathology of MS, but this remains a research hypothesis rather than an established strategy. The randomised, double-blind, placebo-controlled phase II trial of intrathecal MSC-neural progenitor (MSC-NP) treatment in progressive MS reported by Harris et al. (2024) did not meet its primary endpoint; exploratory ambulatory, bladder and grey-matter atrophy findings are hypothesis-generating only, and the trial evaluated MSC-NPs alone rather than a combination regimen. Whether combined approaches outperform monotherapy has not been tested directly.

In a preclinical study, Brown et al. (2021) reported that neural stem cells derived from primitive MSCs reversed disease symptoms and promoted neurogenesis in an EAE mouse model, with effects on regulatory T cell and Th17 cell levels. Combining such cells with systemic neuroprotective compounds is a logical next step but remains to be tested.

Furthermore, recent findings suggest that the integration of stem cell therapy with immunomodulatory agents can enhance neuroprotective mechanisms and promote tissue repair more effectively. The modulation of signalling pathways such as BDNF and FGF, as demonstrated in the study by Brown et al. (2021), can lead to enhanced endogenous neurogenesis and improved functional outcomes in MS models.

Despite promising preclinical findings, questions remain open in translating combination therapies into clinical practice, including optimising treatment protocols, ensuring long-term safety, and overcoming regulatory barriers. Further research is needed to define the most effective combinations, dosages, and administration schedules for maximizing therapeutic benefits in MS patients.

Overall, the integration of intrathecal stem cell therapy with complementary treatment modalities presents an active area of investigation in MS management. Future clinical trials should focus on validating these approaches to establish their safety, efficacy, and long-term benefits.

3.5.4. Optimizing Cell Preparations

Cell source, culture conditions and manufacturing methods can affect MSC characteristics. Tissue-source comparisons inform product development (Bieback et al., 2008). Any proposed preconditioning method requires direct potency, stability and safety testing; manufacturing changes cannot be assumed to improve clinical outcomes.

Additionally, a systematic review by Squillaro et al. (2016) emphasized the importance of standardising cell preparation protocols to improve the reproducibility of stem cell-based therapies. The review concluded that optimisation of cell culture conditions, including the use of hypoxic environments and growth factor supplementation, could enhance the potency and longevity of transplanted cells.

These findings support further investigation of cell-preparation methods for intrathecal stem-cell studies in MS, but they do not establish improved clinical efficacy or broader applicability.

3.6. Future Perspectives

Several key areas warrant further investigation. Delivery protocols require optimisation, including cell dose, injection volume, and rate of administration, in order to maximize the distribution and therapeutic efficacy of intrathecally delivered stem cells, and more sophisticated cell tracking methods, such as advanced molecular imaging techniques, could provide valuable insight into their long-term fate and distribution. The potential of genetically modified or preconditioned stem cells for intrathecal delivery is worth exploring as a means of enhancing survival, migration, and therapeutic effects within the CNS environment, as is the use of biomaterial-based delivery systems capable of providing sustained release of cells and therapeutic factors over extended periods, potentially reducing the need for repeated injections. Larger, well-designed clinical trials are needed to establish the long-term safety and efficacy of intrathecal stem cell therapy in different MS subtypes and stages of disease progression. Combination therapies that leverage the targeted nature of intrathecal delivery alongside systemic treatments could address both focal and diffuse aspects of MS pathology, and personalised approaches based on individual patient characteristics, disease subtypes, and biomarkers may further optimise efficacy.

Intrathecal administration of stem cells offers a possible and fast-developing approach for the treatment of Multiple Sclerosis. By providing a more direct route to the CNS, this method offers the potential for enhanced therapeutic efficacy compared to systemic delivery approaches. While problems remain, ongoing research into optimised delivery protocols, biomaterial-based strategies, and combination therapies continues to push the boundaries of what is

possible with intrathecal stem cell therapy. As our understanding of the interaction between transplanted cells and the host CNS environment grows, intrathecal administration is poised to play an increasingly important role in the range of MS treatments, offering hope for improved outcomes and quality of life for patients living with this challenging neurological disorder.

4. Localized Delivery

4.1. Overview and Rationale

Localized delivery methods represent a recent approach in the administration of stem cell therapy for Multiple Sclerosis (MS), aiming to administer stem cells directly to specific regions of the central nervous system (CNS) affected by the disease. This targeted approach offers the potential to maximize the regenerative and immunomodulatory effects of stem cells at the site of injury or inflammation (Cohen et al., 2023). The rationale behind localised delivery is particularly compelling in the context of MS, where lesions are often focal and distributed throughout the CNS, allowing for precise targeting of therapeutic interventions.

Localized delivery encompasses a range of techniques, including direct intracerebral or intraspinal injections, as well as the use of advanced biomaterial scaffolds to support cell engraftment and survival. These methods aim to overcome some of the limitations associated with systemic delivery approaches, such as poor cell survival and limited engraftment in the target tissues.

4.2. Mechanisms of Action

The mechanisms by which locally delivered stem cells exert their therapeutic effects in MS are varied and continue to be an area of active investigation. Recent studies have provided insights into several key mechanisms:

4.2.1. Direct Cell Replacement and Remyelination

Localized delivery of neural stem cells (NSCs) offers the potential for direct cell replacement and remyelination at sites of central nervous system (CNS) injury. Research has demonstrated that NSCs can migrate to demyelinated areas, differentiate into oligodendrocytes, and promote remyelination. For instance, transplanted NSCs have been observed to migrate to demyelinated regions and contribute to remyelination in experimental autoimmune encephalomyelitis (EAE) models, a commonly used animal model for multiple sclerosis (MS) (Smith et al., 2021).

Advancements in imaging techniques have provided deeper insights into the behaviour of transplanted NSCs. Two-photon microscopy, for example, has enabled real-time visualisation of NSC migration and interaction with damaged axons in the spinal cord. This imaging modality has revealed that transplanted NSCs can physically associate with damaged axons, potentially facilitating remyelination (Greenberg et al., 2014).

These findings show the broad benefits of targeted NSC delivery, including direct cell replacement and the activation of endogenous repair mechanisms, contributing to functional recovery in demyelinating diseases such as MS.

4.2.2. Immunomodulation and Neuroprotection

Locally delivered stem cells have demonstrated significant immunomodulatory and neuroprotective effects in the vicinity of multiple sclerosis (MS) lesions. Studies have shown that mesenchymal stem cells (MSCs) transplanted into the central nervous system (CNS) can modulate the immune response and provide neuroprotective benefits primarily through the secretion of trophic factors and cytokines.

Joyce et al. (2010) highlighted that MSCs could secrete a variety of cytokines and growth factors, including IL-10, TGF-β, and indoleamine 2,3-dioxygenase (IDO), which contribute to reducing local inflammation, promoting endogenous neuronal growth, and supporting neuronal survival. The study indicated that MSCs transplanted into the brain reduced levels of free radicals, inhibited apoptosis, and stimulated the proliferation of resident neural stem cells, thereby enhancing functional recovery.

The controlled study by Petrou et al. (2020), summarized in Section 4.6, reported short-term clinical benefits. Clinical improvement alone cannot identify the biological mechanism or establish new myelin formation.

Additionally, the meta-analysis by Zhou et al. (2019) summarized clinical outcomes of autologous MSC transplantation in MS across small, mostly uncontrolled studies; proposed neuroprotective mechanisms, such as the secretion of brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), remain inferred largely from experimental work.

MSC-based therapies may therefore modify the course of MS by attenuating neuroinflammation and fostering neuroprotection. However, additional large-scale studies are required to further elucidate the long-term benefits and optimise therapeutic protocols.

4.2.3. Stimulation of Endogenous Repair Mechanisms

Localized stem cell delivery has been shown to stimulate endogenous repair mechanisms within the central nervous system (CNS), offering potential therapeutic benefits for multiple sclerosis (MS). Several studies have demonstrated that mesenchymal stem cells (MSCs) and neural stem cells (NSCs) can promote endogenous remyelination and neuroprotection by modulating the microenvironment and secreting trophic factors.

Franklin & Ffrench-Constant (2008) made clear the central role of endogenous oligodendrocyte precursor cells (OPCs) in the remyelination process within the central nervous system (CNS). They detailed how OPCs, which are widely distributed throughout the adult CNS, mediate remyelination by differentiating into myelinating oligodendrocytes. The authors emphasized that successful remyelination not only restores the integrity of demyelinated axons but is also a key determinant of preserving axonal health and function. Furthermore, they discussed the potential of various factors and signalling pathways in enhancing the recruitment, proliferation, and differentiation of OPCs, thereby promoting effective remyelination.

Cohen et al. (2018) primarily examined MSC infusion feasibility and tolerability. Its uncontrolled design cannot establish MRI efficacy or endogenous repair.

Mechanistic interpretation must remain separate from clinical outcomes. The Petrou et al. (2020) findings do not directly demonstrate that BDNF or GDNF secretion caused remyelination in patients.

Localized stem cell therapy may therefore stimulate endogenous repair in MS by enhancing OPC activation and promoting a regenerative microenvironment. However, further large-scale studies are required to optimise treatment protocols and validate long-term clinical benefits.

4.3. Clinical Evidence and Efficacy

While localised delivery approaches for multiple sclerosis (MS) treatment are still primarily in the preclinical stage, several clinical studies have provided promising evidence supporting the feasibility and effectiveness of mesenchymal stem cell (MSC) transplantation. A randomised, placebo-controlled phase II trial conducted by Llufriu et al. (2014) investigated the safety and efficacy of autologous MSC transplantation in patients with relapsing-remitting MS (RRMS). The study demonstrated that MSC therapy was safe and provided a trend towards reduced inflammation, as indicated by a lower mean cumulative number of gadolinium-enhancing lesions (GELs) at 6 months compared to placebo.

The route comparison in Petrou et al. (2020) provides controlled early clinical evidence, summarized in Section 4.6. Differences in patient selection, products, delivery, endpoints, and follow-up limit comparisons with other MSC trials.

Systematic summaries of early MSC studies are limited by heterogeneous products, routes, populations and study designs. Without a randomised head-to-head comparison, differences between intravenous and intrathecal series do not demonstrate route superiority. NEDA estimates from uncontrolled cohorts cannot establish treatment efficacy.

Taken together, these studies leave MSC therapy a credible candidate for MS, demonstrating its safety, neuroprotective effects, and potential to improve functional outcomes. However, further large-scale, long-term trials are necessary to confirm these findings and establish standardised treatment protocols.

4.4. Challenges and Limitations

Despite the early findings observed in preclinical and early clinical studies, localised delivery methods face several challenges in their translation to widespread clinical use.

The first is invasiveness. The invasive nature of direct CNS injections carries inherent risks of tissue damage and potential complications. Minimising the invasiveness of these procedures while maintaining their precision and efficacy remains an ongoing challenge.

Coverage is a second limitation. Given the multifocal nature of MS lesions, treating all affected areas through localised delivery may be impractical or impossible. Developing strategies to maximize the coverage and distribution of locally delivered stem cells is an important area of ongoing research.

Determining the optimal timing for localised stem cell delivery in relation to disease activity and progression is central for maximizing therapeutic efficacy. This is particularly challenging given the unpredictable and heterogeneous nature of MS.

Ensuring the long-term survival and functional integration of locally delivered stem cells in the hostile environment of MS lesions remains a significant challenge.

4.5. Innovative Approaches and Future Directions

4.5.1. Advanced Imaging-Guided Delivery Systems

To address the challenges associated with precise and minimally invasive localised delivery, researchers have explored MRI-guided focused ultrasound (MRgFUS) as an approach for enhancing stem cell delivery. A review by Ahmed et al. (2021) described how MRgFUS can transiently and non-invasively open the blood-brain barrier (BBB) to enable targeted delivery of therapeutic cells to specific CNS regions; the technique combines real-time MRI guidance with focused ultrasound and has so far been applied to cell delivery mainly in preclinical models.

Building on this foundation, Lipsman et al. (2018) showed in a small clinical study that MRgFUS could safely and reversibly open the blood-brain barrier in patients with Alzheimer's disease. Cells were not delivered in that study, and MRgFUS-assisted stem cell delivery in MS has yet to be tested clinically.

4.5.2. Biomaterial-Enhanced Localized Delivery

The integration of advanced biomaterials with localised stem cell delivery has been investigated as an investigational strategy to enhance cell survival, retention, and therapeutic efficacy in multiple sclerosis (MS). Biomaterial-based scaffolds and hydrogels provide structural support and biochemical cues that facilitate stem cell engraftment and promote neuroregeneration.

A study by Jafarisavari et al. (2024) developed a novel nanofibrous conduit composed of polycaprolactone-chitosan-hyaluronic acid for nerve regeneration in a preclinical model. This biomaterial demonstrated enhanced support for neural cells by mimicking the extracellular matrix (ECM) and improving cell survival and differentiation. The results suggested that such biomaterial-based systems could be beneficial for stem cell delivery in CNS disorders like MS.

In another study, Yun et al. (2024) investigated the use of collagen protein-chitosan scaffolds for neural tissue engineering. The study highlighted the importance of ECM-mimicking hydrogels in creating a microenvironment conducive to neural repair, showing increased neural progenitor cell viability and differentiation, leading to enhanced remyelination and axonal growth.

Furthermore, a meta-analysis by Zhou et al. (2019) reviewed various biomaterial applications for MSC delivery, emphasizing the advantages of hydrogel-based systems in enhancing localised delivery. The analysis concluded that hydrogels containing growth factors such as brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) promoted better cell retention, modulated inflammation, and facilitated neural repair.

These studies indicate the potential of biomaterial-enhanced localised stem cell delivery to improve therapeutic outcomes in MS by providing mechanical support, enhancing cell survival, and creating a regenerative microenvironment. However, further research is needed to optimise these systems for clinical translation.

4.5.3. Combinatorial Approaches

Recognising the complex nature of multiple sclerosis (MS) pathology, researchers have explored combinatorial treatment strategies to enhance therapeutic outcomes. Zhang et al. (2017) reported that fingolimod (FTY720) acts directly on neural stem cells (NSCs) and explored its combination with NSC-based repair in a chronic experimental autoimmune encephalomyelitis (EAE) model. Such findings suggest that integrating NSC transplantation with immunomodulatory agents like FTY720 could offer complementary benefits, but they come from animal models and do not establish synergy in people with MS.

Combination therapy requires a design that can distinguish the effects of each component. The Cohen et al. (2018) pilot does not establish superiority of a combined regimen.

4.5.4. Gene-Modified Stem Cells for Enhanced Localized Therapy in MS

The advent of advanced gene editing technologies has opened up new possibilities for modulating immune cell behaviour in multiple sclerosis. A notable study by Kendirli et al. (2023) utilised a genome-wide CRISPR screen in an experimental MS model to identify critical regulators of T cell migration to the CNS. Their work revealed that genetic modulation of pathways involving α4-integrin, CXCR3, and GRK2 significantly impacts the ability of autoreactive CD4+ T cells to infiltrate the CNS. CRISPR-based approaches may therefore be able to target immune cell trafficking, which could complement stem cell therapies by creating a more favourable microenvironment for repair and remyelination.

Islam et al. (2023) summarized heterogeneous MSC studies. Pooled proportions of improvement, stability and worsening describe reported observations, not causal

treatment effects. Differences between intravenous and intrathecal subgroups may reflect patient selection and study design; they do not validate a preferred route.

4.6. Future Perspectives

As research in localised stem cell delivery for MS continues to advance, several key areas warrant further investigation.

Further work is needed to determine the optimal cell doses, injection volumes, and delivery rates for different types of MS lesions and disease stages.

Continued innovation in imaging-guided and robotic delivery systems is important for enhancing the safety and precision of localised stem cell therapy.

The development of next-generation biomaterials that can provide dynamic support to transplanted cells and modulate the local microenvironment has investigational potential for enhancing the efficacy of localised delivery.

Further exploration of synergistic combinations of localised stem cell delivery with other therapeutic modalities, including pharmacological agents and non-invasive neuromodulation techniques, may lead to more comprehensive treatment strategies for MS.

The development of tailored localised delivery strategies based on individual patient characteristics, lesion profiles, and biomarkers may help optimise treatment outcomes.

Conducting extended follow-up studies in both preclinical models and clinical trials is essential for assessing the long-term safety, efficacy, and durability of localised stem cell therapies in MS.

Addressing the challenges of scaling up personalised localised delivery approaches and developing standardised protocols will be important for broader clinical implementation.

Localized stem-cell delivery is an experimental strategy intended to increase exposure at selected CNS sites and potentially improve cell survival or retention. Biomaterials, delivery devices, and combination strategies may improve local delivery, but the relevant dose, distribution, durability, safety, and clinical benefit have not been established for routine MS care.

5. Conclusion

5.1. Comparative Analysis of Administration Routes

Intravenous administration, characterised by its simplicity and minimal invasiveness, offers the advantage of systemic distribution of stem cells. This approach has shown promise in modulating the overall immune response and potentially addressing both focal and diffuse aspects of MS pathology. Recent advancements in cell engineering, preconditioning techniques, and nanoparticle-based approaches have significantly enhanced the homing and therapeutic efficacy of intravenously administered stem cells. However, challenges related to limited CNS engraftment due to the blood-brain barrier and the pulmonary first-pass effect remain areas of ongoing research.

Intrathecal administration provides a more direct route to the CNS, potentially overcoming some of the limitations associated with intravenous delivery. This method has demonstrated encouraging results in terms of cell migration to key areas of the CNS and has shown promise in promoting remyelination and neuroprotection. The development of advanced delivery techniques and biomaterial-based approaches has further enhanced the potential of this route. However, optimising cell distribution within the CNS and addressing the invasiveness of the procedure remain important considerations.

Localized delivery methods offer the most targeted approach, allowing for precise administration of stem cells to specific regions of the CNS affected by MS. This approach has shown measurable effects in experimental systems for direct cell replacement, remyelination, and modulation of the local immune environment. The integration of advanced imaging-guided delivery systems and innovative biomaterials has significantly enhanced the precision and efficacy of localised stem cell therapy. However, the invasive nature of the procedure and the challenge of addressing multiple lesion sites are important limitations that require further investigation.

5.2. Emerging Trends and Future Directions

Several key trends have emerged across all three administration routes, pointing to future directions in the field of stem cell therapy for MS.

The first is the move towards personalised treatment. There is a growing recognition of the need for tailored therapeutic strategies based on individual patient characteristics, disease subtypes, and biomarkers. Future research is likely to focus on developing predictive models to guide the selection of optimal administration routes and cell types for each patient.

The integration of stem cell therapy with other treatment modalities, such as immunomodulatory drugs, neuroprotective agents, and non-invasive neuromodulation techniques, warrants further investigation. These

combinatorial approaches may offer broader treatment strategies that address multiple aspects of MS pathology simultaneously.

The development of sophisticated biomaterials and delivery systems is advancing stem cell therapy across all administration routes. From nanoparticle-based approaches for intravenous delivery to advanced hydrogels for localised administration, these innovations are enhancing cell survival, integration, and therapeutic efficacy.

The application of gene editing technologies to create enhanced stem cell lines is an active area of investigation in MS treatment. Whether improving homing capabilities for intravenous delivery or enhancing neuroprotective factor secretion for localised therapy, genetically modified stem cells offer the potential for more potent and targeted treatments.

The integration of advanced imaging techniques and biomarker monitoring may enable real-time tracking of stem cell fate and therapeutic effects. This could support development of adaptive treatment protocols that can be adjusted based on individual patient responses.

5.3. Challenges and Future Research Priorities

Despite significant progress in stem cell therapy for MS, several difficulties remain that warrant further investigation.

The first is long-term safety and efficacy. Conducting extended follow-up studies to assess the long-term safety, efficacy, and durability of stem cell therapies across all administration routes is key.

Developing standardised protocols for cell preparation, characterisation, and administration will be essential for ensuring reproducibility and facilitating larger-scale clinical implementation.

A third priority is mechanism elucidation. Further work is needed to determine how stem-cell products exert their effects and whether those mechanisms differ by route of administration.

Determining the ideal timing of intervention, as well as optimising cell doses and administration frequencies for each route, remains an important area of investigation.

While most current research has focused on relapsing-remitting MS, developing effective stem cell therapies for progressive forms of the disease represents a critical unmet need.

Large-scale, randomised controlled trials comparing the efficacy of different administration routes and cell types are needed to inform clinical decision-making and treatment guidelines.

Administration route materially affects cell distribution, exposure, procedural risk, and feasibility. Future trials should compare routes and cell products using standardised manufacturing, dosing, and outcome definitions rather than assuming that combining routes or cell types will improve efficacy.

The integration of current technologies, from advanced biomaterials to gene editing techniques, is pushing the boundaries of what is possible in stem cell therapy. While questions remain open, the rapid pace of innovation in this field supports further investigation of more effective, targeted, and personalised treatments for individuals living with MS.

Clinical translation will require coordinated work among researchers, clinicians, trialists, regulators, manufacturers, and people with MS. Priorities are reproducible cell products, transparent reporting, appropriate comparators, prespecified endpoints, and long-term safety follow-up.

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4.8. CHALLENGES AND CONSIDERATIONS

1. Introduction

Multiple Sclerosis (MS) is a chronic autoimmune disorder that affects the central nervous system, causing a wide range of debilitating symptoms and significantly impacting patients' quality of life (Compston & Coles, 2008). Stem cell transplantation has demonstrated potential in improving disability and reducing brain lesion volume in MS patients (Nawar et al., 2024). This innovative approach leverages the regenerative and immunomodulatory properties of various stem cell types to potentially halt disease progression and even reverse some of the damage caused by MS (Genc et al., 2019). See Figure 4.8.1.

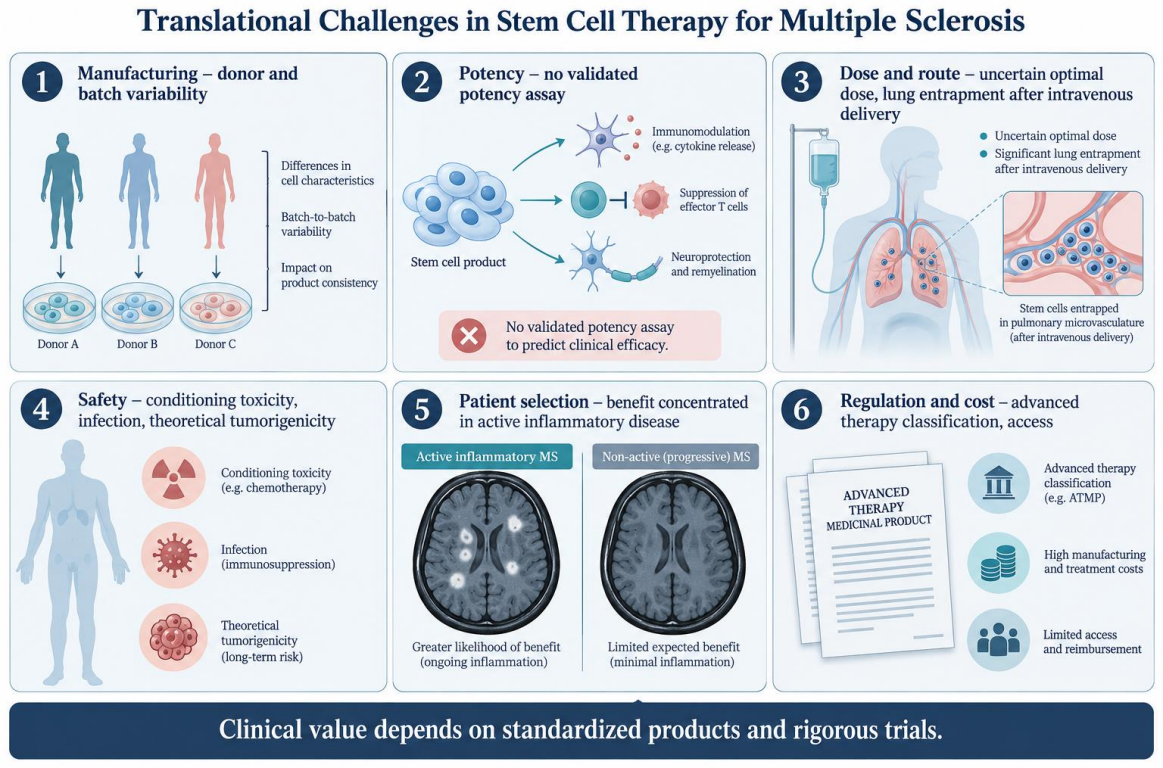


Figure 4.8.1. Translational challenges in stem cell therapy for multiple sclerosis, from manufacturing and potency to patient selection, regulation and cost.

Three recurring concerns require separate consideration in stem-cell studies for MS: ethical issues, graft-versus-host disease in allogeneic transplantation, and tumourigenicity. Their relevance depends on the cell product and transplantation strategy, and they should not be generalized across all forms of cell-based therapy.

Source-cell function cannot be inferred from MSC identity markers alone. In cultures from five people with MS and seven healthy controls, Tanasescu et al. (2025) found that patient-derived MSCs increased selected proinflammatory T-cell responses, whereas control-derived MSCs suppressed them. Differences involving IL-10, osteopontin, and ADAM28 suggest targets for product assessment. This laboratory finding does not demonstrate clinical harm from autologous MSC administration, but it argues for functional testing rather than assuming equivalent immunomodulation.

2. Ethical Concerns

The ethical implications of stem cell therapy for MS are complex and continue to be a subject of intense debate within the scientific and broader communities. These concerns primarily revolve around the sourcing of stem cells, issues of informed consent, and the potential for exploitation of vulnerable patient populations.

2.1. Stem Cell Sourcing

One of the most contentious ethical issues in stem cell therapy for multiple sclerosis (MS) pertains to the source of the stem cells used. Embryonic stem cells (ESCs), derived from human embryos, have been a particular focus of ethical scrutiny due to their origin (Lo & Parham, 2009). The use of ESCs raises questions about the moral status of embryos and whether their destruction for research purposes can be

ethically justified, even in the pursuit of potentially life-changing treatments for conditions like MS (Hyun, 2010).

A review by Hyun (2010) discusses the ethical dilemmas surrounding ESC research, emphasizing concerns related to the moral status of embryos and the justifiability of their destruction for potential therapeutic benefits. He argues that while ESC research has investigational potential for conditions such as MS, it also raises significant ethical concerns regarding embryo commodification and exploitation in biomedical research. Adult stem cells, including mesenchymal stem cells (MSCs) derived from bone marrow or adipose tissue, generally pose fewer ethical concerns. However, their use is not entirely free from ethical considerations. Issues such as donor consent, potential risks to donors, and the commercialization of human tissue still need to be carefully addressed (Hyun, 2010).

Induced pluripotent stem cells (iPSCs), which are created by reprogramming adult cells to an embryonic-like state, have developed into a potential ethical alternative to ESCs. While iPSCs circumvent many of the ethical issues associated with embryonic stem cells, they introduce new ethical considerations related to genetic manipulation and the potential for creating gametes or embryos from reprogrammed cells (Yamanaka, 2020). Yamanaka's notable discovery of iPSCs has significantly shifted the ethical terms of stem cell research by providing an alternative that does not require embryo destruction.

These debates argue for regulatory frameworks that balance scientific progress with ethical responsibility in stem cell research.

2.2. Informed Consent and Patient Autonomy

Ensuring truly informed consent in stem cell therapy for MS is essential yet challenging. The complexity of the treatment, coupled with the often desperate situation of patients with advanced MS, raises concerns about the ability of patients to make fully autonomous decisions (Amezcua & Nelson, 2017). There is a risk that the promise of stem cell therapy might lead patients to underestimate the risks or overestimate the potential benefits.

Kimmelman et al. (2016) argue that the informed consent process for stem cell therapies should be particularly rigorous, including detailed discussions about the experimental nature of the treatment, potential risks, and the likelihood of benefit. They also suggest that patients should be made aware of alternative treatment options and the opportunity costs of participating in stem cell therapy trials.

2.3. Equitable Access and Resource Allocation

The high cost and limited availability of stem cell therapies raise important questions about equitable access to these potentially important treatments. There are concerns that stem cell therapies for MS could exacerbate existing health disparities, with only wealthy individuals or those in high-income countries having access to these advanced treatments (Caulfield et al., 2016).

2.4. Regulation and Oversight

The ethical conduct of stem cell research and therapy for MS requires robust regulatory frameworks and oversight mechanisms. However, striking the right balance between facilitating scientific progress and ensuring patient safety is challenging. Overregulation could stifle innovation, while insufficient oversight could lead to unethical practices or exploitation of patients (Daley et al., 2016).

The International Society for Stem Cell Research (ISSCR) has developed guidelines for stem cell research and clinical translation, which address many of these ethical concerns. These guidelines emphasize the importance of scientific and ethical review of research protocols, transparency in reporting results, and responsible communication of scientific findings to the public (ISSCR, 2021).

2.5. Long-term Follow-up and Responsibility

Ethical considerations in stem cell therapy for MS extend beyond the initial treatment phase. There is an ethical obligation to conduct long-term follow-up studies to assess the safety and efficacy of these treatments over time. This raises questions about the extent and duration of researchers' and clinicians' responsibilities to patients who have undergone experimental stem cell therapies (Sugarman, 2008).

Moreover, the potential for stem cell therapies to have long-term or even transgenerational effects (particularly in the case of germline interventions) introduces complex ethical questions about responsibility and liability that extend far into the future (de Melo-Martín, 2016).

While stem cell therapy has investigational potential for MS treatment, it is imperative that its development and application proceed in an ethically responsible manner. This requires ongoing dialogue between scientists, ethicists, policymakers, and patient advocates to navigate the complex ethical questions and ensure that the pursuit of scientific progress does not come at the expense of ethical principles.

3. Graft-versus-Host Disease in Allogeneic Transplantation

Graft-versus-host disease (GVHD) occurs when donor immune cells attack recipient tissues after an allogeneic transplant (Ferrara et al., 2009). The established haematopoietic transplant approach investigated for MS is autologous AHSCT, using the patient's own cells; conventional allogeneic GVHD is therefore not a principal complication of MS AHSCT. The allogeneic literature below provides transplant context, not estimates of GVHD risk in standard MS care.

3.1. Mechanisms of GVHD in Stem Cell Therapy for MS

The pathophysiology of GVHD in the context of MS stem cell therapy is multifactorial. It involves the activation of donor T cells by host antigen-presenting cells, leading to the release of pro-inflammatory cytokines and the recruitment of additional immune cells (Blazar et al., 2012). This inflammatory cascade can cause damage to various organs, including the skin, liver, and gastrointestinal tract.

Allogeneic GVHD can affect several organ systems, but its incidence and outcomes in other transplant indications should not be extrapolated to people receiving autologous transplantation for MS.

3.2. Incidence and Risk Factors

The often-cited rates of acute GVHD after allogeneic transplantation reflect donor-cell procedures used principally for other indications. They do not estimate risk for autologous AHSCT in MS. Protocol, donor matching, and graft source matter when discussing allogeneic risk.

Several factors influence the risk of GVHD in MS patients undergoing stem cell therapy.

The first is HLA matching. The degree of human leukocyte antigen (HLA) matching between donors and recipients is central. Mismatched transplants carry a higher risk of GVHD (Lee et al., 2007).

The source of stem cells (e.g., bone marrow, peripheral blood, or umbilical cord blood) can affect GVHD risk. Peripheral blood stem cells are associated with a higher incidence of chronic GVHD compared to bone marrow (Anasetti et al., 2012).

The intensity of the pre-transplant conditioning regimen can impact GVHD risk. Reduced-intensity conditioning may lower the risk of acute GVHD but may not significantly affect chronic GVHD rates (Mohty et al., 2002).

Older recipients, and female donors to male recipients are associated with increased GVHD risk (Flowers et al., 2011).

The choice and duration of post-transplant immunosuppression can significantly influence GVHD incidence and severity (Ruutu et al., 2014).

3.3. Prevention and Management Strategies

The following strategies address prevention or management of GVHD in allogeneic transplantation generally; they are not routine prophylaxis for autologous AHSCT in MS.

Removing T cells from the graft before transplantation can reduce GVHD risk but may also increase the risk of graft failure and relapse (Ho & Soiffer, 2001).

Prophylactic use of immunosuppressive drugs such as cyclosporine, tacrolimus, and methotrexate is standard practice to prevent GVHD (Storb et al., 1986).

Agents targeting specific immune pathways, such as antithymocyte globulin (ATG) and post-transplant cyclophosphamide, have shown promise in reducing GVHD risk (Luznik et al., 2008).

Co-transplantation of mesenchymal stem cells (MSCs) with haematopoietic stem cells has been explored as a strategy to reduce GVHD risk due to the immunomodulatory properties of MSCs (Le Blanc et al., 2008).

Infusion of regulatory T cells is being studied in allogeneic transplant settings; possible relevance to other indications is speculative (Brunstein et al., 2011).

3.4. GVHD in Different Stem Cell Therapy Approaches for MS

The risk and management of GVHD vary depending on the specific stem cell therapy approach used for MS.

In autologous AHSCT, the patient's own collected cells are reinfused after conditioning. Classical donor-derived GVHD is not expected. Treatment-related risks instead include infection, organ toxicity, infertility, and other conditioning-related effects; rare GVHD-like phenomena should not be confused with the conventional allogeneic complication.

In allogeneic HSCT, donor-cell transplantation carries a genuine GVHD risk, but it is not a routine transplantation strategy for MS. Allogeneic rates and prophylaxis should be interpreted in their original transplant indication.

In mesenchymal stem cell therapy, MSCs have immunomodulatory properties and are less likely to cause GVHD.

In fact, they are being investigated as a treatment for GVHD itself (Galipeau & Sensébé, 2018).

In neural stem and progenitor cell therapy, while these cells have the potential to promote remyelination and neuroprotection in MS, their use is still largely experimental, and the risk of GVHD needs further evaluation (Pluchino et al., 2020).

3.5. Long-term Consequences and Quality of Life

Chronic GVHD can affect long-term quality of life following allogeneic transplantation. For MS AHSCT, interpretation of longer-term outcomes should instead focus on disease activity, disability, and the specific late toxicities of autologous conditioning.

The MS AHSCT literature cited elsewhere in this chapter should not be used as evidence that chronic GVHD is a common complication of the autologous procedure. Results from allogeneic cohorts must be kept separate from MS-specific outcomes.

3.6. Future Directions

The following research directions concern GVHD after allogeneic transplantation in general and should not be presented as established needs for autologous MS AHSCT.

Identifying reliable biomarkers for GVHD could allow for earlier intervention and more personalised prevention strategies (Paczesny et al., 2009).

Emerging evidence suggests that the gut microbiome plays a role in GVHD development, opening up new avenues for prevention and treatment (Staffas et al., 2017).

Gene editing technologies such as CRISPR-Cas9 can be employed to modify donor T cells in order to reduce their alloreactivity while preserving their beneficial immune functions (Neidemire-Colley et al., 2024).

Machine learning algorithms are being developed to predict GVHD risk and optimise prevention strategies based on individual patient characteristics (Gandelman et al., 2019).

GVHD concerns allogeneic transplantation. Decisions about autologous AHSCT in MS instead depend on patient selection, inflammatory disease activity, conditioning toxicity, infection, fertility, and long-term neurological outcomes.

4. Tumorigenicity

Tumourigenicity, the potential for transplanted stem cells to form tumours, represents a significant concern in the development and application of stem cell therapies for Multiple Sclerosis (MS). This risk is particularly pertinent given the long-term nature of MS treatment and the need for sustained therapeutic effects. Understanding and mitigating the tumorigenic potential of stem cell therapies is important for ensuring patient safety and the overall success of these innovative treatments.

4.1. Mechanisms of Tumorigenesis in Stem Cell Therapy

The tumorigenic potential of stem cells used in MS therapy can be attributed to several factors.

The first is pluripotency and self-renewal. The very properties that make stem cells valuable for therapy – their ability to differentiate into multiple cell types and self-renew – also contribute to their tumourigenic potential. Incomplete differentiation or uncontrolled proliferation can lead to tumor formation (Ben-David & Benvenisty, 2011).

Prolonged ex vivo culture of stem cells, often necessary for expansion before transplantation, can lead to genetic and epigenetic changes that may increase the risk of tumorigenesis (Maitra et al., 2005).

In cases where genetic modification of stem cells is employed, the insertion of transgenes can potentially disrupt normal gene function or activate oncogenes, leading to tumour formation (Hacein-Bey-Abina et al., 2003).

The interaction between transplanted stem cells and the host microenvironment can influence cell behaviour. Factors in the MS-affected central nervous system might promote aberrant cell growth or transformation (Yong et al., 2018).

4.2. Tumorigenicity Risks in Different Stem Cell Types

The risk of tumorigenicity varies depending on the type of stem cell used in MS therapy.

Embryonic stem cells carry the greatest risk. ESCs have the highest tumorigenic potential due to their pluripotency. The risk of teratoma formation is a significant concern with ESC-based therapies (Biehl & Russell, 2009).

While iPSCs offer an alternative to ESCs, they still carry a risk of tumour formation, particularly if residual pluripotent cells remain after differentiation (Yamanaka, 2020).

Generally considered to carry a lower tumorigenic potential, MSCs have been the focus of many MS stem cell therapy studies. However, long-term safety data are still limited (Barkholt et al., 2013).

Neural stem and progenitor cells complete this picture. These cells, which have shown promise in promoting remyelination in MS, have a more restricted differentiation potential compared to pluripotent stem cells. However, the risk of aberrant growth or differentiation still exists, particularly in the complex environment of the MS-affected central nervous system (Pluchino et al., 2003).

4.3. Assessing Tumorigenicity Risk

Evaluating the tumorigenic potential of stem cell therapies for MS involves a multi-faceted approach.

The first group comprises in vitro assays. These include tests for genetic stability, such as karyotyping and genomic sequencing, as well as assays for malignant transformation, like soft agar colony formation assays (Goldring et al., 2011).

Long-term studies in animal models, particularly immunodeficient mice, are important for assessing the tumorigenic potential of stem cell therapies. However, interspecies differences must be considered when extrapolating results to humans (Cunningham et al., 2012).

Advanced techniques such as single-cell RNA sequencing and epigenetic profiling can provide insights into the heterogeneity of stem cell populations and identify potentially tumorigenic subpopulations (Kumar et al., 2017).

Non-invasive imaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), can be used to monitor transplanted cells in vivo and detect any abnormal growth patterns (Kircher et al., 2011).

4.4. Strategies to Mitigate Tumorigenicity Risk

Several approaches are being developed to reduce the risk of tumorigenicity in stem cell therapies for MS.

Implementing stringent purification protocols to remove undifferentiated cells from the final cell product can significantly reduce the risk of tumour formation (Biehl & Russell, 2009).

Developing robust protocols for directed differentiation of stem cells into specific neural cell types relevant to MS treatment can minimise the presence of pluripotent cells in the transplanted population (Goldman et al., 2012).

Introducing suicide genes into stem cells before transplantation allows for selective elimination of transplanted cells if abnormal growth is detected (Liang et al., 2018).

Encapsulating transplanted cells in semi-permeable membranes can allow for the diffusion of therapeutic factors while preventing uncontrolled cell proliferation (Zanotti et al., 2013).

Developing culture protocols that maintain genetic stability and minimise the accumulation of mutations during ex vivo expansion is key for reducing tumorigenic potential (Stacey et al., 2013).

Transplanting pre-differentiated cells rather than stem cells can reduce the risk of tumourigenicity while still providing therapeutic benefits.

4.5. Clinical Experience and Long-term Follow-up

While preclinical studies have highlighted the tumorigenic potential of stem cells, clinical experience with stem cell therapies for MS has so far not revealed a signal of treatment-derived tumours, although follow-up remains limited. A meta-analysis by Sormani et al. (2017) of 764 patients treated with autologous haematopoietic stem cell transplantation for MS focused on transplant-related mortality and disease outcomes; long-term registry data are still needed to define the risk of secondary malignancies after the intensive immunosuppression involved.

The latency period for tumour development can be long, which makes extended follow-up necessary. The International Society for Stem Cell Research (ISSCR) guidelines emphasize long-term monitoring of patients receiving stem cell-based interventions (ISSCR, 2021), and regulatory guidance recommends follow-up of up to 15 years for gene-modified cell products with integrating vectors.

4.6. Regulatory Considerations

Regulatory agencies have recognised the unique challenges posed by the tumorigenic potential of stem cell therapies. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have issued guidance documents outlining the preclinical and clinical data required to assess the tumourigenicity risk of cell-based therapies (U.S. Food and Drug Administration [FDA], 2013; EMA, 2013).

These guidelines emphasize the need for full characterisation of cell products, rigorous preclinical testing in relevant animal models, careful patient selection and monitoring in clinical trials, and long-term follow-up studies to assess late-onset adverse events, including tumour formation.

4.7. Future Directions

Several lines of research are likely to change how tumorigenicity risk is understood and managed.

Identifying reliable biomarkers that can predict the tumorigenic potential of stem cell populations could enhance safety screening protocols (Goldring et al., 2011).

Developing more sensitive and specific imaging modalities for long-term in vivo tracking of transplanted cells could allow for earlier detection of aberrant growth (Bulte & Daldrup-Link, 2018).

Machine learning algorithms could be used to analyse complex datasets and predict tumourigenicity risk based on cell characteristics and patient factors (Zhu et al., 2020).

CRISPR-Cas9 and other genome editing technologies might be employed to enhance the safety profile of stem cells by eliminating genes associated with tumourigenicity or introducing additional safeguards (Doudna, 2020).

Brain organoids derived from human stem cells could provide more accurate models for studying the behaviour of transplanted cells in a human CNS-like environment (Mansour et al., 2018).

Tumour risk needs to be assessed for the specific cell product, including residual undifferentiated cells, culture-associated changes, and any genetic modification. Long-term surveillance remains necessary even when an early trial reports no tumour signal.

5. Conclusion

Cell-based therapy is an active area of MS research, but evidence and risk differ markedly across AHSCT, MSC products, neural progenitor approaches, and other cell types. Translation therefore depends on product-specific evidence rather than general claims about "stem cell therapy."

The ethical concerns surrounding stem cell therapy, particularly regarding the source of stem cells and issues of informed consent, necessitate ongoing dialogue and careful consideration to ensure that scientific progress aligns with societal values and respects patient autonomy. The risk of graft-versus-host disease, especially in allogeneic transplantation approaches, requires sophisticated prevention and management strategies to minimise its impact on treatment outcomes. Finally, the tumorigenic potential of stem cells demands rigorous safety assessments and long-term monitoring to safeguard patient well-being.

Cell engineering and imaging may improve product assessment and monitoring. Their clinical contribution must be demonstrated within the particular treatment protocol.

Further development of stem cell approaches for MS requires collaboration among researchers, regulators, clinicians, ethicists, and patient representatives. Manufacturing quality, prospective safety monitoring, and controlled efficacy assessment should precede routine clinical claims.

The next step is to establish which products, doses, and patient populations justify further clinical testing, with safety and efficacy evaluated separately.

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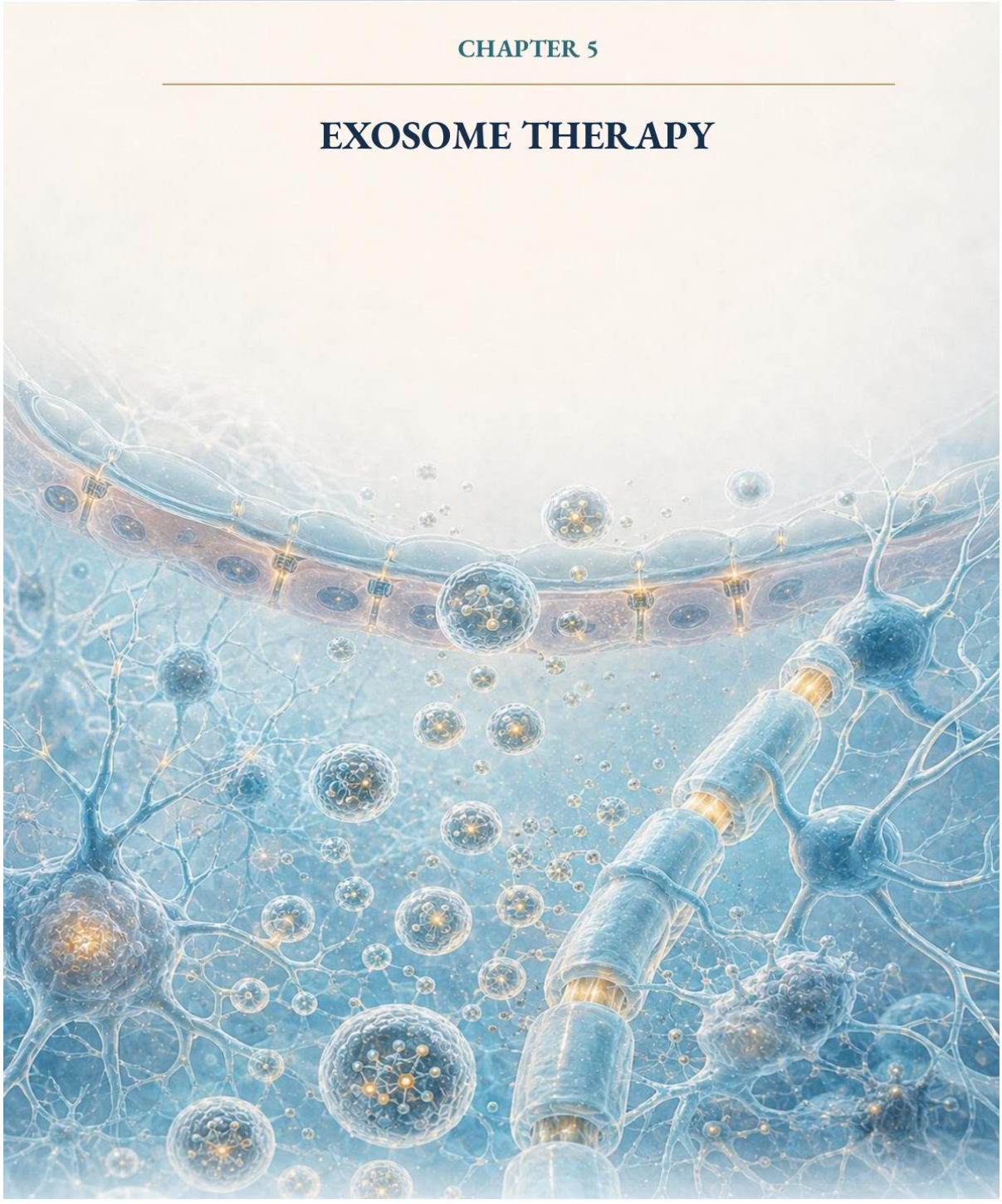
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CHAPTER 5

EXOSOME THERAPY



5.1. DEFINITION AND BIOLOGICAL ROLE OF EXOSOMES

Exosomes are extracellular vesicles of endosomal origin. Size and common EV-associated proteins alone do not establish that origin, and separation methods can co-isolate other vesicles and non-vesicular particles. When biogenesis has not been demonstrated, extracellular vesicle or small extracellular vesicles are the more defensible term. This book retains exosome when identifying the terminology used by a cited study, while interpreting the material according to the reported characterisation (Welsh et al., 2024). Figure 5.1.1 shows the vesicle classes and the practical limits of separating them.

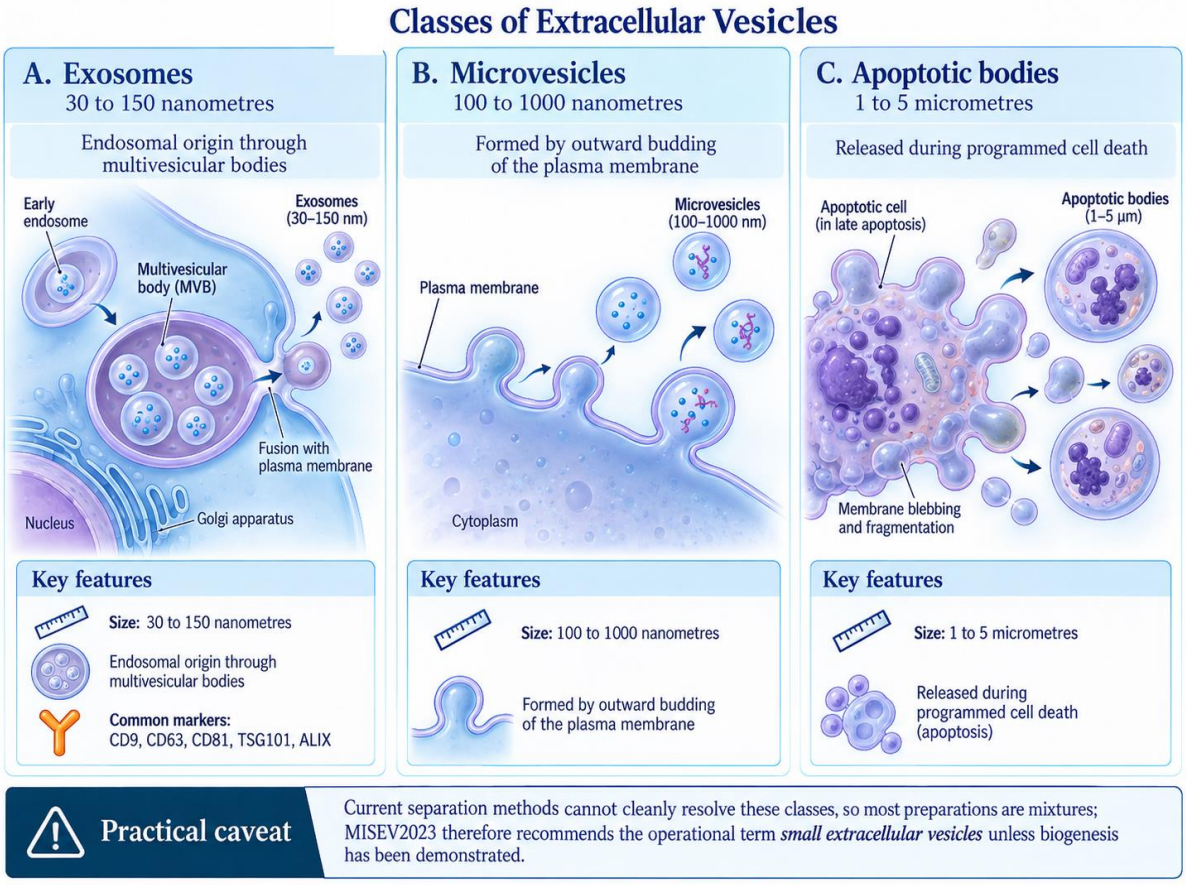


Figure 5.1.1. Classes of extracellular vesicles. Current separation methods cannot cleanly resolve these classes, so most preparations are mixtures and MISEV2023 recommends the operational term small extracellular vesicles.

EV publications have expanded substantially, but a bibliometric growth estimate requires a reproducible search string, database, search date and counting method. Publication volume itself does not establish therapeutic efficacy.

Exosome biogenesis and composition help explain their biological activity. Analytical specificity, manufacturing consistency, and functional validation determine whether that activity can be studied reproducibly or translated into a clinical product.

1. Historical Perspective and Evolution of Exosome Research

Exosome research began in the early 1980s when two independent groups observed the release of membrane-enclosed vesicles during reticulocyte maturation (Harding et al., 1983). Initially considered as cellular waste disposal mechanisms, these vesicles were termed "exosomes" by Rose Johnstone and her colleagues in 1987 (Johnstone et al., 1987).

The field remained relatively quiet for the next decade until a notable study by Raposo et al. (1996) demonstrated that B lymphocytes secrete exosomes carrying MHC class II molecules capable of inducing antigen-specific T cell responses. This discovery sparked renewed interest in exosomes, particularly in the context of immune regulation and cell-cell communication.

The turn of the millennium marked a significant shift in exosome research, with several key discoveries expanding our understanding of their biological significance.

In 2007, Valadi et al. reported that exosomes contain both mRNA and microRNA, which can be transferred to recipient cells and remain functional (Valadi et al., 2007). This finding opened up new avenues for understanding exosome-mediated intercellular communication and their potential as vehicles for RNA-based therapies.

The role of exosomes in cancer progression and metastasis began to be elucidated, with studies demonstrating their involvement in tumour angiogenesis, immune evasion, and pre-metastatic niche formation (Peinado et al., 2012; Skog et al., 2008).

The potential of exosomes as biomarkers for various diseases, including cancer and neurodegenerative disorders, started to be explored, leading to the concept of "liquid biopsies" (Taylor & Gercel-Taylor, 2008; Skog et al., 2008).

The therapeutic potential of exosomes, particularly those derived from mesenchymal stem cells, began to be investigated in various disease models, including myocardial infarction, kidney injury, and stroke (Lai et al., 2010; Bruno et al., 2012; Xin et al., 2013).

Technological advances have been central to what is now known about exosome biology. The development of high-resolution imaging techniques, advanced proteomics and genomics methods, and novel isolation and characterisation technologies has enabled more detailed studies of exosome composition, biogenesis, and function (Théry et al., 2018).

Recent years have seen an explosion of interest in the therapeutic applications of exosomes, with numerous clinical trials exploring their potential in various diseases (Wiklander et al., 2019). The COVID-19 pandemic has further highlighted the relevance of exosome research, with studies investigating the role of exosomes in SARS-CoV-2 infection and their potential as therapeutic agents or vaccine delivery vehicles (Hassanpour et al., 2020).

Exosome and extracellular-vesicle research continues to change rapidly as analytical methods improve and new biological functions are defined. The clinical relevance of these findings will depend on reproducible characterisation, standardised manufacturing, and prospective validation rather than on mechanistic plausibility alone.

2. Exosome Biogenesis and Secretion

The formation and release of exosomes is a complex, multi-step process that involves the coordinated action of numerous cellular mechanisms. Understanding this process is central for developing strategies to manipulate exosome production and cargo loading for therapeutic applications.

2.1. Early Endosome Formation

Exosome biogenesis begins at the plasma membrane, where a portion of the membrane invaginates to form an early endosome. This process is mediated by clathrin-dependent and independent mechanisms, involving various proteins such as the GTPase Rab5 and its effector EEA1 (Early Endosome Antigen 1) (Gruenberg & Stenmark, 2004).

During this early stage, proteins destined for exosomal secretion are sorted into the endosomal membrane. This sorting process involves specific protein-protein interactions and post-translational modifications, such as ubiquitination (Piper & Katzmann, 2007).

2.2. Multivesicular Body Formation

As the early endosome matures, it undergoes a series of changes, including acidification of its lumen and accumulation of intraluminal vesicles (ILVs) in its interior. This process transforms the early endosome into a multivesicular body (MVB) (Huotari & Helenius, 2011).

The first is the ESCRT-dependent mechanism. The Endosomal Sorting Complex Required for Transport (ESCRT) machinery is closely involved in ILV formation. This machinery consists of four multiprotein complexes (ESCRT-0, -I, -II, and -III) and associated proteins such as Alix and VPS4 (Henne et al., 2011). The ESCRT complexes work sequentially to recognize and cluster ubiquitinated cargo proteins, deform the endosomal membrane, and facilitate vesicle scission.

The second comprises ESCRT-independent mechanisms. Recent studies have revealed that ILVs can also form in the absence of ESCRT machinery, through a ceramide-dependent pathway in which the enzyme neutral sphingomyelinase generates ceramide, which can induce membrane curvature and ILV formation (Trajkovic et al., 2008); through tetraspanin-enriched microdomains, in which proteins of the tetraspanin family such as CD63 organize membrane microdomains that facilitate ILV budding (van Niel et al., 2011); and through heat shock proteins, since HSP70 and other chaperones have been implicated

in ESCRT-independent exosome biogenesis (Sahu et al., 2011).

The relative contribution of ESCRT-dependent and independent mechanisms may vary depending on the cell type and physiological conditions, adding to the complexity and heterogeneity of exosome populations.

2.3. Cargo Sorting

The selective incorporation of specific proteins, lipids, and nucleic acids into ILVs is an important aspect of exosome biogenesis. This process involves various sorting mechanisms.

Protein sorting is the best characterised. Ubiquitination is a well-characterised signal for protein sorting into ILVs via the ESCRT pathway. However, other post-translational modifications and protein-protein interactions also play roles in protein sorting (Villarroya-Beltri et al., 2014).

RNA is sorted by a different route: specific sequence motifs and RNA-binding proteins have been implicated in the sorting of RNAs into exosomes. For example, the presence of EXOmotifs in miRNAs and their recognition by RNA-binding proteins like hnRNPA2B1 can direct miRNAs into exosomes (Villarroya-Beltri et al., 2013).

Lipids are sorted too. The lipid composition of exosomes differs from that of the plasma membrane, suggesting active lipid sorting mechanisms. Ceramide and its metabolites play important roles in this process (Kajimoto et al., 2013).

2.4. MVB Trafficking and Fusion

Once formed, MVBs can have different fates within the cell. They can either fuse with lysosomes for degradation of their contents or traffic to the plasma membrane for exosome release. The balance between these pathways is regulated by various factors, including:

Rab GTPases are central. Different Rab proteins control distinct steps of MVB trafficking. For example, Rab27a and Rab27b regulate MVB docking at the plasma membrane, while Rab35 is involved in MVB trafficking and exosome secretion (Ostrowski et al., 2010; Hsu et al., 2010).

The fusion of MVBs with the plasma membrane is mediated by SNARE proteins. The v-SNARE VAMP7 on MVBs interacts with t-SNAREs on the plasma membrane to facilitate fusion and exosome release (Fader et al., 2009).

Intracellular calcium levels can modulate exosome release, with increased calcium promoting MVB fusion with the plasma membrane (Savina et al., 2003).

2.5. Exosome Release

The final step in exosome biogenesis is their release into the extracellular space. This process involves:

Cytoskeleton remodeling is one factor. The actin and microtubule networks play important roles in MVB trafficking and exosome release. Disruption of these networks can affect exosome secretion (Villarroya-Beltri et al., 2014).

Cholesterol-rich microdomains in the plasma membrane have been implicated in exosome release, possibly by facilitating MVB docking and fusion (Trajkovic et al., 2008).

Various regulatory proteins, including members of the synaptotagmin family and the calcium-binding protein Munc13-4, regulate the final steps of exosome release (Messenger et al., 2018).

Understanding the complex process of exosome biogenesis and release is key for developing strategies to manipulate exosome production and cargo loading for therapeutic applications. Recent advances in this field have opened up new possibilities for engineering exosomes with desired properties for drug delivery and other biomedical applications (Liang et al., 2021).

3. Molecular Composition of Exosomes

Exosomes are characterised by a unique and complex molecular composition that reflects their cellular origin and the physiological state of the parent cell. The exosomal cargo comprises a diverse array of biomolecules, including proteins, lipids, and nucleic acids, which contribute to their biological functions and potential as biomarkers (Raposo & Stoorvogel, 2013). Recent advances in high-throughput omics technologies have greatly expanded our understanding of exosome composition, revealing its complexity and heterogeneity.

3.1. Proteins

Exosomes contain a wide variety of proteins, some of which are common to all exosomes regardless of their cellular origin, while others are cell-type specific. The protein composition of exosomes has been extensively studied using proteomic approaches, revealing a complex and diverse protein cargo (Mathivanan et al., 2012).

3.1.1. Common Exosomal Proteins

Among the tetraspanins, CD9, CD63, CD81 and CD82 are abundantly present in exosomes and are often used as exosome markers. These membrane-spanning proteins are involved in exosome biogenesis and can mediate interactions with recipient cells (Andreu & Yáñez-Mó, 2014).

Proteins involved in MVB formation, such as Alix, TSG101, and CHMP4B, are frequently found in exosomes (Colombo et al., 2014).

HSP70, HSP90 and other chaperone proteins are commonly found in exosomes. They play roles in protein folding, cellular stress responses, and potentially in loading proteins into exosomes (Keller et al., 2006).

Annexins, Rab proteins and SNARE proteins involved in membrane trafficking and fusion events are present in exosomes (Théry et al., 2001).

Integrins and cell adhesion molecules facilitate exosome-cell interactions and uptake. Different integrin compositions can direct exosomes to specific target tissues (Hoshino et al., 2015).

Metabolic enzymes complete this picture. Various enzymes involved in glucose, lipid, and amino acid metabolism have been identified in exosomes, suggesting potential roles in metabolic regulation.

3.1.2. Cell-Type Specific Proteins

The protein content of exosomes can vary significantly depending on their cell of origin, reflecting the specialized functions of different cell types. Some examples include:

Immune cell-derived exosomes may contain MHC molecules, costimulatory molecules, and cytokines, reflecting their roles in immune regulation (Théry et al., 2009).

Cancer cell-derived exosomes often carry oncoproteins, growth factor receptors, and immunosuppressive proteins that can promote tumour progression and metastasis (Melo et al., 2015).

Stem cell-derived exosomes may contain proteins involved in self-renewal and differentiation, contributing to their regenerative potential (Lai et al., 2015).

Neuron-derived exosomes can carry neurotransmitter receptors and synaptic proteins, potentially playing roles in synaptic plasticity and neurotransmission (Chivet et al., 2012).

Recent studies have highlighted the heterogeneity of exosome populations, even those derived from a single cell type. Advanced proteomic techniques, such as single-vesicle proteomics, are revealing subpopulations of exosomes with distinct protein profiles, suggesting specialized functions (Kugeratski et al., 2021).

3.2. Lipids

The lipid composition of exosomes is distinct from that of the parent cell plasma membrane, reflecting their endosomal origin and specific lipid sorting mechanisms during biogenesis. Exosomal membranes are generally enriched in:

Exosomes contain high levels of cholesterol, which contributes to membrane rigidity and stability (Llorente et al., 2013).

Sphingomyelin is enriched in exosomes and plays roles in membrane organization and signal transduction (Skotland et al., 2017).

Phosphatidylserine, typically found on the inner leaflet of the plasma membrane, phosphatidylserine is exposed on the outer leaflet of exosomes, potentially facilitating their uptake by recipient cells (Skotland et al., 2017).

Ceramide matters for ESCRT-independent exosome biogenesis and can also act as a bioactive signalling molecule (Trajkovic et al., 2008).

Lysobisphosphatidic acid (LBPA), enriched in the internal membranes of MVBs, is involved in ILV formation and is found in some exosome populations (Subra et al., 2007).

Prostaglandins complete this group. These lipid mediators have been identified in exosomes and may contribute to their immunomodulatory effects (Subra et al., 2010).

The unique lipid composition of exosomes not only contributes to their stability in the extracellular environment but also influences their interactions with recipient cells and their potential as drug delivery vehicles (Skotland et al., 2017). Recent studies have employed advanced lipidomic approaches to provide a more detailed understanding of exosome lipid composition and its functional implications (Haraszti et al., 2016).

The lipid composition of exosomes can also vary depending on the cell type of origin and physiological conditions. For instance, exosomes derived from oligodendrocytes are enriched in myelin lipids, reflecting their role in myelin biogenesis (Krämer-Albers et al., 2007). Similarly, cancer cell-derived exosomes often show altered lipid profiles that may contribute to their pro-tumorigenic effects (Llorente et al., 2013).

3.3. Nucleic Acids

Exosomes carry a diverse repertoire of nucleic acids, including various RNA species and DNA. The discovery of functional RNAs in exosomes has substantially changed our understanding of exosome-mediated intercellular communication and opened up new avenues for biomarker discovery and therapeutic applications.

3.3.1. RNA Content

Functional mRNAs in exosomes can be translated in recipient cells, potentially altering their protein expression profiles. This phenomenon was first demonstrated by Valadi et al. (2007) and has since been confirmed in various biological contexts. The mRNA content of exosomes is often enriched for specific transcripts, suggesting selective packaging mechanisms (Skog et al., 2008).

MicroRNAs are abundantly present in exosomes and play essential roles in exosome-mediated intercellular communication. Exosomal miRNAs can modulate gene expression in recipient cells, influencing various cellular processes including proliferation, differentiation, and apoptosis. The miRNA profile of exosomes often differs from that of their parent cells, indicating specific sorting mechanisms (Guduric-Fuchs et al., 2012).

Long non-coding RNAs in exosomes have been implicated in various cellular processes and disease states. For example, exosomal lncRNAs have been shown to promote tumour progression and metastasis in various cancers (Conigliaro et al., 2015). The functions of many exosomal lncRNAs are still being elucidated, and remain an active area of research.

Circular RNAs are a more recently described class of non-coding RNAs that are highly stable due to their closed-loop structure. Exosomal circRNAs have been implicated in various biological processes and diseases, particularly cancer (Li et al., 2018). Their stability and specificity make them candidates for biomarker development.

3.3.2. DNA Content

Both genomic and mitochondrial DNA have been detected in exosomes, although their functional significance is still being investigated (Thakur et al., 2014). Exosomal DNA has been shown to reflect the mutational status of parent cells, making it a potential source of biomarkers for cancer and other diseases (Kahlert et al., 2014).

The presence of DNA in exosomes has also raised intriguing questions about its potential transfer between cells and its role in shaping the genome of recipient cells. Some studies have suggested that exosomal DNA can be taken up by recipient cells and integrate into their genome, potentially influencing cellular behaviour (Fischer et al., 2016).

3.4. Metabolites

In addition to proteins, lipids, and nucleic acids, exosomes have been found to contain various metabolites. Metabolomic analyses have revealed the presence of amino acids, lipid mediators, and other small molecules in exosomes (Altadill et al., 2016). These metabolites may contribute to the biological effects of exosomes on recipient cells and represent another layer of complexity in exosome-mediated intercellular communication.

4. Biological Roles of Exosomes

Exosomes play varied roles in intercellular communication, influencing a wide array of physiological and pathological processes. Their ability to transfer bioactive molecules between cells enables them to modulate recipient cell behaviour and function across various biological contexts.

4.1. Intercellular Communication

One of the primary functions of exosomes is to facilitate communication between cells, both in local microenvironments and at distant sites within the body. This communication is mediated by the transfer of proteins, lipids, and nucleic acids from donor to recipient cells (Raposo & Stoorvogel, 2013). The specificity of exosome-cell interactions is governed by surface proteins on both the exosome and the target cell, allowing for selective uptake and potential tissue-specific effects (Mulcahy et al., 2014).

Exosome-mediated communication has been implicated in various physiological processes, including:

Exosomes from immune cells can modulate immune responses by presenting antigens, activating T cells, or suppressing immune functions. For instance, dendritic cell-derived exosomes can stimulate antigen-specific T cell responses, while regulatory T cell-derived exosomes can suppress effector T cell proliferation (Théry et al., 2009).

In the nervous system, exosomes have been shown to participate in neuron-glia communication and synaptic plasticity. Neuronal exosomes can transfer proteins and RNAs to astrocytes and oligodendrocytes, potentially influencing their function and myelination processes (Frühbeis et al., 2013; Budnik et al., 2016).

Exosomes also help to regulate stem cell niches and directing cell fate decisions. For example, exosomes from the stem cell niche can maintain stem cell properties, while those from differentiated cells can induce differentiation (Nawaz et al., 2016; Ratajczak et al., 2006).

Exosomes from various cell types, particularly mesenchymal stem cells, have demonstrated regenerative properties in different tissues. These effects are mediated by the transfer of proteins, mRNAs, and miRNAs that can promote cell survival, proliferation, and angiogenesis (Zhang et al., 2020).

Exosomes have been implicated in various aspects of embryonic development, including implantation, gastrulation, and organogenesis. They can mediate communication between the embryo and maternal tissues, as well as between different cell types within the developing embryo (Desrochers et al., 2016).

In bone, exosomes mediate communication between osteoblasts, osteoclasts, and osteocytes, regulating bone formation and resorption. They can transfer proteins and miRNAs that modulate osteoblast differentiation and osteoclast activity (Cappariello et al., 2018).

Exosomes can also act as endocrine signals, carrying hormones and other regulatory molecules to distant target tissues. For instance, adipocyte-derived exosomes can influence insulin sensitivity in other tissues, potentially contributing to metabolic regulation (Ying et al., 2017).

4.2. Role in Disease Pathogenesis

While exosomes are central for normal physiological functions, they also play significant roles in the development and progression of various diseases, particularly cancer.

4.2.1. Cancer

In the context of cancer, exosomes have been implicated in multiple aspects of tumor biology.

The first is tumour growth and metastasis. Cancer cell-derived exosomes can promote tumour growth by transferring oncogenic proteins and nucleic acids to neighboring cells (Tickner et al., 2014). They also contribute to the formation of pre-metastatic niches by modifying distant tissue microenvironments to favor metastatic colonization. For example, melanoma-derived exosomes can educate bone marrow progenitor cells to create a pro-metastatic environment in the lung (Peinado et al., 2012).

4.2.2. Neurodegenerative Diseases

Exosomes have been implicated in the pathogenesis of various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). They may contribute to the spread of pathogenic proteins, such as amyloid-β, α-synuclein, and tau, between neurons (Soria et al., 2017).

In Alzheimer's disease, exosomes have been shown to carry amyloid-β and tau proteins, potentially contributing to their spread throughout the brain. However, exosomes may also play a protective role by facilitating the clearance of these toxic proteins (Rajendran et al., 2006; Dinkins et al., 2014).

In Parkinson's disease, α-Synuclein, the main component of Lewy bodies, can be transmitted between neurons via exosomes, potentially explaining the progressive nature of the disease (Emmanouilidou et al., 2010).

In ALS, exosomes carrying misfolded SOD1 protein have been implicated in the spread of pathology in ALS models (Grad et al., 2014).

Conversely, exosomes have also been investigated for their potential neuroprotective effects and as vehicles for therapeutic interventions in these diseases. For example, exosomes derived from mesenchymal stem cells have shown promise in reducing neuroinflammation and promoting neuronal survival in models of various neurodegenerative diseases (Jarmalavičiūtė & Pivoriūnas, 2016).

4.2.3. Cardiovascular Diseases

In cardiovascular pathologies, exosomes play dual roles. They can contribute to the progression of atherosclerosis by promoting inflammation and lipid accumulation in vessel walls (Boulanger et al., 2017). Exosomes derived from endothelial cells, platelets, and leukocytes can transfer pro-inflammatory molecules and tissue factor, contributing to plaque formation and thrombosis (Giró et al., 2021).

However, exosomes derived from certain cell types, such as cardiac progenitor cells and mesenchymal stem cells, have shown cardioprotective effects and potential for cardiac repair following injury. These exosomes can promote angiogenesis, reduce fibrosis, and enhance cardiomyocyte survival (Ibrahim & Marbán, 2016; Barile et al., 2014).

4.2.4. Infectious Diseases

Exosomes play complex roles in host-pathogen interactions. They can facilitate the spread of pathogens, including viruses and intracellular bacteria, by transferring pathogenic components between cells (Schorey et al., 2015).

In HIV infection, exosomes can carry HIV proteins and RNA, potentially contributing to viral spread and immune dysfunction (Madison & Okeoma, 2015).

In hepatitis C virus infection, exosomes can transfer viral RNA and proteins between hepatocytes, facilitating viral infection (Ramakrishnaiah et al., 2013).

In Mycobacterium tuberculosis infection, exosomes from infected macrophages can carry mycobacterial proteins, potentially modulating immune responses (Giri & Schorey, 2008).

However, exosomes can also contribute to host defense by presenting pathogen-derived antigens to immune cells and modulating immune responses. They can carry pathogen-

associated molecular patterns (PAMPs) that activate innate immune responses and stimulate adaptive immunity (Rodrigues et al., 2018).

In the context of the recent COVID-19 pandemic, exosomes have gained attention for their potential roles in SARS-CoV-2 infection and as possible therapeutic agents. Studies have suggested that exosomes may be involved in the transmission of SARS-CoV-2 between cells and in the modulation of immune responses to the virus (Hassanpour et al., 2020; Elrashdy et al., 2020).

4.2.5. Autoimmune Diseases

Exosomes have been implicated in the pathogenesis of various autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus (SLE), and multiple sclerosis. They can contribute to disease progression by:

Exosomes can carry autoantigens and present them to immune cells, potentially triggering or exacerbating autoimmune responses (Turpin et al., 2016).

Exosomes from immune cells can carry pro-inflammatory cytokines and microRNAs that promote inflammation and autoimmune reactions (Lee et al., 2016).

In rheumatoid arthritis, synovial fibroblast-derived exosomes can promote cartilage degradation by activating osteoclasts and stimulating the release of matrix-degrading enzymes (Ding et al., 2020).

4.3. Physiological Roles

Beyond their involvement in disease processes, exosomes play important roles in various physiological functions.

Platelet-derived exosomes carry tissue factor and other procoagulant molecules, contributing to hemostasis and potentially to pathological thrombosis (Aatonen et al., 2012).

In wound healing, exosomes from platelets, endothelial cells, and mesenchymal stem cells, contribute to different stages of wound healing by promoting angiogenesis, modulating inflammation, and stimulating cell proliferation and migration (Guo et al., 2017).

Exosomes also play important roles in embryo implantation, placental development, and fetal-maternal communication throughout pregnancy. They can modulate maternal immune responses to prevent rejection of the fetus and facilitate nutrient and waste exchange (Mitchell et al., 2015).

Osteoblast-derived exosomes contain calcium-binding proteins and enzymes involved in matrix mineralization, contributing to bone formation (Cui et al., 2016). These exosomes can also regulate osteoclast differentiation, playing a role in bone remodeling (Cappariello et al., 2018).

Exosomes released by myoblasts and muscle stem cells contribute to muscle repair and regeneration by promoting myoblast proliferation and differentiation (Choi et al., 2016).

Hepatocyte-derived exosomes participate in various aspects of liver physiology, including lipid metabolism, regeneration, and detoxification. They can transfer enzymes and other functional proteins between hepatocytes, contributing to liver homeostasis (Nojima et al., 2016).

Exosomes are involved in several aspects of kidney physiology, including tubular function, blood pressure regulation, and stone formation. For example, exosomes from renal tubular cells can transfer aquaporins and other transporters, influencing water and solute reabsorption (Knepper & Pisitkun, 2007).

In the skin, exosomes from keratinocytes, melanocytes, and dermal fibroblasts contribute to skin barrier function, pigmentation, and extracellular matrix remodeling (Lo Cicero et al., 2015).

5. Exosomes in Diagnostics and Therapeutics

The unique properties of exosomes, including their stability in biological fluids, their ability to cross biological barriers, and their cargo of biomolecules reflective of their cellular origins, have made them attractive candidates for both diagnostic and therapeutic applications.

5.1. Diagnostic Applications

Exosomes have come to be seen as a promising source of biomarkers for various diseases, particularly cancer. The concept of "liquid biopsy" using exosomes isolated from bodily fluids offers several advantages over traditional tissue biopsies.

The first is non-invasiveness. Exosomes can be isolated from easily accessible biofluids such as blood, urine, saliva, and cerebrospinal fluid, allowing for repeated sampling with minimal patient discomfort (Lin et al., 2015).

The ability to sample exosomes frequently allows for continuous monitoring of disease progression and treatment response, enabling more personalised and adaptive treatment strategies (Melo et al., 2015).

Exosomal cargo provides a snapshot of the molecular landscape of their cells of origin, potentially offering more thorough information than circulating tumour cells or cell-free DNA (Kalluri, 2016).

The lipid bilayer of exosomes protects their cargo from degradation, making them more stable than other circulating biomarkers and allowing for easier storage and transport of clinical samples (Kalra et al., 2013).

Exosomal biomarkers have shown promise for early detection of various diseases, including cancers that are difficult to diagnose in their early stages (Melo et al., 2015).

Exosome-based diagnostics are being explored for a range of diseases. In cancer, exosomal biomarkers have shown potential for early detection, prognosis, and monitoring of treatment response: glypican-1 positive exosomes have been identified as a potential early biomarker in pancreatic cancer (Melo et al., 2015), exosomal PCA3 and TMPRSS2:ERG fusion transcripts have shown promise for the non-invasive diagnosis of prostate cancer (McKiernan et al., 2016), exosomal EGFRvIII has been detected in the serum of glioblastoma patients (Skog et al., 2008), and miRNA profiles of circulating exosomes have been associated with tumour progression and metastasis in breast cancer (Hannafon et al., 2016). In neurodegenerative disease, exosomal levels of amyloid-β and tau proteins have been studied as potential biomarkers of Alzheimer's disease (Fiandaca et al., 2015), while α-synuclein levels in neurally derived plasma exosomes have been explored as a diagnostic tool in Parkinson's disease (Shi et al., 2014). Exosomal miRNAs are being studied as biomarkers for various cardiovascular conditions, including myocardial infarction and heart failure (Danielson & Das, 2014), urinary exosomes are being investigated as a source of biomarkers for renal pathologies such as acute kidney injury and chronic kidney disease (Miranda et al., 2010), and exosomes in the maternal circulation are being studied as potential biomarkers for conditions such as preeclampsia and gestational diabetes (Salomon et al., 2016).

5.2. Therapeutic Applications

The natural ability of exosomes to transfer biological cargo between cells has inspired their development as drug delivery vehicles and therapeutic agents in their own right.

5.2.1. Drug Delivery

Exosomes offer several advantages as drug delivery vehicles.

Researchers have explored loading exosomes with various therapeutic cargoes.

Chemotherapeutic agents such as doxorubicin and paclitaxel have been successfully loaded into exosomes, showing improved efficacy and reduced toxicity compared to free drugs (Kim et al., 2016; Saari et al., 2015).

Proteins and peptides have been carried in the same way: catalase-loaded exosomes have shown neuroprotective effects in Parkinson's disease models (Haney et al., 2015).

siRNAs and miRNAs loaded into exosomes have demonstrated therapeutic potential in various disease models, including cancer and neurodegenerative disorders (Alvarez-Erviti et al., 2011).

Exosomes have been used to deliver CRISPR-Cas9 components for genome editing, offering a potential alternative to viral vectors (Lin et al., 2018).

These engineered exosomes have shown promise in preclinical studies for treating various diseases, including cancer, neurodegenerative diseases, and inflammatory disorders (Kamerkar et al., 2017; Haney et al., 2015; Zhuang et al., 2011).

5.2.2. Regenerative Medicine

Exosomes, particularly those derived from mesenchymal stem cells (MSCs), have shown promise in various regenerative applications. In cardiovascular repair they promote cardiac regeneration and reduce inflammation following myocardial infarction (Arslan et al., 2013), and in the nervous system they enhance functional recovery in models of stroke and traumatic brain injury (Xin et al., 2013). In wound healing they accelerate cutaneous wound closure and reduce scar formation (Shabbir et al., 2015), while in liver and kidney regeneration they ameliorate organ damage in models of acute injury and chronic disease (Tan et al., 2014; Bruno et al., 2012). In osteoarthritis they reduce cartilage degradation and promote tissue repair in joint diseases (Cosenza et al., 2017), and in lung injuries they attenuate inflammation and fibrosis in models of acute lung injury and pulmonary fibrosis (Cruz & Rocco, 2020).

The therapeutic effects of these exosomes are attributed to their complex cargo of proteins, lipids, and RNAs, which can modulate multiple cellular processes simultaneously. In regenerative medicine, exosomes are attractive partly for practical reasons: they are easier to store and handle than living cells, and because they do not replicate they cannot themselves form a tumour (Phinney & Pittenger, 2017). Immunogenicity and overall safety are harder to generalize, since both depend on how a given preparation is made and what it happens to contain.

5.2.3. Immunomodulation

Exosomes have shown potential as immunotherapeutic agents, both for enhancing anti-tumour immunity and for suppressing autoimmune responses.

Dendritic cell-derived exosomes loaded with tumour antigens have been explored as cancer vaccines in clinical trials (Besse et al., 2016). These exosomes can stimulate antigen-specific T cell responses, potentially enhancing anti-tumour immunity.

The immunomodulatory effects of exosomes are mediated by their complex cargo of proteins and RNAs, which can influence various aspects of immune cell function, including activation, differentiation, and cytokine production (Robbins & Morelli, 2014).

6. Challenges in Exosome Research and Clinical Translation

Despite the investigational potential of exosomes in diagnostics and therapeutics, several challenges need to be addressed to facilitate their successful clinical translation.

6.1. Isolation and Purification

Obtaining pure, well-characterised exosome preparations remains a significant challenge. Current isolation methods, including ultracentrifugation, size-exclusion chromatography, and immunoaffinity capture, each have limitations in terms of purity, yield, or scalability (Théry et al., 2018). The heterogeneity of extracellular vesicle populations further complicates this issue, as it can be difficult to separate exosomes from other types of vesicles with similar physical properties (Witwer et al., 2013).

Recent advances in microfluidic technologies and nanomaterial-based separation methods offer promising solutions for more efficient and selective exosome isolation (Liu et al., 2017). However, standardisation of isolation protocols across the field remains a need to ensure reproducibility and comparability of results between studies.

6.2. Characterization

Accurate characterisation of exosome populations is important for understanding their biological functions and developing them as therapeutic agents. However, the small size of exosomes poses challenges for their visualisation and analysis. While techniques such as nanoparticle tracking analysis, transmission electron microscopy, and flow cytometry provide valuable information, each has limitations in terms of resolution, specificity, or throughput (Gardiner et al., 2016).

6.3. Cargo Loading and Engineering

For therapeutic applications, efficient and reproducible methods for loading exosomes with desired cargo molecules are essential. While various approaches have been developed, including electroporation, sonication, and chemical transfection, each has limitations in terms of loading efficiency, cargo stability, or potential damage to the exosome structure (Luan et al., 2017).

Additionally, engineering exosomes to enhance their targeting specificity or therapeutic efficacy presents both opportunities and challenges. Strategies such as surface modification with targeting ligands or expression of therapeutic proteins have shown promise but optimising these approaches while maintaining the natural properties of exosomes requires careful consideration (Kooijmans et al., 2013).

6.4. Scalability and Manufacturing

The translation of exosome-based therapies to the clinic requires the development of scalable, reproducible, and cost-effective manufacturing processes. Current methods for large-scale production of clinical-grade exosomes are limited, and issues such as batch-to-batch variability and quality control need to be addressed (Whitford & Guterstam, 2019).

Advances in bioreactor technologies and cell engineering approaches are being explored to enhance exosome production and quality (Watson et al., 2018). However, establishing robust and standardised manufacturing processes that meet regulatory requirements remains a significant challenge for the field.

6.5. Pharmacokinetics and Biodistribution

Understanding the in vivo behaviour of exosomes is key for their development as therapeutic agents. While studies have provided insights into the biodistribution and clearance of exosomes, many questions remain regarding their tissue-specific targeting, cellular uptake mechanisms, and long-term fate in the body (Wiklander et al., 2015).

Improved methods for tracking exosomes in vivo, such as advanced imaging techniques and novel labeling strategies, are needed to fully elucidate their pharmacokinetic properties and optimise their therapeutic applications (Lai et al., 2015).

6.6. Regulatory Considerations

The complex and heterogeneous nature of exosomes presents unique challenges for regulatory agencies. Developing appropriate guidelines for characterisation, quality control, and clinical testing of exosome-based therapeutics is an ongoing process that requires close collaboration between researchers, industry, and regulatory bodies (Lener et al., 2015).

7. Future Directions and Emerging Trends

Several trends are now shaping exosome research.

7.1. Artificial Intelligence and Machine Learning in Exosome Research

The integration of artificial intelligence (AI) and machine learning (ML) approaches is poised to advance various aspects of exosome research.

7.2. Single-Exosome Analysis

Emerging technologies in this area include nanoscale flow cytometry for the high-throughput analysis of individual exosomes (Morales-Kastresana et al., 2017), single-vesicle Raman spectroscopy for the label-free characterisation of exosome composition (Lee et al., 2018), and single-molecule fluorescence techniques for studying exosome uptake and intracellular trafficking at the individual vesicle level (Verweij et al., 2021).

These single-exosome analysis techniques are likely to provide new insights into exosome heterogeneity and function, potentially leading to more precise diagnostic and therapeutic applications.

7.3. Exosome-Inspired Synthetic Nanovesicles

Drawing inspiration from natural exosomes, researchers are developing synthetic nanovesicles that mimic the beneficial properties of exosomes while offering greater control over composition and function (Jang et al., 2013). These "artificial exosomes" or "exosome mimetics" could overcome some of the challenges associated with natural exosomes, such as heterogeneity and scalability, while retaining their biocompatibility and targeting capabilities.

Recent developments in this area include exosome-mimetic nanovesicles produced by serial extrusion of cells through membrane filters (Jang et al., 2013) and exosome–liposome hybrid nanoparticles capable of carrying large cargoes such as the CRISPR/Cas9 system (Lin et al., 2018).

These engineered nanovesicles allow more controlled and scalable production of exosome-like therapeutics.

7.4. Exosomes in Personalized Medicine

Emerging approaches in this area include tumour-derived exosomes as personalised cancer vaccines, which leverage the unique antigen profile of an individual's tumour (Escudier et al., 2005); engineered exosomes carrying patient-specific neoantigens for cancer immunotherapy (Meng et al., 2025); and customized exosome-based delivery of gene therapies tailored to an individual's genetic profile (Usman et al., 2018).

Better knowledge of exosome biology, together with improved engineering methods, should widen the scope for individualized exosome-based treatment.

7.5. Exosome-Mediated Tissue Engineering

The role of exosomes in intercellular communication is being harnessed for tissue engineering applications. Exosomes derived from specific cell types or engineered to carry particular signalling molecules could be incorporated into biomaterials to guide tissue regeneration and promote functional recovery (Qin et al., 2016).

Recent developments in this area include exosome-functionalized scaffolds for bone and cartilage regeneration (Diomede et al., 2018), hydrogels incorporating stem cell-derived exosomes for cardiac tissue engineering, and exosome-based approaches for promoting angiogenesis in engineered tissues (Shabbir et al., 2015).

These exosome-based tissue engineering strategies may enhance the efficacy of regenerative medicine approaches across a range of applications.

7.6. Exosomes in Gene Therapy

The natural ability of exosomes to transfer nucleic acids between cells makes them attractive vehicles for gene therapy applications. Ongoing research is exploring the use of exosomes for delivering therapeutic genes, gene-editing tools like CRISPR-Cas9, and RNA-based therapeutics (Usman et al., 2018).

Emerging approaches in this area include exosome-mediated delivery of CRISPR-Cas9 components for targeted gene editing (Lin et al., 2018), engineered exosomes for the delivery of therapeutic mRNAs and proteins (Yang et al., 2020), and exosome-based delivery of siRNAs and miRNAs for gene silencing therapies (Alvarez-Erviti et al., 2011).

As gene therapy approaches continue to advance, exosomes may play an increasingly important role as delivery vehicles, potentially offering advantages over viral vectors in terms of safety and targeting.

7.7. Exosomes in Neurodegenerative Disease Therapy

Given their ability to cross the blood-brain barrier, exosomes are being intensively studied as potential therapeutic agents for neurodegenerative diseases. Recent developments include exosomes engineered to deliver

neuroprotective proteins or RNAs to the brain (Qu et al., 2018), stem cell-derived exosomes for promoting neuroregeneration and reducing neuroinflammation (Webb et al., 2018), and exosome-based approaches for targeting and clearing pathogenic proteins in Alzheimer's and Parkinson's diseases (Yuyama et al., 2014).

These approaches could advance the treatment of neurodegenerative disorders, which have long been challenging to target with traditional therapeutics.

7.8. Exosomes in COVID-19 Research

The COVID-19 pandemic has spurred new interest in the role of exosomes in viral infections and in their potential as therapeutic agents. Emerging areas of research include investigating the role of exosomes in SARS-CoV-2 transmission and pathogenesis (Hassanpour et al., 2020), exploring exosomes as delivery vehicles for antiviral drugs or RNA-based therapeutics against COVID-19 (Sengupta et al., 2020), and studying exosomal biomarkers for COVID-19 diagnosis and prognosis (Barberis et al., 2021).

This research may not only contribute to our understanding and management of COVID-19 but also provide insights applicable to other viral diseases.

8. Conclusion

Exosomes have become key players in intercellular communication and candidates for diagnostic and therapeutic applications across a wide range of diseases. Their complex cargo of proteins, lipids, and nucleic acids enables them to modulate diverse cellular processes and influence both physiological and pathological states. The unique properties of exosomes, including their stability, biocompatibility, and ability to cross biological barriers, make them attractive as natural nanocarriers for drug delivery and as sources of biomarkers for liquid biopsy-based diagnostics.

Significant progress has been made in understanding exosome biogenesis, composition, and function, as well as in developing methods for their isolation, characterisation, and engineering. Exosome-based liquid biopsies are showing promise for early disease detection and monitoring, particularly in cancer. In therapeutic terms, exosomes are being explored as drug delivery vehicles, regenerative agents, and immunomodulators, with several clinical trials underway.

However, significant problems remain in translating the potential of exosomes into clinical reality. Addressing issues related to isolation, characterisation, engineering, and large-scale production of exosomes will be essential for their successful development as therapeutic agents and diagnostic tools. Standardisation of methods and regulatory considerations also present important hurdles to overcome.

Looking ahead, the field of exosome research is likely to move quickly. Emerging technologies such as single-exosome analysis, AI-driven approaches, and advanced engineering techniques are likely to deepen our understanding of exosome biology and expand their applications. The development of exosome-inspired synthetic nanovesicles and the exploration of exosomes in personalised medicine, tissue engineering, and gene therapy offer promising new directions.

As our knowledge of exosome biology continues to grow, and new technologies emerge to overcome current limitations, the coming years are likely to see significant advances in exosome-based therapies and diagnostics. The versatility and potential of these vesicles suggest that they will play an increasingly important role in the future of personalised medicine, tissue engineering, and targeted therapies for a wide range of diseases.

Overall, while obstacles persist, the field of exosome research has investigational potential for advancing our approach to disease diagnosis and treatment. Continued interdisciplinary collaboration and investment in this area will be essential to fully realize the potential of exosomes in improving human health.

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5.2. BIOGENESIS AND RELEASE OF EXOSOMES

Exosomes, small extracellular vesicles ranging from 30 to 150 nm in diameter, have attracted attention as central mediators of intercellular communication, playing key roles in both physiological processes and pathological conditions (Xu et al., 2016). These vesicles act as carriers for the transport of a diverse array of bioactive molecules, including proteins, lipids, various species of RNA (such as mRNA, miRNA, and long non-coding RNA), and even DNA fragments, thereby facilitating the exchange of genetic and molecular information between cells (Valadi et al., 2007; Thakur et al., 2014). Size alone does not establish endosomal origin; EV or small EV is the operational term when that origin has not been demonstrated.

The biogenesis and release of exosomes involve intricate cellular processes that are tightly regulated by various molecular mechanisms. Understanding these processes is not only important for elucidating the fundamental biology of intercellular communication but also for developing potential therapeutic strategies that harness or target exosome biology. This broad synthesis provides an in-depth overview of the current knowledge regarding exosome biogenesis and release, exploring the molecular machinery involved, the regulatory mechanisms at play, and the implications for health and disease. Figure 5.2.1 shows biogenesis, release and uptake as a single sequence.

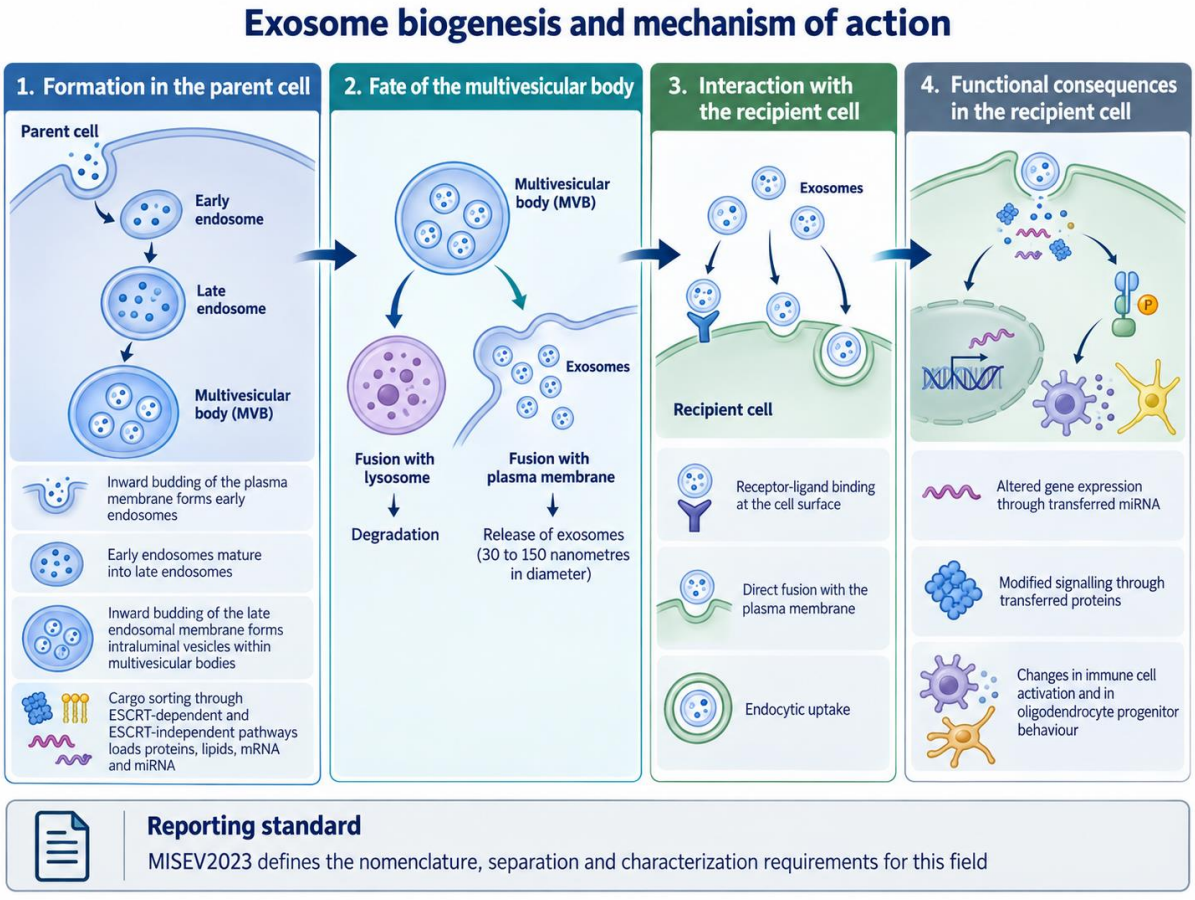


Figure 5.2.1. Exosome biogenesis, release and interaction with recipient cells. Nomenclature and characterisation follow the MISEV2023 recommendations.

1. The Endosomal Origin of Exosomes

1.1. Early Endosome Formation

Exosome biogenesis begins with the formation of early endosomes, a process that involves the invagination of the plasma membrane (Klumperman & Raposo, 2014). This initial step is mediated by clathrin-dependent and independent endocytosis mechanisms, involving various adaptor proteins and lipid rafts (Kirchhausen et al., 2014).

The formation of early endosomes is facilitated by an interaction of proteins, including Rab5, a small GTPase that drives endosome biogenesis and maturation (Zeigerer et al., 2012). Rab5 recruits effector proteins such as EEA1 (Early Endosome Antigen 1) and phosphatidylinositol 3-kinases,

which are essential for endosome fusion and maturation (Christoforidis et al., 1999).

Recent studies have also highlighted the role of BAR (Bin/Amphiphysin/Rvs) domain-containing proteins in the early stages of endosome formation. These proteins can sense and induce membrane curvature, contributing to the initial invagination of the plasma membrane (Simunovic et al., 2019).

1.2. Endosome Maturation

As early endosomes mature, they undergo a series of transformations, including changes in their protein composition, luminal pH, and morphology (Huotari & Helenius, 2011). This maturation process involves the gradual replacement of Rab5 with Rab7, a key marker of late endosomes (Rink et al., 2005).

The maturation of endosomes is accompanied by changes in their phosphoinositide composition. Early endosomes are enriched in phosphatidylinositol 3-phosphate (PI3P), while late endosomes accumulate phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2) (Schink et al., 2016). These changes in lipid composition are important for the recruitment of specific proteins involved in cargo sorting and intraluminal vesicle formation.

Another important aspect of endosome maturation is the progressive acidification of the endosomal lumen. This is achieved through the action of vacuolar ATPases, which pump protons into the endosome, lowering the pH from about 6.8 in early endosomes to around 5.5 in late endosomes (Huotari & Helenius, 2011). This acidification is essential for the activation of hydrolytic enzymes and the dissociation of receptor-ligand complexes.

2. Formation of Multivesicular Bodies (MVBs)

2.1. The ESCRT Machinery

The formation of multivesicular bodies (MVBs) is a critical step in exosome biogenesis. This process involves the inward budding of the endosomal membrane to form intraluminal vesicles (ILVs), which will eventually become exosomes upon fusion of the MVB with the plasma membrane (Henne et al., 2011).

The endosomal sorting complex required for transport (ESCRT) machinery is central to this process. The ESCRT machinery consists of four main protein complexes (ESCRT-0, -I, -II, and -III) and associated proteins that work in concert to mediate the formation of ILVs and the sorting of cargo into these vesicles (Colombo et al., 2013).

ESCRT-0 initiates the process by recognising and clustering ubiquitinated proteins on the endosomal membrane. This complex contains ubiquitin-binding domains and interacts with PI3P, allowing it to bind to both cargo proteins and the endosomal membrane (Raiborg & Stenmark, 2009).

ESCRT-I and ESCRT-II are then recruited to the endosomal membrane, where they work together to induce membrane deformation and initiate the budding process. These complexes form a supercomplex that stabilizes the neck of the budding vesicle (Wollert & Hurley, 2010).

ESCRT-III, the final complex in the pathway, assembles into spiral filaments at the neck of the budding vesicle, facilitating membrane scission and the release of the ILV into the MVB lumen. The ATPase Vps4 then disassembles the ESCRT-III complex, allowing it to be recycled for further rounds of vesicle formation (Adell et al., 2014).

Recent studies have provided new insights into the mechanics of ESCRT-mediated membrane budding and scission. For example, Schöneberg et al. (2017) reviewed how ESCRT-III filaments drive reverse-topology membrane deformation and scission.

2.2. ESCRT-Independent Mechanisms

While the ESCRT machinery is essential to ILV formation, ESCRT-independent mechanisms of exosome biogenesis have also been identified. One such pathway involves the lipid ceramide, which can induce spontaneous vesicle budding due to its cone-shaped structure (Trajkovic et al., 2008).

The enzyme neutral sphingomyelinase 2 (nSMase2) contributes substantially to this process by catalyzing the production of ceramide from sphingomyelin (Menck et al., 2017). Inhibition of nSMase2 has been shown to reduce exosome release in various cell types, demonstrating the importance of this lipid-mediated pathway.

Another ESCRT-independent mechanism involves tetraspanins, a family of transmembrane proteins that are enriched in exosomes. Tetraspanins, including CD63, CD9, and CD81, can form specialized membrane microdomains called tetraspanin-enriched microdomains (TEMs), which may serve as platforms for the clustering of cargo proteins and the initiation of ILV budding (Andreu & Yáñez-Mó, 2014).

Recent research has suggested that these different mechanisms of ILV formation may not be mutually exclusive. For example, Colombo et al. (2013) demonstrated that

subpopulations of ILVs within the same MVB could be generated by both ESCRT-dependent and ESCRT-independent mechanisms, suggesting an interaction between these pathways.

3. Cargo Sorting into Exosomes

3.1. Protein Sorting Mechanisms

The sorting of specific proteins into exosomes is a highly selective process that involves various molecular mechanisms. The most well-characterised sorting mechanism is ubiquitination, which targets proteins for incorporation into ILVs via the ESCRT machinery (Raiborg & Stenmark, 2009).

However, not all proteins found in exosomes are ubiquitinated, indicating the presence of alternative sorting mechanisms. For instance, tetraspanins and lipid raft domains have been shown to play critical roles in protein sorting into intraluminal vesicles (ILVs), independently of ubiquitination (Kowal et al., 2016; Villarroya-Beltri et al., 2014). These mechanisms may be particularly important for cytosolic proteins that lack transmembrane domains, facilitating their incorporation into exosomes through interactions with specific membrane-associated proteins and lipids.

Specific sequence motifs within proteins can also target them for exosomal sorting. For instance, the YXXΦ motif (where X is any amino acid and Φ is a hydrophobic residue) has been shown to direct proteins to ILVs through interaction with the ESCRT machinery (Bonifacino & Traub, 2003).

Recent studies have also highlighted the role of post-translational modifications and protein interactions in protein sorting to exosomes. For example, the syndecan–syntenin–ALIX pathway has been shown to regulate exosome biogenesis (Baietti et al., 2012).

3.2. RNA Sorting Mechanisms

The sorting of RNA species into exosomes is another area of intense research. Several RNA-binding proteins have been implicated in the sorting of specific miRNAs into exosomes. For example, hnRNPA2B1 has been shown to recognize specific sequence motifs in miRNAs and facilitate their packaging into exosomes (Villarroya-Beltri et al., 2014).

Another RNA-binding protein, YBX1, has been implicated in the sorting of both miRNAs and mRNAs into exosomes. YBX1 can bind to specific sequence motifs in RNAs and may act as a general regulator of RNA sorting into exosomes (Shurtleff et al., 2016).

Post-transcriptional modifications of RNAs can also influence their sorting into exosomes. For instance, 3′-end adenylation or uridylation of miRNAs has been shown to affect their incorporation into exosomes (Koppers-Lalic et al., 2014).

3.3. Lipid-Mediated Sorting

The role of lipids in cargo sorting to exosomes is an emerging area of research. Lipid rafts, cholesterol-rich microdomains in the membrane, have been implicated in the sorting of specific proteins and lipids into exosomes (de Gassart et al., 2004).

Ceramide, in addition to its role in ILV formation, may also play a role in cargo sorting. The cone-shaped structure of ceramide can induce membrane curvature and potentially facilitate the incorporation of certain lipids and proteins into budding vesicles (Trajkovic et al., 2008).

Recent studies have also highlighted the role of specific lipid-modifying enzymes in exosome cargo sorting. For example, the lipid kinase PIKfyve, which generates PI(3,5)P2, has been shown to regulate the sorting of specific proteins into exosomes (Hessvik et al., 2016).

4. Regulation of Exosome Release

4.1. Rab GTPases in Exosome Secretion

The release of exosomes is tightly regulated by various molecular mechanisms, with Rab GTPases playing a central role in this process. Rab proteins are small GTPases that act as molecular switches, cycling between active (GTP-bound) and inactive (GDP-bound) states to regulate various aspects of membrane trafficking (Stenmark, 2009).

Rab27a and Rab27b have been identified as key regulators of exosome secretion (Ostrowski et al., 2010). These proteins control different steps in the exosome secretion pathway, with Rab27a promoting the docking of MVBs to the plasma membrane and Rab27b facilitating their peripheral distribution.

Other Rab proteins have also been implicated in exosome release.

Rab11 is involved in the recycling endosome pathway and has been shown to regulate the release of exosomes containing the transferrin receptor (Savina et al., 2005). Rab35 participates in the release of proteolipid protein-containing exosomes from oligodendroglial cells, playing a role in myelin biogenesis (Hsu et al., 2010). Rab7 has been

implicated in the maturation of late endosomes and their fusion with lysosomes, potentially influencing the balance between exosome secretion and degradation (Vanlandingham & Ceresa, 2009).

Recent studies have highlighted the critical role of Rab proteins in exosome biogenesis and secretion. Among these, Rab27a and Rab27b are key regulators involved in the trafficking and docking of multivesicular bodies (MVBs) to the plasma membrane, enabling the release of exosomes. These processes are particularly key in immune cells, where exosome-mediated intercellular communication is a key determinant of immune regulation and response (Gurung et al., 2021).

4.2. SNARE Proteins and Membrane Fusion

The fusion of MVBs with the plasma membrane, leading to exosome release, is mediated by SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor) proteins. These proteins are essential components of the membrane fusion machinery in eukaryotic cells (Jahn & Scheller, 2006).

Several SNARE proteins have been implicated in exosome release.

VAMP7, a v-SNARE protein, has been shown to regulate the fusion of MVBs with the plasma membrane, thereby facilitating exosome secretion (Fader et al., 2009). YKT6, another SNARE protein, has been implicated in the release of exosomes containing Wnt proteins, which are important signalling molecules in development and disease (Gross et al., 2012). Syntaxin-4 and SNAP-23, two plasma membrane SNAREs, have been shown to be involved in the docking and fusion of MVBs during exosome release (Puri & Roche, 2008).

Recent studies have provided new insights into the regulation of SNARE-mediated exosome release. For example, Wei et al. (2017) demonstrated that pyruvate kinase type M2 (PKM2) promotes exosome secretion in tumour cells by phosphorylating SNAP-23, potentially contributing to cancer progression.

4.3. Calcium Signaling in Exosome Release

Calcium signalling is closely involved in regulating exosome release. Increased intracellular calcium levels have been shown to promote exosome secretion in various cell types (Savina et al., 2003). This effect is mediated, in part, by calcium-dependent activation of protein kinase C (PKC) and synaptotagmin-7, which facilitate the fusion of MVBs with the plasma membrane (Kreutzberger et al., 2017).

Recent research has provided new insights into the mechanisms by which calcium regulates exosome release.

Messenger et al. (2018) identified a Ca^{2+}-stimulated exosome release pathway in cancer cells that is regulated by Munc13-4, which primes MVBs for fusion with the plasma membrane. Catalano and O'Driscoll (2020) reviewed pharmacological inhibitors of EV formation and release, including agents that act on calcium-dependent steps.

4.4. Cytoskeleton Involvement in Exosome Release

The cytoskeleton matters for exosome release, particularly in the transport of MVBs to the plasma membrane and in facilitating membrane fusion events. The actin cytoskeleton, in particular, has been shown to be essential for efficient exosome secretion.

Recent studies have provided new insights into the role of cytoskeleton in exosome release.

Sinha et al. (2016) showed that cortactin promotes exosome secretion by controlling branched actin dynamics at MVB docking sites. Hoshino et al. (2013) found that invadopodia are specialized sites of exosome secretion and that this secretion drives invasive behaviour.

Microtubules also play a role in exosome release, primarily in the long-range transport of MVBs within the cell, and Rab27 GTPases, which control MVB docking, have been linked to invasive growth and metastasis (Hendrix & De Wever, 2013).

5. Regulatory Mechanisms of Exosome Biogenesis and Release

5.1. Transcriptional Regulation

The biogenesis and release of exosomes are subject to regulation at multiple levels, including transcriptional control. Several transcription factors have been implicated in regulating the expression of genes involved in exosome biogenesis and secretion.

One of the most well-studied transcriptional regulators of exosome release is p53, a tumour suppressor protein. Yu et al. (2006) demonstrated that p53 enhances exosome secretion through its transcriptional target TSAP6. This regulation is particularly important in response to cellular stress, such as DNA damage, suggesting a role for exosomes in the cellular stress response.

Other transcription factors involved in regulating exosome biogenesis and release include:

Hypoxia-inducible factor signalling is one route: King et al. (2012) showed that hypoxia enhances exosome release by breast cancer cells. NF-κB has also been proposed to influence exosome secretion in cancer, and Giordano et al. (2020) reviewed the roles of exosomes in breast cancer dissemination, immune evasion, and metastatic colonization. In adipocytes, Durcin et al. (2017) characterised large and small extracellular vesicle subtypes and identified distinct protein and lipid signatures, pointing to links between vesicle release and metabolic regulation.

5.2. Post-Transcriptional Regulation

Post-transcriptional regulation, particularly through miRNAs, may also play a role in controlling exosome biogenesis and release.

MicroRNAs could regulate exosome secretion by targeting components of the secretory machinery, such as Rab27A, although evidence for specific miRNAs remains limited. Rab27a itself supports exosome-dependent and -independent mechanisms that modify the tumour microenvironment and can promote tumour progression (Bobrie et al., 2012).

5.3. Post-Translational Modifications

Post-translational modifications and protein interactions of the exosome machinery provide another layer of regulation. Some examples include:

Hsu et al. (2010) showed that Rab35 and its GTPase-activating proteins TBC1D10A–C regulate exosome secretion. Colombo et al. (2013) found that depleting ESCRT-0 components such as Hrs, together with other ESCRT proteins, altered exosome secretion and composition, highlighting the heterogeneity of extracellular vesicles. Baietti et al. (2012) showed that the syndecan–syntenin–ALIX pathway regulates the biogenesis of exosomes.

5.4. Metabolic Regulation

Cellular metabolism governs exosome biogenesis and release. Recent studies have revealed intricate connections between metabolic pathways and exosome production.

AMPK signalling is one proposed link: as a key regulator of cellular energy homeostasis, AMP-activated protein kinase could couple exosome secretion to energy availability, although direct evidence remains limited. Increased glycolysis, a hallmark of cancer cells, has been shown to promote exosome secretion; Wei et al. (2017) found that upregulation of pyruvate kinase type M2 (PKM2) in cancer cells enhances exosome release, potentially contributing to tumour progression. Lipid metabolism is a further factor. The role of lipids in exosome biogenesis extends beyond their structural components; Llorente et al. (2007) showed that cholesterol regulates the release of prostasomes, exosome-like vesicles, from PC-3 prostate cancer cells, supporting a role for cholesterol-rich membrane domains in exosome biogenesis.

6. Exosome Release in Response to Cellular Stress

Exosome release can be modulated by various forms of cellular stress, often serving as a mechanism for cell-to-cell communication under challenging conditions.

6.1. Oxidative Stress

Oxidative stress has been shown to influence exosome release in various cell types.

Hedlund et al. (2011) showed that thermal and oxidative stress enhance the release of immunosuppressive exosomes bearing NKG2D ligands from leukemia and lymphoma cells, potentially as a mechanism of immune evasion. Atienzar-Aroca et al. (2016) showed that oxidative stress promotes the release of angiogenic exosomes from retinal pigment epithelial cells, which may contribute to pathological angiogenesis in the eye.

6.2. Hypoxia

Hypoxia, or low oxygen conditions, is another important modulator of exosome release.

King et al. (2012) found that hypoxia enhances exosome release by breast cancer cells. Kucharzewska et al. (2013) showed that exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumour development.

6.3. DNA Damage

DNA damage can also trigger changes in exosome release.

Yu et al. (2006) showed that DNA damage-induced activation of p53 enhances exosome secretion through its target gene TSAP6. Lehmann et al. (2008) found that DNA damage-induced senescence increases exosome release from human prostate cancer cells.

6.4. Endoplasmic Reticulum (ER) Stress

ER stress has been implicated in modulating exosome release.

Kanemoto et al. (2016) showed that endoplasmic reticulum (ER) stress enhances multivesicular body formation and exosome release. Hosoi et al. (2018) found that the spliced form of XBP1 mRNA, generated by the IRE1α branch of the unfolded protein response, is incorporated into exosomes during ER stress.

7. Exosome Release in Different Cell Types

While the basic mechanisms of exosome biogenesis and release are conserved across cell types, there are notable differences in the regulation and function of exosome secretion in different cellular contexts.

7.1. Immune Cells

Exosome release plays central roles in immune cell function and communication.

Théry et al. (2002) showed that exosomes released by dendritic cells, which carry MHC class II molecules, can indirectly activate naïve CD4+ T cells. Mittelbrunn et al. (2011) demonstrated that T cells secrete exosomes containing microRNAs that can modulate gene expression in antigen-presenting cells. Saunderson et al. (2008) found that stimulation of primary B cells through CD40 and the IL-4 receptor induces exosome release.

7.2. Cancer Cells

Exosome release from cancer cells has been extensively studied due to their potential roles in tumour progression and metastasis.

Skog et al. (2008) demonstrated that glioblastoma cells secrete vesicles containing mRNA, miRNA, and angiogenic proteins that can stimulate tumour growth and provide diagnostic biomarkers. Peinado et al. (2012) found that melanoma-derived exosomes can educate bone marrow progenitor cells towards a pro-metastatic phenotype through MET, facilitating metastasis.

7.3. Neurons and Glial Cells

Exosome release in the nervous system is central to neuron-glia communication and neurological function.

Lachenal et al. (2011) showed that neurons release exosomes in a synaptic activity-dependent manner, suggesting a role for exosomes in synaptic plasticity. Frühbeis et al. (2013) demonstrated that neurotransmitter-triggered transfer of exosomes from oligodendrocytes mediates oligodendrocyte–neuron communication and can support neurons under stress. Potolicchio et al. (2005) performed a proteomic analysis of microglia-derived exosomes and identified a metabolic role for the aminopeptidase CD13 in neuropeptide catabolism.

7.4. Stem Cells

Exosome release from stem cells has garnered significant interest due to their potential therapeutic applications.

Mesenchymal stem cells provide one example. Lai et al. (2010) showed that MSC-derived exosomes reduce myocardial ischemia/reperfusion injury. Neural stem cells provide another: Webb et al. (2018) found that human neural stem cell extracellular vesicles improved tissue and functional recovery in a murine thromboembolic stroke model. For induced pluripotent stem cells, Santoso et al. (2020) found that exosomes from iPSC-derived cardiomyocytes promote autophagy and myocardial repair in a mouse model of myocardial infarction.

8. Technological Advances in Studying Exosome Biogenesis and Release

Recent technological advancements have greatly enhanced our ability to study exosome biogenesis and release:

8.1 High-Resolution Microscopy

Techniques such as STORM (Stochastic Optical Reconstruction Microscopy) and PALM (Photoactivated Localization Microscopy) have allowed researchers to visualise exosome biogenesis and release in unprecedented detail. Cryo-electron microscopy (cryo-EM) has significantly advanced our understanding of the structural organization of the ESCRT-III complex, which is important for intraluminal vesicle (ILV) formation during exosome biogenesis. A study by Huber et al. (2020) used cryo-EM to elucidate the helical assembly of Saccharomyces cerevisiae Vps24, a component of the ESCRT-III complex, revealing insights into its role in membrane remodeling. These structural insights have deepened our understanding of the molecular mechanisms underlying cargo sorting and membrane deformation within multivesicular bodies (MVBs).

8.2 Live-Cell Imaging

Advances in live-cell imaging have allowed researchers to observe exosome biogenesis and release in real-time.

Bebelman et al. (2020) developed a pH-sensitive optical reporter system to visualise the dynamics of MVB-plasma membrane fusion events during exosome release. Sung et al. (2015) used total internal reflection fluorescence (TIRF) microscopy to track the movement of MVBs and their fusion with the plasma membrane during exosome secretion.

8.3 High-Throughput Screening

High-throughput screening approaches have been employed to identify new regulators of exosome biogenesis and release.

Ostrowski et al. (2010) used an siRNA screen to identify Rab27a and Rab27b as key regulators of exosome secretion. Datta et al. (2018) performed a high-throughput screen of FDA-approved drugs to identify compounds that modulate exosome biogenesis and secretion, potentially leading to new therapeutic strategies.

8.4 Single-Vesicle Analysis

Techniques for analysing individual exosomes have provided new insights into exosome heterogeneity and function.

van der Vlist et al. (2012) described fluorescent labeling of nano-sized vesicles followed by high-resolution flow cytometric analysis, allowing vesicle subpopulations to be characterised, and Théry et al. (2018) set out the MISEV2018 minimal information requirements for studies of extracellular vesicles. Smith et al. (2015) used single-exosome Raman analysis to reveal subpopulations distributed among cell lines, with variability related to membrane content.

9. Exosome Biogenesis and Release in Pathological Conditions

The dysregulation of exosome biogenesis and release has been implicated in various pathological conditions, which suggests how far these processes contribute to cellular homeostasis.

9.1. Cancer

Altered exosome biogenesis and release play important roles in cancer progression and metastasis.

One change is increased exosome secretion: Logozzi et al. (2009) found high levels of exosomes expressing CD63 and caveolin-1 in the plasma of melanoma patients. Cargo processing is also altered: Melo et al. (2014) showed that cancer exosomes can perform cell-independent microRNA biogenesis and promote tumorigenesis. Exosomes also transfer drug resistance: Chen et al. (2014) found that exosomes from drug-resistant breast cancer cells can transfer resistance to sensitive cells through the delivery of specific miRNAs.

9.2. Neurodegenerative Diseases

Exosomes have been implicated in the spread of pathogenic proteins in various neurodegenerative disorders.

Alzheimer's disease provides one example. Rajendran et al. (2006) demonstrated that β-amyloid peptides are released in association with exosomes, potentially contributing to the formation of amyloid plaques. Parkinson's disease provides another. Danzer et al. (2012) showed that α-synuclein oligomers can be transmitted between neurons via exosomes, potentially explaining the progressive nature of the disease. In prion disease, Fevrier et al. (2004) found that prion proteins can be released in association with exosomes, suggesting a mechanism for the intercellular spread of prions.

9.3. Cardiovascular Diseases

Exosome biogenesis and release play important roles in various cardiovascular pathologies.

In atherosclerosis, Hergenreider et al. (2012) demonstrated that endothelial cell-derived exosomes can transfer atheroprotective miRNAs to smooth muscle cells, potentially influencing plaque formation. Gupta and Knowlton (2007) showed that cardiac myocytes release heat shock protein 60 via exosomes in response to hypoxia, which may contribute to inflammation following myocardial infarction.

9.4. Infectious Diseases

Exosomes play complex roles in host-pathogen interactions.

Viral infections provide one example. Narayanan et al. (2013) found that cells infected with HIV-1 release exosomes containing viral proteins and RNA, which can enhance viral spread and modulate host immune responses. Bacterial infections provide another. Bhatnagar et al. (2007) demonstrated that Mycobacterium tuberculosis-infected macrophages release exosomes containing mycobacterial components, which can modulate recipient cell functions.

10. Therapeutic Potential of Modulating Exosome Biogenesis and Release

Understanding the mechanisms of exosome biogenesis and release has opened up new avenues for therapeutic interventions in various diseases.

10.1. Inhibiting Exosome Release in Cancer

Several strategies have been explored to inhibit exosome release in cancer.

One strategy targets Rab GTPases. Ostrowski et al. (2010) showed that Rab27a and Rab27b control different steps of the exosome secretion pathway, and Bobrie et al. (2012) found that knockdown of Rab27a reduced exosome secretion and tumour growth in a mouse breast carcinoma model. A second inhibits nSMase2. Kosaka et al. (2013) demonstrated that inhibition of neutral sphingomyelinase 2 (nSMase2) reduces exosome release and metastasis in breast cancer models. A third blocks MVB fusion, for example by targeting SNARE proteins such as VAMP7, although the effects of such interventions on tumour growth require further study.

10.2. Enhancing Exosome Release for Regenerative Medicine

In contrast to cancer, enhancing exosome release has shown promise in regenerative medicine applications.

Cardiac repair is one application. Ibrahim et al. (2014) demonstrated that exosomes derived from cardiosphere-derived cells can promote cardiac regeneration and improve heart function after myocardial infarction. Neuroprotection is another. Xin et al. (2013) showed that exosomes derived from mesenchymal stem cells can promote functional recovery in stroke models by enhancing neurite remodeling and neurogenesis.

10.3. Engineering Exosome Biogenesis for Drug Delivery

Researchers are exploring ways to engineer the exosome biogenesis pathway to create tailored drug delivery vehicles.

One approach is the loading of therapeutic cargo. Alvarez-Erviti et al. (2011) loaded exosomes with siRNA by electroporation and targeted them to the brain by fusing the exosomal membrane protein Lamp2b with a neuron-specific rabies virus glycoprotein peptide, achieving delivery to the mouse brain after systemic injection. A second modifies surface proteins. Tian et al. (2014) engineered exosomes to display a targeting peptide on their surface, enhancing doxorubicin delivery to tumour tissues.

11. Future Directions and Challenges

While significant progress has been made in understanding exosome biogenesis and release, several challenges and open questions remain:

11.1. Heterogeneity of Exosome Populations

Exosomes are heterogeneous in terms of their size, cargo, and functional properties. Future research should focus on:

Developing methods to isolate and characterize specific subpopulations of exosomes. Understanding the mechanisms that determine exosome heterogeneity and its functional significance.

11.2. Tissue-Specific Regulation of Exosome Biogenesis

While general mechanisms of exosome biogenesis have been elucidated, tissue-specific regulation remains poorly understood. Future studies should investigate:

How exosome biogenesis and release are regulated in different tissue contexts. The role of tissue-specific transcription factors and signalling pathways in modulating exosome production.

11.3. Exosome Biogenesis in In Vivo Systems

Most of our current knowledge about exosome biogenesis comes from in vitro studies. Future research should focus on:

Developing tools to study exosome biogenesis and release in living organisms. Understanding how systemic factors influence exosome production in different tissues.

11.4. Therapeutic Modulation of Exosome Biogenesis

While targeting exosome biogenesis and release shows therapeutic promise, several challenges need to be addressed.

Developing more specific inhibitors of exosome biogenesis and release. Understanding the potential side effects of modulating exosome production systemically. Optimising methods for engineering exosomes for therapeutic applications.

11.5. Integration with Other Cellular Processes

Exosome biogenesis and release are intimately connected with other cellular processes. Future research should explore:

The interplay between exosome biogenesis and autophagy pathways. How exosome production is integrated with cellular stress responses and metabolism. The role of exosome biogenesis in cellular homeostasis and development.

12. Evolutionary Aspects of Exosome Biogenesis and Release

The evolutionary conservation of exosome-mediated communication across diverse species suggests that this mechanism of intercellular signalling emerged early in the evolution of life. Comparative studies of exosome biology across

different species can provide insights into the evolution of multicellularity and intercellular communication.

12.1. Exosomes in Single-Cell Organisms

Interestingly, exosome-like vesicles have been observed in single-cell organisms, suggesting an ancient origin for this form of cellular communication.

Fungi provide one example. Rodrigues et al. (2008) demonstrated that the pathogenic fungus Cryptococcus neoformans produces extracellular vesicles that contain virulence factors, suggesting a role in host-pathogen interactions. Silverman et al. (2010) showed that the parasitic protozoan Leishmania donovani releases exosome-like vesicles that can modulate host immune responses.

12.2. Exosomes in Plants

While most exosome research has focused on animal cells, recent studies have revealed that plants also produce exosome-like vesicles.

Rutter & Innes (2017) demonstrated that plants secrete extracellular vesicles that share many characteristics with mammalian exosomes, including their size, density, and the presence of specific marker proteins. Cai et al. (2018) showed that Arabidopsis thaliana releases exosome-like vesicles containing small RNAs that can be taken up by fungal pathogens, resulting in cross-kingdom RNA interference and enhanced plant immunity.

12.3. Exosomes in Lower Metazoans

Exosome-mediated communication has been observed in lower metazoans, providing insights into the evolution of this process.

Nematodes offer one case. Liégeois et al. (2006) showed that in Caenorhabditis elegans the V0-ATPase mediates the apical secretion of exosomes containing Hedgehog-related proteins from epidermal cells.

12.4. Conservation of Exosome Biogenesis Machinery

Many of the key proteins involved in exosome biogenesis and release are highly conserved across species.

The ESCRT machinery is conserved from archaea to humans, suggesting an ancient origin for this vesicle formation mechanism (Hurley & Hanson, 2010). Rab GTPases, key regulators of exosome release, are found in all eukaryotes and show strong conservation in their functions (Klöpper et al., 2012).

13. Exosome Biogenesis and Release in the Context of Cellular Metabolism

The biogenesis and release of exosomes are intimately connected with cellular metabolism, with recent research revealing complex interactions between these processes.

13.1. Metabolic Regulation of Exosome Release

Cellular metabolism is essential to regulating exosome biogenesis and release.

AMPK signalling has been proposed to couple exosome secretion to cellular energy status, although direct evidence remains limited. mTOR pathway: Zou et al. (2019) showed that inhibition of mTORC1, a central regulator of cell growth and metabolism, enhances exosome release. Increased glycolysis, a hallmark of cancer cells, has been shown to promote exosome secretion; Wei et al. (2017) found that pyruvate kinase M2 (PKM2) regulates exosome release through phosphorylation of synaptosome-associated protein 23 (SNAP-23). Metabolic state also shapes exosome cargo: Kumar et al. (2021) found that high-fat diet-induced upregulation of exosomal phosphatidylcholine contributes to insulin resistance.

13.2. Exosomes in Metabolic Communication

Exosomes play a role in metabolic communication between cells and tissues.

Thomou et al. (2017) demonstrated that adipose tissue secretes exosomes containing microRNAs that can regulate gene expression in distant tissues, potentially contributing to systemic metabolic regulation. Liver-derived exosomes contribute substantially to inter-organ metabolic communication. Recent studies indicate that hepatocyte-derived exosomes can transfer various biomolecules, including proteins, lipids, mRNAs, and microRNAs, to other tissues, potentially influencing systemic metabolic processes and contributing to metabolic regulation (Pan et al., 2022).

13.3. Mitochondrial DNA in Exosomes

Recent studies have revealed that exosomes can contain mitochondrial DNA (mtDNA).

Sansone et al. (2017) demonstrated that cancer-associated fibroblasts can transfer mtDNA to breast cancer cells via exosomes, promoting exit from metabolic quiescence and enhancing tumour growth. Guescini et al. (2010) showed that astrocytes release exosomes containing mtDNA, suggesting a potential mechanism for intercellular mitochondrial gene transfer.

14. Conclusion

The biogenesis and release of exosomes represent a fascinating and complex aspect of cellular biology with far-reaching implications for health and disease. From their evolutionary origins to their roles in modern multicellular organisms, exosomes have been investigated as key mediators of intercellular communication.

The integration of advanced technologies, including super-resolution microscopy, microfluidic devices, and artificial intelligence, is accelerating our ability to unravel the mysteries of exosome biogenesis and release.

However, many questions remain unanswered, particularly regarding the tissue-specific regulation of exosome production, the heterogeneity of exosome populations, and the interaction between exosome biogenesis and cellular metabolism. Addressing these challenges will require continued interdisciplinary research and technological innovation.

By deepening our understanding of exosome biogenesis and release, we stand to gain not only insights into fundamental cellular processes but also powerful new tools for diagnosing and treating a wide range of diseases. The coming years are likely to see significant advances in our ability to modulate exosome biogenesis and release, potentially leading to novel therapeutic strategies for conditions ranging from neurodegenerative disorders to cancer.

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5.3. CARGO OF EXOSOMES: PROTEINS, LIPIDS, RNA

Vesicle cargo varies with the source cell and its environment. Figure 5.3.1 outlines the principal cargo classes and the factors that influence them.

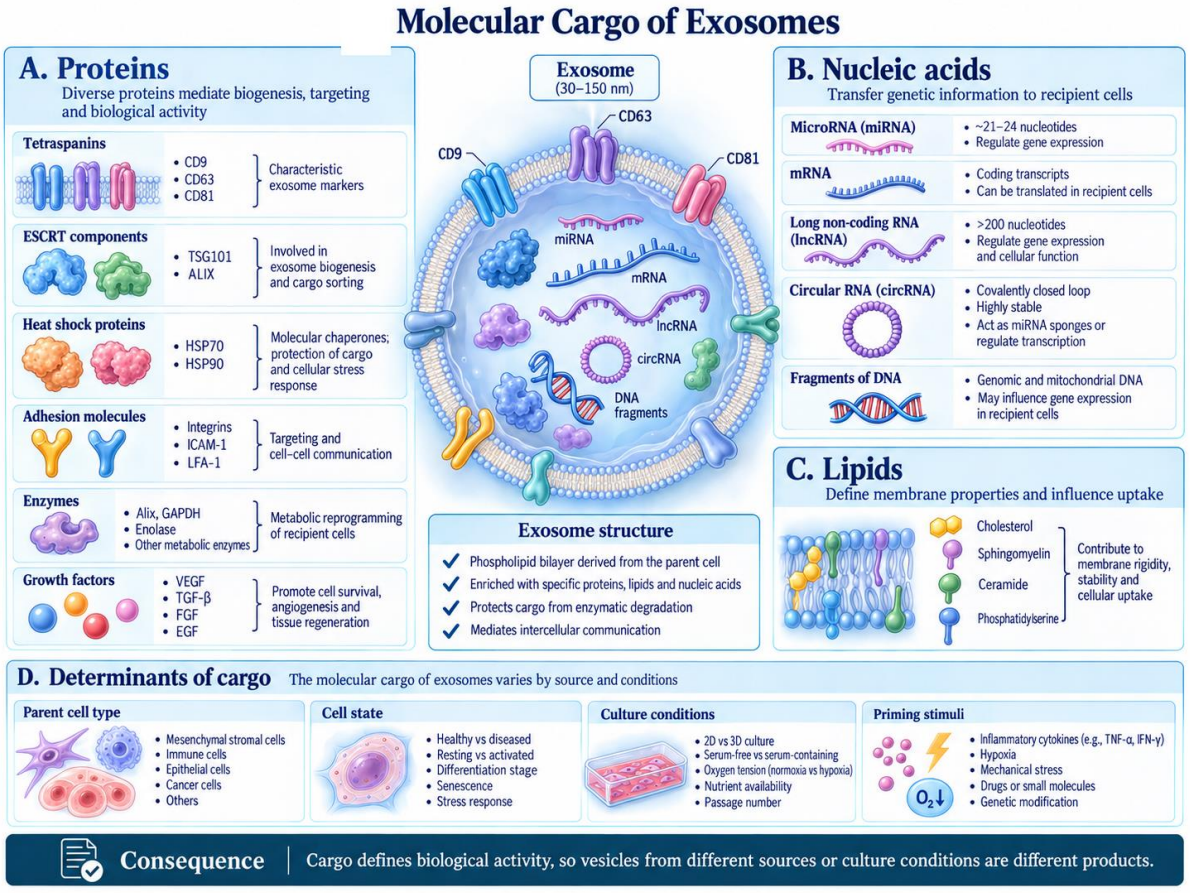


Figure 5.3.1. Molecular cargo of exosomes and the factors that determine it. Because cargo defines biological activity, vesicles from different sources or culture conditions are different products.

1. Introduction to Exosomes

1.1. Definition and Characteristics

Exosomes are small extracellular vesicles ranging from 30 to 150 nm in diameter that have developed into essential mediators of intercellular communication (Théry et al., 2018). These nanoscale vesicles, secreted by virtually all cell types, have garnered substantial attention in the scientific community due to their unique ability to transport a diverse array of biological molecules between cells. The cargo carried by exosomes includes proteins, lipids, various forms of RNA, and even DNA fragments, making them potent vehicles for the transfer of molecular information (Colombo et al., 2014; Kalluri & LeBleu, 2020). Size alone does not establish endosomal origin; EV or small EV is the operational term when that origin has not been demonstrated.

Exosomes are characterised by their distinct biophysical and biochemical properties. Their size range distinguishes them from larger extracellular vesicles such as microvesicles (100-1000 nm) and apoptotic bodies (500-2000 nm) (van Niel et al., 2018). Exosomes have a density ranging from 1.13 to 1.19 g/mL, which allows for their separation using density gradient centrifugation (Théry et al., 2006). The lipid bilayer membrane of exosomes is enriched in cholesterol, sphingomyelin, and ceramide, contributing to their stability in extracellular environments (Skotland et al., 2017a).

1.2. Historical Perspective

The discovery of exosomes can be traced back to the early 1980s when two seminal studies laid the foundation for this field. In 1983, Harding et al. observed the release of small vesicles containing transferrin receptors during

reticulocyte maturation (Harding et al., 1983). Concurrently, Pan and Johnstone reported the externalization of transferrin receptors via small vesicles in sheep reticulocytes (Pan & Johnstone, 1983). These vesicles were initially thought to be a mechanism for cellular waste disposal.

The term "exosome" was first coined by Johnstone et al. in 1987 to describe these small vesicles of endosomal origin (Johnstone et al., 1987). However, it wasn't until the late 1990s that the potential biological significance of exosomes began to be recognized. An important study by Raposo et al. in 1996 demonstrated that B lymphocytes secrete exosomes carrying MHC class II molecules capable of inducing antigen-specific T cell responses (Raposo et al., 1996). This finding sparked interest in the immunological functions of exosomes.

The field of exosome research experienced a fundamental change in 2007 when Valadi et al. reported the presence of mRNA and miRNA in exosomes, which could be transferred to recipient cells and remain functional (Valadi et al., 2007). This discovery opened up new avenues for understanding exosome-mediated intercellular communication and their potential role in various physiological and pathological processes.

1.3. Biogenesis of Exosomes

The biogenesis of exosomes is a complex, multi-step process that occurs within the endosomal system. The process begins with the formation of early endosomes through the invagination of the plasma membrane (Hessvik & Llorente, 2018). These early endosomes mature into late endosomes, also known as multivesicular bodies (MVBs), through a series of changes in their protein composition and morphology.

During MVB formation, the endosomal membrane invaginates inward, forming intraluminal vesicles (ILVs) within the lumen of the MVB. This process is tightly regulated and involves various molecular machineries, including the Endosomal Sorting Complexes Required for Transport (ESCRT) proteins (Colombo et al., 2013). The ESCRT machinery consists of four multiprotein complexes (ESCRT-0, -I, -II, and -III) and associated proteins that work together to recognize ubiquitinated proteins and facilitate their sorting into ILVs.

In addition to the ESCRT-dependent pathway, ESCRT-independent mechanisms of ILV formation have been identified. These involve lipid-driven processes, such as the generation of ceramide by neutral sphingomyelinase 2 (nSMase2), which can induce membrane curvature and promote ILV budding (Trajkovic et al., 2008). Tetraspanins, a family of membrane proteins enriched in exosomes, have also been implicated in ESCRT-independent exosome biogenesis (van Niel et al., 2011).

Once formed, MVBs can either fuse with lysosomes for degradation of their contents or fuse with the plasma membrane to release their ILVs as exosomes into the extracellular space (Mathieu et al., 2019). The fate of MVBs is regulated by various factors, including Rab GTPases (e.g., Rab27a, Rab27b, and Rab35) and SNARE proteins, which play central roles in membrane trafficking and fusion events (Ostrowski et al., 2010; Hsu et al., 2010).

1.4. Significance in Intercellular Communication

Exosomes have come to be seen as key players in intercellular communication, capable of transferring a diverse array of bioactive molecules between cells over both short and long distances (Tkach & Théry, 2016). This mode of communication offers several unique advantages.

The lipid bilayer of exosomes provides protection for their cargo against degradation in the extracellular environment, allowing for the stable transfer of sensitive molecules such as RNA (Kamerkar et al., 2017). Exosomes can also exhibit cellular tropism, preferentially interacting with specific recipient cells based on the presence of surface proteins and receptors (Hoshino et al., 2015). The cargo delivered by exosomes can remain functional in recipient cells, potentially altering their phenotype or behaviour (Valadi et al., 2007). Exosomes can travel through biological fluids, enabling communication between distant organs and tissues (Thomou et al., 2017). The diverse cargo of exosomes allows for the simultaneous transfer of multiple signalling molecules, enabling more complex and nuanced communication than single-molecule signalling (Patel et al., 2019).

The significance of exosome-mediated communication has been demonstrated in various physiological and pathological contexts. In cancer, tumour-derived exosomes have been shown to promote metastasis by preparing pre-metastatic niches in distant organs (Peinado et al., 2012). In the immune system, exosomes play important roles in antigen presentation, T cell activation, and modulation of inflammatory responses (Robbins & Morelli, 2014). Exosomes have also been implicated in neurodegenerative diseases, potentially contributing to the spread of pathogenic proteins (Soria et al., 2017).

Furthermore, the discovery of exosomes as carriers of functional RNA molecules has substantially changed our

understanding of gene regulation and opened up new possibilities for therapeutic applications. Exosomal miRNAs, for instance, have been shown to modulate gene expression in recipient cells, influencing various cellular processes and potentially serving as biomarkers for disease (Melo et al., 2014).

The unique properties of exosomes make them attractive candidates for diagnostic and therapeutic applications, driving intense research efforts to apply them in clinical settings.

2. Composition of Exosomal Cargo

2.1. Proteins

2.1.1 Types of Exosomal Proteins

Exosomes carry a diverse array of proteins that reflect the physiological state of their cell of origin and play important roles in the biological functions of these vesicles (Li et al., 2021). The protein composition of exosomes includes both conserved and cell-type-specific proteins, which can be broadly categorized into several groups. The major protein categories found in exosomes include membrane transport and fusion proteins, cytoskeletal proteins, stress response proteins, and enzymes involved in metabolic pathways. These proteins contribute to various functions such as cell signalling, intercellular communication, and regulation of the tumour microenvironment.

Studies have shown that exosomal proteins such as tetraspanins (CD9, CD63, CD81), heat shock proteins (HSP70, HSP90), and Rab family proteins are commonly found in exosomes, highlighting their conserved nature across different cell types (Li et al., 2021). Additionally, exosomal protein profiles can serve as potential biomarkers for various diseases, including cancer and neurodegenerative disorders.

The protein composition of exosomes includes both conserved and cell-type-specific proteins, which can be broadly categorized into several groups.

CD9, CD63, and CD81 are among the most commonly found tetraspanins in exosomes. These proteins are involved in exosome biogenesis and are often used as exosomal markers (Andreu & Yáñez-Mó, 2014). Tetraspanins form tetraspanin-enriched microdomains (TEMs) on the exosomal membrane, which may play a role in exosome formation and cargo sorting (Zimmerman et al., 2016). HSP70 and HSP90 are frequently found in exosomes and play roles in protein folding and cellular stress responses (Zhu et al., 2014). These proteins may also contribute to the immunomodulatory effects of exosomes (Chalmin et al., 2010). ESCRT-associated proteins such as TSG101 and ALIX are also present, which are involved in the formation and sorting of exosomes (Colombo et al., 2014). The presence of these proteins in exosomes reflects their endosomal origin and the involvement of the ESCRT machinery in exosome biogenesis. Proteins such as integrins and intercellular adhesion molecule 1 (ICAM-1) are present in exosomes and may be involved in cellular uptake and targeting (Clayton et al., 2004). Integrins, in particular, have been shown to determine the organotropic nature of exosome targeting in metastasis (Hoshino et al., 2015). Various enzymes, including glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate kinase, have been identified in exosomes (Mathivanan et al., 2010). Some of these enzymes may retain their catalytic activity within exosomes or upon delivery to recipient cells. Growth factors, cytokines and other signalling molecules can be packaged into exosomes, potentially mediating paracrine effects (Peinado et al., 2012). For example, transforming growth factor-β (TGF-β) has been found in tumour-derived exosomes and can promote immunosuppression and metastasis (Webber et al., 2010). Major histocompatibility complex (MHC) class I and II molecules have been found in exosomes, suggesting a role in immune modulation (Théry et al., 2002). Exosomes derived from antigen-presenting cells can directly activate T cells or transfer antigens to other antigen-presenting cells (Raposo et al., 1996). Proteins involved in membrane transport, such as sodium/potassium ATPases and aquaporins, have been identified in exosomes (Graner et al., 2009). These proteins may play a role in maintaining exosome integrity or facilitating the transfer of small molecules. Actin, tubulin and intermediate filament proteins have been found in exosomes (Théry et al., 2001). These proteins may be involved in exosome formation or contribute to the structural integrity of the vesicles. RNA-binding proteins complete this picture. Proteins such as Argonaute2 (AGO2), which is involved in miRNA function, have been identified in exosomes (Melo et al., 2014). These proteins may play a role in the packaging and function of exosomal RNA cargo.

2.1.2 Functional Implications of Exosomal Proteins

The protein composition of exosomes can vary significantly depending on the cell type of origin and the physiological or pathological state of the cell. This variability in protein content contributes to the diverse functional roles of exosomes in different biological contexts.

One context is cancer progression and metastasis. Tumour-derived exosomes often carry proteins involved in cell proliferation, angiogenesis, and metastasis (Lazar et al., 2016). For example, exosomal integrins have been shown to determine organotropic metastasis by preparing pre-metastatic niches in specific organs (Hoshino et al., 2015). Additionally, matrix metalloproteinases (MMPs) found in cancer-derived exosomes can promote extracellular matrix degradation and facilitate tumour invasion (Shimoda & Khokha, 2017). Exosomal proteins play key roles in both innate and adaptive immune responses. MHC molecules and co-stimulatory proteins on exosomes can directly activate T cells or transfer antigens to other antigen-presenting cells (Théry et al., 2002). Exosomal HSPs have been shown to activate natural killer (NK) cells and potentially contribute to tumour immunogenicity (Gobbo et al., 2016). Conversely, tumour-derived exosomes carrying immunosuppressive proteins like PD-L1 can inhibit T cell responses and promote immune evasion (Chen et al., 2018). In neurodegenerative disorders, exosomes may contain proteins associated with pathological processes. For instance, α-synuclein-containing exosomes have been implicated in the spread of Parkinson's disease pathology (Stuendl et al., 2016), while tau-containing exosomes may contribute to the progression of Alzheimer's disease (Wang et al., 2017). Conversely, exosomes may also play a neuroprotective role by delivering functional proteins that support neuronal survival and regeneration (Xin et al., 2013). Exosomal proteins contribute to various aspects of cardiovascular physiology and pathology. For example, exosomes derived from cardiomyocytes under stress conditions can carry functional HSP60, which may serve as a danger signal to surrounding cells (Gupta & Knowlton, 2007). Endothelial cell-derived exosomes containing delta-like 4 (DLL4) can modulate angiogenesis by transferring Notch signalling to recipient cells (Sheldon et al., 2010). Mesenchymal stem cell (MSC)-derived exosomes have been shown to promote tissue repair in various contexts. These exosomes often carry growth factors, cytokines, and enzymes that can stimulate cell proliferation, reduce inflammation, and enhance tissue regeneration (Phinney & Pittenger, 2017). For instance, MSC-derived exosomes containing VEGF, HGF, and MMPs have been shown to promote angiogenesis and wound healing (Shabbir et al., 2015).

2.1.3 Proteomics of Exosomes

Recent advances in proteomic technologies have greatly enhanced our understanding of the exosomal proteome. High-resolution mass spectrometry-based approaches have enabled the identification of thousands of proteins across various exosome preparations. Kalra et al. (2012) established Vesiclepedia, a continuously annotated compendium of molecules identified in extracellular vesicles, which documents the diversity of the vesicular proteome across thousands of proteins.

Comparative proteomics have revealed both common and cell type-specific exosomal proteins. A core set of proteins, including tetraspanins, ESCRT components, and cytoskeletal proteins, are found in exosomes from various sources. However, the relative abundance of these proteins can vary significantly between different cell types and conditions (Kowal et al., 2016).

Quantitative proteomics approaches, such as SILAC (stable isotope labeling by amino acids in cell culture) and iTRAQ (isobaric tags for relative and absolute quantitation), have enabled the comparison of exosomal protein profiles under different conditions. For example, Hurwitz et al. (2016) used nanoparticle analysis across cell lines to investigate genetic drivers of extracellular vesicle biogenesis.

Proteomic analysis has also revealed post-translational modifications (PTMs) of exosomal proteins, which may influence their function and sorting into exosomes. Phosphorylation, ubiquitination, and glycosylation have been identified on various exosomal proteins (Moreno-Gonzalo et al., 2014). These PTMs may play essential roles in protein-protein interactions, protein stability, and the recognition of cargo proteins by the exosomal sorting machinery.

The field of exosome proteomics continues to evolve, with new technologies enabling more detailed and sensitive analyses. Single-vesicle proteomics approaches are being developed to address heterogeneity within exosome populations (Chiang & Chen, 2019). Additionally, the integration of proteomics data with other -omics approaches, such as transcriptomics and lipidomics, is providing a more holistic view of exosome composition and function (Abramowicz et al., 2016).

2.2. Lipids

2.2.1 Types of Exosomal Lipids

Lipids are essential components of exosomes, not only forming the structural basis of their membrane but also playing central roles in exosome biogenesis, stability, and function (Skotland et al., 2017a). The lipid composition of exosomes is distinct from that of the plasma membrane of

their cells of origin, suggesting a selective enrichment process during exosome formation (Llorente et al., 2013).

The lipid content of exosomes typically includes:

Phospholipids are the principal class. These are the main structural components of the exosomal membrane. The most abundant phospholipids in exosomes are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI).

The relative proportions of these phospholipids can vary depending on the cell type of origin and cellular conditions (Subra et al., 2007).

Sphingomyelin and ceramide are enriched in exosomes and play important roles in exosome biogenesis and stability (Trajkovic et al., 2008). Glycosphingolipids, including gangliosides, are also found in exosomes and may be involved in cellular recognition and signalling (Tan et al., 2013). Exosomes are typically enriched in cholesterol compared to their parent cells, contributing to membrane rigidity and stability (Llorente et al., 2013). The high cholesterol content of exosomes is thought to be a key factor in their ability to withstand harsh extracellular environments. These bioactive lipid mediators have been identified in exosomes, suggesting a role in intercellular signalling (Subra et al., 2010). Prostaglandins can modulate various cellular processes, including inflammation and immune responses. Both saturated and unsaturated fatty acids have been detected in exosomes. These can be present as free fatty acids or as components of more complex lipids (Record et al., 2014). Lysophospholipids, which have lost one fatty acid chain, are also present. Lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) have been identified in exosomes and may contribute to membrane curvature and fluidity (Llorente et al., 2013). These are a special class of phospholipids with an ether linkage at the sn-1 position. Plasmalogens have been found to be enriched in some exosome populations and may play a role in protecting exosomal contents from oxidative stress (Skotland et al., 2019). Bis(monoacylglycero)phosphate (BMP), also known as lysobisphosphatidic acid (LBPA), is enriched in the internal membranes of multivesicular bodies and has been detected in exosomes. It may play a role in exosome formation and cargo sorting (LAULAGNIER et al., 2004).

2.2.2 Roles of Lipids in Exosome Biology

Lipids play important roles in various aspects of exosome biology.

The first is biogenesis. Ceramide, generated by neutral sphingomyelinase 2 (nSMase2), has been shown to promote the inward budding of multivesicular bodies (MVBs), a key step in exosome formation (Trajkovic et al., 2008). The cone-shaped structure of ceramide is thought to induce negative curvature in the membrane, facilitating vesicle budding. The high content of cholesterol and sphingomyelin in exosomes contributes to the rigidity and stability of their membrane. This unique lipid composition allows exosomes to maintain their integrity in the extracellular environment and potentially travel long distances in the body (LAULAGNIER et al., 2004). Lipid rafts, cholesterol-rich microdomains in membranes, have been implicated in the sorting of proteins and RNA into exosomes (de Gassart et al., 2003). These specialized membrane domains may serve as platforms for the concentration of specific cargo molecules during exosome formation. The lipid composition of exosomes can also influence their uptake by recipient cells. For example, phosphatidylserine exposure on exosomes has been shown to enhance their uptake by macrophages through interactions with PS receptors (Matsumoto et al., 2017). Additionally, the fluidity of the exosomal membrane, which is influenced by its lipid composition, can affect fusion with target cell membranes. Bioactive lipids carried by exosomes, such as prostaglandins and sphingosine-1-phosphate, can act as signalling molecules in recipient cells (Subra et al., 2010). These lipid mediators can modulate various cellular processes, including inflammation, cell growth, and differentiation. The lipid bilayer provides a protective barrier for their cargo, shielding sensitive molecules like RNA from degradation in the extracellular environment (Kamerkar et al., 2017). Lipid composition may also influence the ability of exosomes to fuse with the plasma membrane of recipient cells. For instance, the presence of fusogenic lipids like lysobisphosphatidic acid (LBPA) may facilitate membrane fusion events (Chevallier et al., 2008). Exosomal lipids can directly affect the properties of recipient cell membranes upon fusion or uptake. This can potentially alter membrane fluidity, organization of lipid rafts, and the function of membrane-associated proteins in target cells (Record et al., 2014).

2.2.3 Lipidomics of Exosomes

Advances in lipidomics technologies, particularly high-resolution mass spectrometry, have enabled thorough characterisation of the exosomal lipidome. These studies have revealed both commonalities and differences in lipid composition across exosomes from various sources.

Studies comparing the lipid profiles of exosomes to their parent cells have consistently shown enrichment of certain lipid species in exosomes. For example, Llorente et al. (2013) found that exosomes derived from PC-3 prostate cancer cells were enriched in cholesterol, sphingomyelin, glycosphingolipids, and phosphatidylserine compared to the cellular membranes. The lipid composition of exosomes can vary significantly depending on the cell type of origin. Skotland et al. (2017a) reviewed the lipid composition of exosomes from different sources and highlighted how cellular origin shapes exosomal lipid profiles. Changes in exosomal lipid profiles have been observed in various pathological conditions. For instance, exosomes derived from cancer cells often show alterations in their lipid composition compared to those from normal cells, potentially reflecting the metabolic changes associated with malignancy (Lydic et al., 2015). Lipidomic analyses have also provided insights into the potential functional roles of exosomal lipids. For example, the enrichment of specific lipid species in exosomes has been linked to their ability to fuse with target cell membranes or to activate certain signalling pathways (LAULAGNIER et al., 2004). The distinct lipid profiles of exosomes from different cellular origins and disease states have raised interest in their potential as biomarkers. Lipid-based signatures of exosomes are being explored for diagnostic and prognostic applications in various diseases, including cancer and neurodegenerative disorders (Skotland et al., 2017b, 2019).

New technologies are making lipidomic analyses both broader and more sensitive. For instance, imaging mass spectrometry techniques are being developed to visualise the spatial distribution of lipids within individual exosomes (Li et al., 2023). Additionally, the integration of lipidomics data with other -omics approaches is providing a more holistic view of exosome composition and function.

The study of exosomal lipids has also opened new possibilities for therapeutic applications. Manipulation of exosomal lipid composition could enhance drug delivery efficiency or alter the targeting of exosomes to specific cell types (Skotland et al., 2017a). Furthermore, understanding the role of lipids in exosome biogenesis and function may lead to new strategies for modulating exosome production and uptake in various disease contexts.

2.3. Nucleic Acids

2.3.1 Types of Exosomal RNA

RNA molecules constitute a significant and functionally important component of exosomal cargo. The RNA content of exosomes includes various species of RNA, each with potential regulatory functions in recipient cells (Valadi et al., 2007). The major types of RNA found in exosomes include:

Full-length and fragmented mRNAs have been identified in exosomes. These mRNAs can potentially be translated into proteins in recipient cells, suggesting a mechanism for the transfer of genetic information between cells (Valadi et al., 2007). The presence of functional mRNAs in exosomes has been demonstrated in various contexts, including cancer progression and stem cell-mediated tissue repair (Ratajczak et al., 2006). MicroRNAs, small non-coding RNAs typically 20–22 nucleotides in length, are abundantly present in exosomes and can regulate gene expression in recipient cells by targeting specific mRNAs for degradation or translational repression (Pegtel et al., 2010). Exosomal miRNAs have been implicated in various biological processes, including immune regulation, cancer progression, and neurodegenerative diseases (Alexander et al., 2015; Melo et al., 2014). Long non-coding RNAs, longer than 200 nucleotides, have been found in exosomes and can potentially modulate various cellular processes in recipient cells, including transcriptional regulation and chromatin remodeling (Gezer et al., 2014). For example, the lncRNA HOTAIR has been detected in exosomes from cancer cells and may contribute to tumour progression and metastasis (Qu et al., 2016). Fragments of transfer RNAs have been identified in exosomes and may have regulatory functions distinct from their role in translation (Bellingham et al., 2012). These tRNA fragments have been shown to modulate gene expression and cellular stress responses in recipient cells (Sharma et al., 2016). Circular RNAs, closed-loop molecules, have been detected in exosomes and may act as miRNA sponges or regulators of gene expression (Li et al., 2015). Exosomal circRNAs have been implicated in various physiological and pathological processes, including cancer progression and neurological disorders (Wang et al., 2019). Piwi-interacting RNAs, typically 24–32 nucleotides in length, have been identified in exosomes from cancer cells and may play a role in epigenetic regulation and transposon silencing (Tosar et al., 2015). Small nuclear and small nucleolar RNAs, involved in splicing and ribosomal RNA modification, respectively, have been detected in exosomes and may contribute to the regulation of gene expression in recipient cells (Koppers-Lalic et al., 2014). Y RNAs complete this list. These small non-coding RNAs, involved in DNA replication and RNA quality

control, have been found in exosomes and may serve as biomarkers for certain diseases (Chakrabortty et al., 2015).

2.3.2 DNA in Exosomes

While RNA has been the primary focus of nucleic acid studies in exosomes, DNA has also been detected in these vesicles.

Fragments of genomic DNA, ranging from a few hundred base pairs to several kilobases, have been identified in exosomes (Thakur et al., 2014). These DNA fragments can represent the entire genome of the parent cell and may carry important genetic information, including oncogenes and tumour suppressor genes in cancer-derived exosomes. Mitochondrial DNA has been detected in exosomes from various cell types and may serve as a damage-associated molecular pattern (DAMP) in certain pathological conditions (Guescini et al., 2010). Exosomal mtDNA has been implicated in inflammatory responses and may contribute to the pathogenesis of autoimmune diseases. Some studies have suggested that RNA within exosomes can be reverse-transcribed into complementary DNA (cDNA) by reverse transcriptase activity present in the vesicles (Balaj et al., 2011). This process could generate new genetic material that can be transferred to recipient cells.

The presence of DNA in exosomes raises intriguing questions about its potential functional roles and implications for intercellular communication and disease progression.

2.3.3 Functional Implications of Exosomal Nucleic Acids

The transfer of nucleic acids via exosomes has significant functional implications in various biological contexts.

The first is gene regulation. Exosomal miRNAs can modulate gene expression in recipient cells, potentially altering cellular phenotypes and functions (Zhang et al., 2015a). For example, tumour-derived exosomal miRNAs have been shown to promote metastasis by reprogramming stromal cells in the tumour microenvironment (Fabbri et al., 2012). Transfer of exosomal RNAs between immune cells can influence immune responses and inflammation. Alexander et al. (2015) showed that exosome-delivered microRNAs, including miR-155 and miR-146a, modulate the inflammatory response to endotoxin, highlighting the role of exosomal RNA in immune regulation. Tumour-derived exosomal RNAs have been implicated in promoting metastasis, angiogenesis, and drug resistance. For instance, exosomal transfer of lncRNA HOTTIP has been shown to enhance chemoresistance in small cell lung cancer by promoting BCL-2 expression and sponging miR-216a (Sun et al., 2018). Exosomal RNAs may contribute to the spread of pathogenic proteins in diseases such as Alzheimer's and Parkinson's. Additionally, exosomal miRNAs have been explored as potential biomarkers for neurodegenerative disorders (Soria et al., 2017). Exosomal RNAs are involved in several aspects of cardiovascular biology, including angiogenesis, cardiac remodeling, and atherosclerosis. For example, exosomal transfer of miR-126 from endothelial cells to smooth muscle cells has been shown to promote vascular healing (Jansen et al., 2013). Exosomal RNAs from stem cells have shown potential in promoting tissue repair and regeneration. MSC-derived exosomes carrying miR-133b have been demonstrated to enhance neurite outgrowth and functional recovery after stroke in rats (Xin et al., 2013). The presence of mRNA and DNA in exosomes suggests a potential mechanism for horizontal gene transfer between cells. This could have implications for cellular plasticity, tumour evolution, and the spread of drug resistance genes (Cai et al., 2013). Exosomal transfer of non-coding RNAs, such as lncRNAs and circRNAs, may contribute to epigenetic modifications in recipient cells, potentially leading to long-term alterations in gene expression patterns (Chen et al., 2019).

2.3.4 Genomics and Transcriptomics of Exosomes

Advanced sequencing technologies have substantially changed our understanding of the nucleic acid content of exosomes.

High-throughput RNA sequencing has revealed the complexity of the exosomal transcriptome. Studies have shown that exosomes contain a diverse array of RNA species, with small non-coding RNAs, particularly miRNAs, being highly enriched (Huang et al., 2013). The exosomal RNA profile often differs from that of the parent cell, suggesting selective packaging mechanisms. Emerging technologies are enabling the analysis of RNA content in individual exosomes, providing insights into the heterogeneity within exosome populations. For example, droplet-based microfluidic devices have been developed to isolate and analyse the RNA content of single exosomes (Shao et al., 2018). Whole-genome sequencing of exosomal DNA has revealed that it can represent the entire genome of the parent cell. In cancer, exosomal DNA has been shown to reflect the mutational status of tumour cells, suggesting potential applications in liquid biopsy approaches (Kahlert et al., 2014). Studies comparing the nucleic acid profiles of

exosomes from different cell types or under various conditions have provided insights into the selectivity of exosomal cargo packaging. For instance, Skog et al. (2008) compared the mRNA profiles of glioblastoma-derived exosomes to those of their parent cells, identifying specific mRNAs that were enriched in exosomes. The integration of genomics and transcriptomics data with proteomics and lipidomics provides a broader understanding of exosome composition and function. These multi-omics approaches are revealing complex relationships between different types of exosomal cargo and their potential functional implications (Abramowicz et al., 2016). Advanced computational methods are being applied to analyse the large datasets generated by exosome sequencing studies. Machine learning algorithms are being developed to predict exosomal RNA cargo based on sequence features and to identify potential functional interactions between exosomal RNAs and their targets (Santangelo et al., 2016). Evolutionary genomics completes this group. Comparative analyses of exosomal nucleic acids across species provide insights into the evolutionary conservation of exosome-mediated communication mechanisms. These studies reveal both conserved and species-specific features of exosomal RNA cargo.

The field of exosome genomics and transcriptomics continues to expand, driven by technological advancements and increasing recognition of the importance of exosome-mediated communication in various biological processes. Future research directions may include:

Elucidating the mechanisms of selective nucleic acid packaging into exosomes with greater precision. Investigating the functional significance of less-studied RNA species in exosomes, such as circRNAs and piRNAs. Developing more sensitive and specific methods for detecting and quantifying low-abundance nucleic acids in exosomes. Exploring the potential of exosomal nucleic acids as biomarkers for early disease detection and monitoring. Investigating the role of exosomal nucleic acids in long-distance signalling within organisms, particularly in the context of systemic responses to localised stimuli. Developing exosome-based therapies that leverage the natural ability of these vesicles to transfer functional nucleic acids between cells.

The unique properties of exosomes as natural carriers of nucleic acids make them attractive candidates for drug delivery and gene therapy approaches, driving intense research efforts to translate these findings into clinical settings.

3. Mechanisms of Cargo Loading

The selective packaging of specific molecules into exosomes is a complex process involving various cellular mechanisms. Understanding these mechanisms is important for elucidating the functional roles of exosomes in intercellular communication and for developing strategies to manipulate exosomal cargo for therapeutic purposes.

3.1. Protein Loading Mechanisms

Several mechanisms have been identified for the sorting of proteins into exosomes.

The first is the ESCRT-dependent mechanism. The Endosomal Sorting Complexes Required for Transport (ESCRT) machinery is closely involved in recognising ubiquitinated proteins and sorting them into intraluminal vesicles (ILVs) that eventually become exosomes. This process involves four ESCRT complexes (ESCRT-0, -I, -II, and -III) and associated proteins such as Alix and VPS4 (Colombo et al., 2014). The ESCRT pathway is particularly important for the sorting of transmembrane proteins and cytosolic proteins that undergo ubiquitination. ESCRT-0 recognises and sequesters ubiquitinated cargo proteins on the endosomal membrane. ESCRT-I and ESCRT-II are recruited to the endosomal membrane and initiate membrane budding. ESCRT-III forms spiral filaments that constrict the neck of the budding vesicle, leading to its scission. VPS4, an AAA-ATPase, disassembles and recycles the ESCRT machinery. Tetraspanins such as CD63, CD9 and CD81 can form microdomains in the membrane and facilitate the clustering and sorting of specific proteins into exosomes (van Niel et al., 2018). These tetraspanin-enriched microdomains (TEMs) may act as platforms for protein-protein interactions and cargo sorting. Some proteins associate instead with lipid rafts, cholesterol-rich microdomains in membranes that can be incorporated into ILVs during exosome formation (de Gassart et al., 2003). Lipid rafts may serve as sorting platforms for specific proteins and lipids, contributing to the unique composition of exosomes. Modifications such as ubiquitination, SUMOylation, and phosphorylation can influence protein sorting into exosomes (Villarroya-Beltri et al., 2014). These modifications may act as sorting signals or alter protein-protein interactions involved in exosome biogenesis. Ubiquitination is a well-established signal for ESCRT-dependent sorting. SUMOylation has been shown to promote the sorting of specific proteins, such as α-synuclein, into exosomes (Kunadt et al., 2015). Phosphorylation can modulate protein interactions and

localization, potentially influencing their incorporation into exosomes. Certain proteins may be sorted into exosomes through their interactions with other exosomal proteins or with components of the sorting machinery (Sahu et al., 2011). These interactions may be transient or form stable complexes that are incorporated into exosomes. Some proteins may be sorted into exosomes through the formation of higher-order oligomers that are too large to be accommodated in the narrow membrane necks of budding ILVs. This mechanism has been proposed for the sorting of syntenin and syndecans (Baietti et al., 2012). The high curvature of the ILV membrane may preferentially accommodate certain protein domains, contributing to their enrichment in exosomes (Roux et al., 2005). Other proteins may be sorted through their association with RNA molecules that are targeted for exosomal packaging. For example, the RNA-binding protein hnRNPA2B1 has been shown to bind specific miRNAs and facilitate their loading into exosomes (Villarroya-Beltri et al., 2013).

Recent studies have exposed the complexity of protein sorting into exosomes. For instance, Stuffers et al. (2009) demonstrated that while the ESCRT machinery is important for exosome biogenesis, ESCRT-independent mechanisms also exist, highlighting the redundancy and robustness of exosome formation pathways. Additionally, the relative contribution of different sorting mechanisms may vary depending on the cell type, physiological state, and specific cargo proteins involved.

Understanding the mechanisms of protein loading into exosomes has important implications for both basic science and translational research.

Biomarker development is one area. Knowledge of protein sorting mechanisms can inform the identification and validation of exosomal protein biomarkers for various diseases. Therapeutic targeting is another. Manipulating protein sorting pathways could be used to alter the protein composition of exosomes for therapeutic purposes. Understanding protein loading mechanisms also enables the development of strategies to load specific therapeutic proteins into exosomes for drug delivery applications. Elucidating protein sorting mechanisms provides insights into the broader process of exosome formation and release. Protein sorting into exosomes may play a role in cellular protein quality control and homeostasis by removing excess or damaged proteins from the cell.

This knowledge will be key for realizing the potential of exosomes in both diagnostic and therapeutic applications.

3.2. Lipid Loading Mechanisms

The enrichment of specific lipids in exosomes involves both the biogenesis of these vesicles and active sorting mechanisms.

Lipid rafts, which are enriched in cholesterol and sphingolipids, may serve as platforms for the formation of intraluminal vesicles (ILVs) and the concentration of specific lipids in exosomes (Tan et al., 2013). These microdomains can facilitate the clustering of proteins and lipids destined for exosomal secretion. The first is enzyme-mediated synthesis. The activity of specific enzymes can generate lipids that promote exosome formation and influence their composition: Neutral sphingomyelinase 2 generates ceramide from sphingomyelin, which promotes the inward budding of multivesicular bodies (MVBs) and influences the lipid composition of exosomes (Trajkovic et al., 2008). Ceramide's cone-shaped structure induces negative membrane curvature, facilitating vesicle budding. Phospholipase D2 is another. This enzyme produces phosphatidic acid, which may contribute to membrane curvature and exosome formation (LAULAGNIER et al., 2004). A second mechanism is flippase and scramblase activity. These enzymes regulate the distribution of phospholipids between the inner and outer leaflets of the membrane, potentially influencing the lipid composition of exosomes (Llorente et al., 2013): Flippases are one class. ATP-dependent enzymes that transport specific phospholipids from the outer to the inner leaflet of the membrane. Floppases are ATP-dependent enzymes that transport phospholipids from the inner to the outer leaflet. Scramblases are ATP-independent enzymes that move phospholipids in both directions across the membrane. The lysosome-associated ABC transporter ABCA3 has been shown to positively modulate exosome biogenesis in Bcell lymphoma, increasing the quantity of exosomes released; which carry CD20, bind therapeutic antibodies like rituximab, and enhance resistance to antibody-mediated cytolysis. Pharmacological inhibition or ABCA3 silencing reduced exosome secretion and sensitized cells to immune attack (Aung et al., 2011). A third mechanism involves lipid-protein interactions. Certain lipids may be sorted into exosomes through their interactions with proteins that are targeted for exosomal secretion. For example, the association of ceramide with the protein syntenin has been shown to promote exosome formation (Baietti et al., 2012). The high curvature of ILV membranes may preferentially accommodate certain lipid species, contributing to their enrichment in exosomes. Cone-shaped lipids like ceramide and

phosphatidic acid may be favored in the inner leaflet of the highly curved ILV membrane (McMahon & Boucrot, 2015). pH-dependent lipid sorting: The acidic environment of late endosomes/MVBs may influence lipid distribution and contribute to the sorting of specific lipids into ILVs. For instance, the formation of bis(monoacylglycero)phosphate (BMP) is promoted in acidic conditions and may facilitate ILV formation (Matsuo et al., 2004). Some lipid modifications made in the Golgi apparatus may influence the sorting of lipids into exosomes. For example, glycosphingolipid synthesis in the Golgi may contribute to their enrichment in exosomes (Halter et al., 2007). Intracellular lipid transfer proteins may facilitate the transport of specific lipids to sites of ILV formation, influencing the lipid composition of exosomes. For instance, ceramide transfer protein (CERT) may play a role in ceramide enrichment in exosomes (Hanada et al., 2003).-

The lipid composition of exosomes can have significant implications for their stability, uptake by recipient cells, and even their cargo-sorting capabilities. For instance, LAULAGNIER et al. (2004) showed that mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization, features that have been linked to their stability in extracellular environments.

Understanding lipid loading mechanisms in exosomes has several important implications.

Manipulating lipid composition could enhance the stability, targeting, or uptake of engineered exosomes for therapeutic applications. Biomarker development is one area. Changes in exosomal lipid profiles may serve as biomarkers for various diseases, particularly those involving altered lipid metabolism. The unique lipid composition of exosomes may be exploited to enhance the delivery of lipophilic drugs or to design exosome-mimetic nanoparticles for drug delivery. A further consideration is cellular lipid homeostasis. Exosomal lipid secretion may play a role in cellular lipid homeostasis, potentially offering insights into lipid-related disorders. Targeting lipid metabolism pathways could be a strategy to modulate exosome production in therapeutic contexts.

Future studies may uncover additional mechanisms and provide more detailed insights into the interaction between lipids, proteins, and nucleic acids in exosome formation and function.

3.3. Nucleic Acid Loading Mechanisms

The packaging of RNA and DNA into exosomes is a selective process that involves various molecular mechanisms.

The first is sequence-specific sorting. Certain RNA sequence motifs have been associated with preferential packaging into exosomes: Villarroya-Beltri et al. (2013) identified a GGAG motif in miRNAs that promotes their sorting into exosomes through interaction with the protein hnRNPA2B1. Binding by RNA-binding proteins is another route. Shurtleff et al. (2016) found that Y-box protein 1 (YBX1) is required to sort microRNAs into exosomes, both in cells and in a cell-free reaction. A second mechanism involves RNA-binding proteins. Several proteins have been implicated in the sorting of specific RNAs into exosomes: hnRNPA2B1: Binds to specific miRNAs and facilitates their loading into exosomes (Villarroya-Beltri et al., 2013). YBX1 is involved in the sorting of miRNAs and mRNAs into exosomes (Shurtleff et al., 2016). SYNCRIP recognises specific motifs in miRNAs and mediates their exosomal packaging (Santangelo et al., 2016). Major vault protein is involved in the sorting of specific miRNAs into exosomes (Statello et al., 2018). A third mechanism is post-transcriptional modification. RNA modifications can influence their sorting into exosomes: 3′-end adenylation: Associated with cellular retention of miRNAs. 3′-end uridylation: Promotes the packaging of miRNAs into exosomes (Koppers-Lalic et al., 2014). Modifications such as N6-methyladenosine (m6A) may influence RNA sorting into exosomes (Zietzer et al., 2020). A fourth mechanism involves membrane-associated processes. The association of RNAs with membrane microdomains or specific lipids may contribute to their incorporation into exosomes: Janas et al. (2015) proposed that the affinity of RNA for these domains could facilitate RNA sorting into exosomes. Annexin A2 provides another. This membrane-associated protein has been shown to bind specific RNAs and facilitate their loading into exosomes (Hagiwara et al., 2015). While primarily associated with protein sorting, the ESCRT machinery may also play a role in RNA loading into exosomes. The ESCRT-II subunit VPS36 binds directly to RNA, potentially facilitating its incorporation into intraluminal vesicles (ILVs) (Hurley, 2010). The VPS36 subunit, through its GLUE (GRAM-Like Ubiquitin-binding in EAP45) domain, interacts with phosphoinositides and ubiquitin, suggesting a potential role in RNA sorting and packaging within exosomes (Hurley, 2010). This ESCRT-associated protein has been implicated in the sorting of miRNAs into exosomes, acting as a

critical mediator in RNA cargo selection and exosomal release (IAVELLO et al., 2016). Studies have shown that Alix interacts with specific exosomal components and contributes to the selective enrichment of miRNAs in exosomal cargo (IAVELLO et al., 2016). A sixth factor is RNA secondary structure. The folding pattern of RNAs may influence their sorting into exosomes. For example, G-quadruplex structures in lncRNAs have been associated with their enrichment in exosomes (Wang et al., 2014). Some RNAs may also be sorted through their association with proteins that are targeted for exosomal secretion. For instance, miRNAs associated with Argonaute 2 (AGO2) protein complexes have been found in exosomes (McKenzie et al., 2016). Some RNAs, particularly abundant cellular ones,, may be incorporated into exosomes through passive mechanisms based on their concentration in the cytoplasm (Squadrito et al., 2014).

For DNA loading into exosomes, fewer mechanisms have been elucidated, but some proposed pathways include:

The first is cytoplasmic DNA sensing. Cytosolic DNA, which can arise from nuclear or mitochondrial genome damage, may be recognised by DNA sensors and targeted for exosomal secretion as a cellular defense mechanism (Takahashi et al., 2017). Nuclear envelope blebbing, which occurs during cell division or in some pathological conditions, may allow nuclear DNA fragments to enter the cytoplasm and subsequently be incorporated into exosomes (Yokoi et al., 2019). Damaged mitochondrial DNA may be selectively packaged into exosomes as a means of cellular quality control (Sansone et al., 2017). Some studies suggest that DNA in exosomes is protected from DNase digestion, indicating that it may be associated with proteins or have specific structural features that promote its packaging and protection (Thakur et al., 2014).

Understanding nucleic acid loading mechanisms in exosomes has several important implications.

Biomarker development is one area. Knowledge of RNA and DNA sorting mechanisms can inform the identification and validation of exosomal nucleic acid biomarkers for various diseases. Insights into RNA loading mechanisms can be exploited to develop exosome-based delivery systems for therapeutic RNAs, such as siRNAs or miRNAs. Understanding nucleic acid sorting allows for the development of strategies to load specific therapeutic nucleic acids into exosomes for targeted delivery. Elucidating the mechanisms of nucleic acid sorting provides insights into the role of exosomes in genetic and epigenetic regulation between cells. Studying these mechanisms may provide clues about the evolutionary development of exosome-mediated intercellular RNA transfer.

This knowledge will be essential for realizing the potential of exosomes in both diagnostic and therapeutic applications.

4. Exosome Biogenesis and Secretion

4.1. Formation of Multivesicular Bodies

The biogenesis of exosomes begins with the formation of multivesicular bodies (MVBs), which are late endosomal structures containing intraluminal vesicles (ILVs). This process involves several steps and molecular mechanisms.

The process starts with the invagination of the plasma membrane to form early endosomes. These early endosomes serve as sorting stations for internalized material and membrane components (Huotari & Helenius, 2011). Early endosomes mature into late endosomes through a series of changes in their protein composition, pH, and morphology. This maturation process involves the recruitment of specific proteins, such as Rab7, and the accumulation of intraluminal vesicles (Piper & Katzmann, 2007). The hallmark of MVB formation is the inward budding of the limiting membrane to form ILVs. This process can occur through several mechanisms: The first is the ESCRT-dependent mechanism. The Endosomal Sorting Complexes Required for Transport (ESCRT) machinery matters for ILV formation. The process involves four ESCRT complexes (ESCRT-0, -I, -II, and -III) and associated proteins: ESCRT-0 recognises and clusters ubiquitinated cargo proteins on the endosomal membrane. ESCRT-I and ESCRT-II are recruited and initiate membrane budding. ESCRT-III forms spiral filaments that constrict the neck of the budding vesicle. VPS4, an AAA-ATPase, disassembles and recycles the ESCRT machinery (Henne et al., 2011). A second group comprises ESCRT-independent mechanisms. Several ESCRT-independent pathways for ILV formation have been identified: The ceramide-driven pathway is one of these. The enzyme neutral sphingomyelinase 2 (nSMase2) generates ceramide, which can induce membrane curvature and promote ILV formation (Trajkovic et al., 2008). Tetraspanin-enriched microdomains act by a second route: tetraspanins such as CD63 can facilitate the clustering of cargo and promote membrane invagination (van Niel et al., 2011). The syntenin-ALIX pathway is a third. This mechanism involves the interaction between syntenin, ALIX, and syndecans to promote ILV formation (Baietti et al., 2012). During MVB formation, specific cargo molecules are sorted into ILVs. This

sorting process involves various mechanisms, including: Ubiquitination-dependent sorting via the ESCRT machinery Interaction with tetraspanin-enriched microdomains Association with lipid rafts RNA-binding protein-mediated sorting of nucleic acids As endosomes mature into MVBs, their lumen becomes progressively acidified through the action of vacuolar ATPases. This acidification is important for the function of various hydrolytic enzymes and can influence the sorting and processing of cargo molecules (Huotari & Helenius, 2011). The distribution of cholesterol within the endosomal system plays a role in MVB formation and cargo sorting. Cholesterol accumulation in MVBs can influence their fate, promoting their fusion with the plasma membrane and exosome release (Strauss et al., 2010). Rab GTPases form a further group; various Rab proteins are involved in the regulation of MVB formation and trafficking. For example, Rab7 is associated with late endosome/MVB maturation, while Rab27a and Rab27b are involved in MVB trafficking to the plasma membrane (Ostrowski et al., 2010). Cytoskeletal interactions form a third. The formation and trafficking of MVBs involve interactions with the cytoskeleton. Actin and microtubules play roles in the spatial organization and movement of MVBs within the cell (Granger et al., 2014). Calcium signalling is a further factor. Intracellular calcium levels can influence MVB formation and exosome release. Elevated calcium levels have been shown to promote exosome secretion in some cell types (Savina et al., 2005). Various post-translational modifications of proteins, such as ubiquitination, SUMOylation, and phosphorylation, can influence their sorting into ILVs and subsequent incorporation into exosomes (Villarroya-Beltri et al., 2014).

The formation of MVBs is a complex and highly regulated process that involves the coordinated action of numerous proteins, lipids, and signalling pathways. Understanding the mechanisms of MVB formation is central for elucidating the biogenesis of exosomes and their cargo selection.

4.2. Fusion with Plasma Membrane

Once MVBs are formed, they can either fuse with lysosomes for degradation of their contents or fuse with the plasma membrane to release their ILVs as exosomes into the extracellular space. The fusion of MVBs with the plasma membrane is a critical step in exosome secretion and involves several key components and processes:

1. Rab GTPases: Several Rab proteins play decisive roles in MVB trafficking and fusion:

Rab27a and Rab27b are the best characterised. These proteins are involved in MVB docking at the plasma membrane. Knockdown of Rab27a or Rab27b has been shown to reduce exosome secretion in various cell types (Ostrowski et al., 2010). Rab35, a second, regulates the docking of MVBs to the plasma membrane in oligodendrocytes (Hsu et al., 2010). Rab11, a third, is involved in the recycling endosome pathway and has been implicated in exosome secretion in some cell types (Savina et al., 2002).

2. SNARE proteins: Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are essential for membrane fusion events.

VAMP7 is an example: a v-SNARE that has been shown to mediate the fusion of MVBs with the plasma membrane in K562 cells (Fader et al., 2009). YKT6 is a second: another v-SNARE protein implicated in exosome release in HEK293 cells (Gross et al., 2012). Syntaxin-4 and SNAP-23 form a third pair: t-SNARE proteins that have been associated with MVB fusion in some cell types (Puri & Roche, 2008).

3. Synaptotagmin family proteins: These calcium-sensing proteins can regulate membrane fusion events.

Synaptotagmin-7 acts as a calcium sensor here; it has been implicated in calcium-dependent exosome release in neuronal cells. Cytoskeletal components: The actin cytoskeleton plays a role in MVB trafficking and fusion. Cortical actin remodeling is often required for exosome release (Sinha et al., 2016). Myosin motors are involved as well. Myosin Va has been implicated in the docking and fusion of multivesicular bodies (MVBs) with the plasma membrane, working in coordination with Rab27a and Rab27b to regulate different steps of the exosome secretion pathway (Ostrowski et al., 2010). Calcium signalling is a further factor. Intracellular calcium levels can regulate MVB fusion and exosome release: Increased cytosolic calcium levels often promote exosome secretion (Savina et al., 2005). Calcium ionophores have been used experimentally to stimulate exosome release in various cell types. Lipid dynamics: The cholesterol content of MVBs can influence their fate, with cholesterol-rich MVBs being more likely to fuse with the plasma membrane (Strauss et al., 2010). Phosphatidic acid, generated by phospholipase D2 (PLD2), has been implicated in promoting

MVB fusion with the plasma membrane (LAULAGNIER et al., 2004). Tethering factors: This octameric protein complex has been implicated in tethering vesicles to the plasma membrane prior to fusion. Components of the exocyst complex have been shown to be involved in exosome secretion in some cell types (Hyenne et al., 2015). Small GTPases: ARF6 is a further regulator. This small GTPase has been shown to regulate the release of exosomes containing syndecans and CD63 (Friand et al., 2015). Among post-translational modifications, ISGylation, a ubiquitin-like modification, has been shown to promote MVB fusion with the plasma membrane and exosome release (Villarroya-Beltri et al., 2016). pH regulation: V-ATPase activity is another. The activity of this proton pump, which regulates the pH of intracellular compartments, can influence MVB fusion and exosome release (Parolini et al., 2009).

The fusion of MVBs with the plasma membrane is a highly regulated process that can be modulated by various cellular signals and environmental factors. Understanding these mechanisms is important for developing strategies to manipulate exosome release for therapeutic purposes or to use exosome secretion as a biomarker for cellular states.

4.3. Regulatory Mechanisms of Exosome Release

The release of exosomes is not a constitutive process but is regulated by various cellular mechanisms and external stimuli. Understanding these regulatory mechanisms is key for manipulating exosome production in therapeutic contexts and for interpreting exosome-based biomarkers. Key regulatory mechanisms include:

Stress responses: Cellular stress, such as heat shock, hypoxia, or oxidative stress, can modulate exosome release (King et al., 2012). p53 activation has been shown to enhance exosome secretion in some cell types (Yu et al., 2006). Oncogenic signalling: Activation of oncogenic pathways, such as KRAS signalling, can increase exosome secretion in cancer cells (McKenzie et al., 2016). Inflammatory stimuli: Exposure to pro-inflammatory cytokines or pathogen-associated molecular patterns (PAMPs) can alter exosome release in immune cells (Bhatnagar & Schorey, 2007). Neurotransmitter signalling: In neurons, depolarization and neurotransmitter release can trigger exosome secretion (Lachenal et al., 2011). Hormone signalling: Various hormones, including estrogen and cortisol, have been shown to modulate exosome release in specific cell types (Stefanski et al., 2019). Cell cycle regulation: Exosome secretion can be influenced by the cell cycle stage, with increased release often observed during G1 phase (Blanc & Vidal, 2018). Autophagy: Crosstalk between autophagy and exosome biogenesis pathways governs exosome release. Autophagy-related proteins have been found to contribute to exosome biogenesis, influencing their formation and secretion. In some contexts, inhibition of autophagy can enhance exosome secretion by preventing the degradation of multivesicular bodies (MVBs) and redirecting their contents towards secretion pathways. Studies suggest that autophagy and exosome biogenesis share common molecular machinery, such as ATG5 and ATG16L1, which are involved in regulating the fusion of MVBs with the plasma membrane for exosome release (Xu et al., 2018). Furthermore, emerging evidence highlights the role of amphisomes, hybrid organelles formed through the fusion of autophagosomes and MVBs, in exosome-mediated intercellular communication, particularly in disease contexts such as neurodegeneration and cancer.

Cellular metabolism and energy availability significantly influence exosome biogenesis and secretion. Notably, glucose deprivation alters the composition and release of exosomes, suggesting a metabolic control mechanism over vesicle trafficking and signalling (Kowal et al., 2016).

Epigenetic regulation: Epigenetic mechanisms, such as DNA methylation and histone modifications, can influence the expression of genes involved in exosome biogenesis and release (Vardaki et al., 2016). microRNA-mediated regulation: Certain microRNAs can modulate the expression of proteins involved in exosome biogenesis and release (Squadrito et al., 2014).

Understanding these regulatory mechanisms provides opportunities for therapeutic interventions and diagnostic applications.

Several applications follow from an understanding of these pathways. Manipulating them could enhance or suppress exosome release in particular cell types for therapeutic purposes, and changes in release patterns could serve as indicators of cellular stress, disease progression or treatment response. The same knowledge could be used to improve the loading and release of therapeutic cargoes in engineered exosomes. In cancer, targeting the pathways that regulate exosome release might modulate tumour progression and metastasis; in neurodegenerative disease, understanding how release is regulated in neurons and glial cells could inform both prognosis and treatment.

This knowledge will be essential for developing more targeted and effective exosome-based therapies and diagnostic tools.

4.4. Cell Type-Specific Differences in Exosome Production

While the basic mechanisms of exosome biogenesis are conserved across different cell types, there are significant variations in the quantity, composition, and functional properties of exosomes produced by different cells. Understanding these cell type-specific differences is central for interpreting exosome-based biomarkers and developing targeted therapeutic strategies. Some key differences include:

Immune cells illustrate this variation. Dendritic cells produce exosomes rich in MHC class I and II molecules that contribute to antigen presentation (Théry et al., 2002); B cells secrete exosomes carrying B cell receptor-derived antigens and MHC class II molecules (Raposo et al., 1996); T cells release exosomes containing T cell receptor components and miRNAs that modulate immune responses (Mittelbrunn et al., 2011); and macrophages produce exosomes that may carry pathogen-associated molecules and act in both pro- and anti-inflammatory directions (Bhatnagar & Schorey, 2007). Cancer cells often release more exosomes than their normal counterparts (Logozzi et al., 2009), and these frequently contain oncoproteins, oncogenic miRNAs and factors that promote metastasis and angiogenesis (Melo et al., 2014). In the nervous system, neurons release exosomes in an activity-dependent manner, often containing neurotransmitter receptors and synaptic proteins (Lachenal et al., 2011); oligodendrocytes produce exosomes carrying myelin proteins and stress-protective factors (Krämer-Albers et al., 2007); and astrocytes secrete exosomes that modulate neuronal function and contribute to neuroinflammation (Wang et al., 2017). Among stem cells, mesenchymal stem cells produce exosomes with regenerative and immunomodulatory properties, rich in growth factors and anti-inflammatory molecules (Phinney & Pittenger, 2017), while neural stem cells release exosomes that promote neurogenesis and neuroprotection (Webb et al., 2018). Epithelial cells show the same specialization: intestinal epithelial cells secrete exosomes that modulate gut immunity and interact with the microbiome (Deng et al., 2015), and renal epithelial cells produce exosomes involved in nephron function that can serve as biomarkers of kidney disease (Borges et al., 2013). Endothelial cells release exosomes that promote angiogenesis and regulate vascular function (van Balkom et al., 2013), often carrying factors that influence vascular permeability and inflammation (Hromada et al., 2017). Hepatocytes produce exosomes involved in liver homeostasis and regeneration (Nojima et al., 2016), containing liver-specific enzymes and metabolic regulators (Royo et al., 2013). Adipocytes secrete exosomes that influence systemic metabolism and insulin sensitivity (Thomou et al., 2017) and often carry adipokines and regulators of lipid metabolism (Zhang et al., 2015a). Placental cells produce exosomes central to fetal-maternal communication and the maintenance of pregnancy (Salomon et al., 2014), including factors that modulate maternal immune responses and vascular function (Mitchell et al., 2015). Finally, skeletal muscle cells release exosomes that influence metabolism and muscle regeneration (Rome et al., 2019), and cardiac myocytes produce exosomes involved in cardiac remodelling and the response to stress (Malik et al., 2013).

Understanding these cell type-specific differences in exosome production has important implications.

Cell type-specific exosome signatures open several lines of work. They can be used to develop more precise biomarkers, and knowledge of cell-specific properties can be turned into targeted therapeutic strategies, such as using neural stem cell-derived exosomes for neurological disorders, or into engineered exosomes with defined properties. Differences in exosome production between normal and diseased cells also point to pathological processes and possible therapeutic targets, while the natural tropism of cell type-specific exosomes could be exploited for delivery to particular tissues or organs.

5. Exosome Uptake and Internalization

The ability of exosomes to transfer their cargo to recipient cells is important for their role in intercellular communication. The process of exosome uptake and internalization by target cells involves several mechanisms and is influenced by various factors. Understanding these processes is essential for developing exosome-based therapies and interpreting the biological effects of exosomes.

5.1. Mechanisms of Exosome Uptake

Several mechanisms have been identified for the uptake of exosomes by recipient cells.

Clathrin-mediated endocytosis: This process involves the formation of clathrin-coated pits on the plasma membrane, which invaginate and pinch off to form intracellular vesicles containing exosomes (Tian et al., 2014). Dynamin,

a GTPase involved in vesicle scission, is central to this process. Caveolin-dependent endocytosis: Caveolae are small, flask-shaped invaginations of the plasma membrane rich in cholesterol and sphingolipids. Some studies have shown that exosomes can be internalized via caveolin-dependent endocytosis (Svensson et al., 2013). Macropinocytosis: This process involves the formation of large, irregular vesicles through actin-driven membrane ruffling. Macropinocytosis has been observed as a mechanism for exosome uptake in various cell types (Costa Verdera et al., 2017). Phagocytosis: Primarily observed in professional phagocytes like macrophages and dendritic cells. Exosomes can be recognised and engulfed through phagocytic processes (Feng et al., 2010). Lipid raft-mediated endocytosis: Lipid rafts are cholesterol- and sphingolipid-rich microdomains in the plasma membrane. Some studies suggest that exosome uptake can occur through lipid raft-mediated endocytosis (Svensson et al., 2013). Direct fusion with plasma membrane: Although less common, some studies have reported direct fusion of exosomes with the plasma membrane of recipient cells (Parolini et al., 2009). This mechanism would allow for direct delivery of exosomal contents into the cytoplasm. Receptor-mediated endocytosis: Specific interactions between exosomal surface proteins and receptors on target cells can mediate exosome uptake. For example, exosomal tetraspanins can interact with integrins on recipient cells to facilitate internalization (Morelli et al., 2004).

5.2. Cellular Tropism of Exosomes

Exosomes can exhibit preferential uptake by specific cell types, a phenomenon known as cellular tropism. This tropism is influenced by various factors.

Surface protein composition: The presence of specific proteins on the exosome surface can determine their interaction with target cells. For example, exosomes bearing MHC molecules may preferentially interact with T cells (Théry et al., 2002). Integrin profile: The integrin composition of exosomes can direct them to specific target tissues. Hoshino et al. (2015) demonstrated that exosomal integrins can determine organotropic metastasis by preparing pre-metastatic niches in specific organs. Lipid composition: The lipid profile of exosomes can influence their interaction with target cell membranes. For instance, phosphatidylserine exposure on exosomes can enhance their uptake by macrophages (Matsumoto et al., 2017). Glycosylation patterns: Surface glycans on exosomes can mediate specific interactions with lectins or other glycan-binding proteins on target cells (Williams et al., 2018). Microenvironmental factors: The presence of specific extracellular matrix components or soluble factors in the target tissue can influence exosome-cell interactions (Whiteside, 2018). Cell type-specific receptors: The expression of specific receptors on target cells can determine their affinity for certain exosome populations (French et al., 2017).

5.3. Factors Influencing Exosome Uptake

Several factors can modulate the efficiency and specificity of exosome uptake.

Exosome size and concentration: Smaller exosomes tend to be internalized more efficiently than larger ones. Higher concentrations of exosomes generally lead to increased uptake, but saturation can occur (Mulcahy et al., 2014). Surface charge: The overall surface charge of exosomes can influence their interaction with cell membranes. Positively charged exosomes tend to have higher uptake rates due to electrostatic interactions with negatively charged cell surfaces (Nakase & Futaki, 2015). pH and ionic strength: Environmental pH and ionic conditions can affect exosome stability and their interaction with cell membranes (Parolini et al., 2009). Temperature: Exosome uptake is generally an energy-dependent process and is reduced at lower temperatures (Escrevente et al., 2011). Cellular activation state: The activation or differentiation state of recipient cells can influence their capacity to internalize exosomes (Obregon et al., 2006). Presence of competing molecules: Soluble factors or other extracellular vesicles in the microenvironment can compete with exosomes for uptake (Mulcahy et al., 2014). Cytoskeletal dynamics: The actin and microtubule networks play key roles in various endocytic pathways. Disruption of these networks can affect exosome internalization (Svensson et al., 2013). Membrane fluidity: The fluidity of both the exosomal and cellular membranes can influence fusion and uptake processes (Parolini et al., 2009). Exosome surface modifications: Natural or engineered modifications to the exosome surface, such as the addition of targeting peptides or antibodies, can alter uptake patterns (Kooijmans et al., 2016). Cell cycle stage: The uptake efficiency of exosomes can vary depending on the cell cycle stage of the recipient cell (Mulcahy et al., 2014).

Understanding the mechanisms of exosome uptake and the factors that influence this process has important implications for both basic science and translational research.

Knowledge of exosome tropism and uptake mechanisms can be exploited to develop more efficient and targeted drug delivery systems. Modifying exosome surface

properties could enhance uptake by particular cell types or tissues, while elucidating how pathogens or tumour cells exploit these pathways may reveal disease mechanisms and therapeutic targets. The same knowledge could yield more sensitive exosome-based diagnostic tools, improve the efficacy of exosome-based therapies by optimising the conditions for uptake, and refine our understanding of how cells communicate in health and disease.

This knowledge will be essential for realizing the potential of exosomes in both diagnostic and therapeutic applications.

6. Functional Implications of Exosomal Cargo Transfer

The transfer of exosomal cargo between cells has considerable implications for intercellular communication and various physiological and pathological processes. The functional consequences of this cargo transfer can be observed in multiple contexts:

6.1. Cancer Progression and Metastasis

Exosomes play critical roles in various aspects of cancer biology, largely mediated by their diverse cargo.

Promoting metastasis: Exosomal integrins have been shown to determine organotropic metastasis by preparing pre-metastatic niches in specific organs. Hoshino et al. (2015) demonstrated that exosomes from different cancer types carry distinct integrin profiles that direct them to specific organ sites, initiating pre-metastatic niche formation. Exosomal matrix metalloproteinases (MMPs) can remodel the extracellular matrix, facilitating tumour cell invasion (Shimoda & Khokha, 2017). Angiogenesis: Exosomal cargo can stimulate angiogenesis, promoting tumour growth. For instance, glioblastoma-derived exosomes carrying VEGF and miR-21 have been shown to stimulate angiogenesis in endothelial cells (Wang et al., 2019). Exosomal miR-210 from hypoxic tumour cells can promote angiogenesis by targeting ephrin A3 in endothelial cells (King et al., 2012). Immune modulation: Cancer-derived exosomes can suppress anti-tumour immune responses. Chalmin et al. (2010) demonstrated that tumour-derived exosomes carrying Hsp72 can activate myeloid-derived suppressor cells, leading to immunosuppression. Exosomal PD-L1 can inhibit T cell activation and promote tumour immune evasion (Chen et al., 2018). Drug resistance: Exosomes can transfer drug resistance traits between cancer cells. Qu et al. (2016) showed that exosome-transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. Exosomal transfer of P-glycoprotein, a drug efflux pump, can confer multidrug resistance to recipient cells (Bebawy et al., 2009). Metabolic reprogramming: Cancer-derived exosomes can alter the metabolism of recipient cells in the tumour microenvironment. For example, pancreatic cancer-derived exosomes can induce lipid synthesis in liver cells, promoting metastatic growth (Fonseca et al., 2016). Epithelial-mesenchymal transition (EMT): Exosomes can transfer factors that promote EMT, a key process in cancer progression and metastasis. Exosomal TGF-β and miRNAs such as miR-200 family members have been implicated in this process (Le et al., 2014). Cancer stem cell maintenance: Exosomes can contribute to the maintenance and propagation of cancer stem cells. Glioblastoma-derived exosomes may also carry miRNAs that act on the tumour microenvironment. Intercellular communication within the tumour: Exosomes mediate communication between different cell populations within the tumour, contributing to tumour heterogeneity and adaptation. For instance, exosomal transfer of oncogenic EGFR from mutant to wild-type cells can promote tumour growth (Al-Nedawi et al., 2008). Modulation of distant organs: Cancer-derived exosomes can influence distant organs, preparing them for metastatic colonization. Pancreatic cancer-derived exosomes have been shown to induce liver pre-metastatic niche formation through macrophage-mediated fibrosis (Costa-Silva et al., 2015). Diagnostic and prognostic biomarkers: The cargo of cancer-derived exosomes can serve as liquid biopsy biomarkers for early detection, disease monitoring, and prognosis. For example, exosomal GPC1 has been proposed as a diagnostic marker for pancreatic cancer (Melo et al., 2015).

Understanding the diverse roles of exosomes in cancer progression and metastasis has important implications for both basic cancer biology and translational oncology.

The implications for oncology run in several directions. Inhibiting exosome production or uptake, or neutralising specific cargoes, has been proposed as an anti-cancer strategy, while engineered exosomes could deliver therapeutic agents to cancer cells or modulate the tumour microenvironment. Exosomal cargoes may also serve as minimally invasive biomarkers for early detection, treatment monitoring and prediction of metastasis, and analysis of exosomal integrins might help anticipate likely metastatic sites, informing surveillance. Studying exosome-mediated communication within tumours helps explain how heterogeneity develops and is maintained, and understanding the

contribution of exosomes to drug resistance could inform combination therapy. Characterisation of patient-derived exosomes, finally, could guide treatment tailored to individual tumour biology.

6.2. Immune Regulation

Exosomes play central roles in both innate and adaptive immune responses, mediating complex interactions between various immune cell types and contributing to both immune activation and suppression. The diverse functions of exosomes in immune regulation include:

Antigen presentation: Exosomes from antigen-presenting cells can carry MHC-peptide complexes and co-stimulatory molecules, potentially activating T cells directly or being taken up by other antigen-presenting cells (Théry et al., 2002). Dendritic cell-derived exosomes can stimulate antigen-specific T cell responses, even in the absence of intact dendritic cells (Zitvogel et al., 1998). Cytokine and chemokine transfer: Exosomes can carry cytokines and chemokines, influencing immune cell activation and migration. For example, dendritic cell-derived exosomes carrying TNF-α have been shown to activate synovial fibroblasts in rheumatoid arthritis (Zhang et al., 2006). Exosomal transfer of CCL2 can promote monocyte migration and differentiation (Viaud et al., 2009). miRNA-mediated regulation: Exosomal miRNAs can modulate immune responses. Alexander et al. (2015) demonstrated that exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Exosomal miR-155 has been shown to promote inflammatory M1 macrophage polarisation (Wang et al., 2017). Pathogen defense: Exosomes can participate in host defense against pathogens by transferring antimicrobial proteins and peptides. Neutrophil-derived exosomes containing myeloperoxidase and elastase can kill bacteria (Timar et al., 2013). Exosomes can also transfer antiviral factors, such as APOBEC3G, conferring resistance to HIV infection (Khatua et al., 2009). Tolerance induction: Exosomes can contribute to the maintenance of immune tolerance. Placenta-derived exosomes carrying immunosuppressive molecules like Fas ligand can promote maternal-fetal tolerance (Stenqvist et al., 2013). Regulatory T cell-derived exosomes can suppress effector T cell responses and promote tolerance (Okoye et al., 2014). Autoimmune regulation: Exosomes can play both pathogenic and protective roles in autoimmune diseases. In systemic lupus erythematosus, exosomes carrying autoantigens can stimulate autoantibody production (J.-H. Lee et al., 2016). Conversely, mesenchymal stem cell-derived exosomes have shown immunomodulatory effects in autoimmune conditions (Riazifar et al., 2017). Tumour immunity: Exosomes can modulate anti-tumour immune responses. Tumour-derived exosomes can suppress NK cell function through TGF-β1 and other immunosuppressive molecules (Clayton et al., 2007). Allergic responses: Exosomes can contribute to allergic sensitization and inflammation. Bronchial epithelial cell-derived exosomes carrying thymic stromal lymphopoietin (TSLP) can promote Th2 responses in allergic asthma (Levänen et al., 2013). Innate immune signalling: Exosomes can transfer pattern recognition receptors (PRRs) and their ligands, modulating innate immune responses. Exosomal transfer of mitochondrial DNA can activate cGAS-STING signalling in recipient cells, promoting inflammatory responses (Kitai et al., 2017). Immune cell differentiation and function: Exosomes can influence the differentiation and functional properties of immune cells. Exosomes from activated platelets can promote differentiation of monocytes into pro-inflammatory macrophages (Sadallah et al., 2011). B cell-derived exosomes can modulate T cell responses and germinal centre reactions (Saunderson et al., 2008).

Understanding the diverse roles of exosomes in immune regulation has important implications for both basic immunology and translational medicine.

Several applications follow from this. Exosome-based vaccines could enhance antigen presentation and immune responses against pathogens or tumours, and engineered exosomes could deliver immunomodulatory molecules or strengthen anti-tumour immunity. Targeting exosome-mediated pathways may lead to new treatments for autoimmune disorders, and manipulating these responses could help to promote transplant tolerance or prevent rejection. Exosomal immune markers could also serve as indicators of immune status or disease activity, while studying exosome-mediated communication between immune cells offers a route into the mechanisms of immune-related disorders. Finally, targeting exosome-mediated allergic sensitization may suggest preventive or therapeutic approaches in allergic disease.

A clearer picture of how exosomes act in immune regulation should lead to new diagnostic tools and therapeutic strategies, potentially advancing the management of immune-related disorders.

6.3. Neurodegenerative Diseases

Exosomes have been implicated in the progression of various neurodegenerative disorders, playing both pathogenic and potentially protective roles.

Protein aggregate spreading: Exosomes may contribute to the spread of pathogenic proteins in diseases such as Alzheimer's and Parkinson's. Stuendl et al. (2016) showed that exosomes from cerebrospinal fluid of Parkinson's disease patients could transmit α-synuclein to recipient cells. In Alzheimer's disease, exosomes have been implicated in the spread of tau and amyloid-β proteins (Rajendran et al., 2006). Neuroinflammation: Exosomes can mediate communication between neurons, glia, and immune cells in the central nervous system, influencing inflammatory responses. Microglial exosomes carrying pro-inflammatory cytokines can exacerbate neuroinflammation in neurodegenerative conditions (Joshi et al., 2015). Neuroprotection: Conversely, exosomes may also play protective roles in the central nervous system.

Xin et al. (2013) demonstrated that exosomes derived from mesenchymal stem cells could promote functional recovery after stroke in rats, partly through the transfer of miR-133b. Exosomes from astrocytes can transfer neuroprotective proteins to neurons, potentially mitigating neurodegenerative processes (Guitart et al., 2016). Synaptic plasticity: Neuronal exosomes can modulate synaptic function and plasticity. Exosomal transfer of Arc protein between neurons has been shown to regulate synaptic plasticity (Ashley et al., 2018). Blood-brain barrier (BBB) integrity: Exosomes can influence BBB permeability and function. Endothelial cell-derived exosomes can transfer claudin-5, a tight junction protein, potentially reinforcing BBB integrity (Yamamoto et al., 2015). Neurogenesis and neural repair: Exosomes from neural stem cells and other sources can promote neurogenesis and neural repair. Neural stem and progenitor cell-derived exosomal miR-210 is one example: Hypoxia-triggered secretion of exosomes by neural progenitor cells, enriched in miR-210, has been shown to enhance the survival and proliferation of recipient neural progenitor cells; suggesting a potential mechanism for promoting neurogenesis following traumatic brain injury (Zhao et al., 2020). Diagnostic biomarkers: Exosomal content from cerebrospinal fluid or blood can serve as potential biomarkers for neurodegenerative diseases. Levels of exosomal α-synuclein have been proposed as a biomarker for Parkinson's disease (Shi et al., 2014). Therapeutic delivery: Exosomes can potentially be used as vehicles for delivering therapeutic agents across the blood-brain barrier. Alvarez-Erviti et al. (2011) delivered siRNA to the mouse brain by systemic injection of targeted exosomes, knocking down BACE1, a therapeutic target in Alzheimer's disease. Glial communication: Exosomes mediate communication between different glial cell types, influencing their roles in neurodegenerative processes. Oligodendrocyte-derived exosomes can transfer protective factors to neurons under stress conditions (Frühbeis et al., 2013). Mitochondrial dysfunction: Exosomes may play a role in the intercellular transfer of dysfunctional mitochondria or mitochondrial DNA in neurodegenerative diseases.

Understanding the roles of exosomes in neurodegenerative diseases has important implications for both basic neuroscience and translational research.

Several implications follow. Inhibiting the exosome-mediated spread of pathogenic proteins could slow disease progression, and harnessing the neuroprotective potential of certain exosome populations could yield new treatments. Exosomal cargo may serve as a minimally invasive biomarker for early detection and for monitoring progression, and engineered exosomes could carry therapeutic agents across the blood-brain barrier. Studying exosome-mediated communication in the nervous system also illuminates the cellular interactions that underlie neurodegeneration, while characterising patient-derived exosomes could guide individualized treatment. Targeting exosome-mediated inflammatory pathways is a further possible strategy for reducing neuroinflammation.

Better characterisation of exosome function in neurodegenerative disease may yield new diagnostic tools and therapeutic strategies, potentially advancing the management of these challenging conditions.

6.4. Cardiovascular Function

Exosomes play diverse roles in cardiovascular physiology and pathology, mediating communication between various cell types in the heart and blood vessels.

Cardioprotection: Exosomes from various cell types, including cardiomyocytes and stem cells, have been shown to confer cardioprotection. For instance, exosomes from cardiac progenitor cells carrying miR-210 can protect cardiomyocytes from oxidative stress (Wang et al., 2015). Mesenchymal stem cell-derived exosomes can reduce infarct size and improve cardiac function after myocardial infarction (Lai et al., 2010). Angiogenesis: Exosomes can promote or inhibit angiogenesis, depending on their cellular origin and cargo. Endothelial cell-derived exosomes containing miR-214 can stimulate angiogenesis by suppressing ataxia

telangiectasia mutated in recipient cells (van Balkom et al., 2013). Conversely, exosomes derived from mesenchymal stem cells have been shown to inhibit tumour angiogenesis by downregulating VEGF expression via miR-16 delivery, thereby impairing neovascularization in a breast cancer model (J.-K. Lee et al., 2013). Atherosclerosis: Exosomes may contribute to the development of atherosclerosis through various mechanisms. Zheng et al. (2017) showed that exosome-mediated transfer of miR-155 from smooth muscle cells to endothelial cells induces endothelial injury and promotes atherosclerosis. Macrophage-derived exosomes can transfer pro-inflammatory mediators, exacerbating atherosclerotic inflammation (Nguyen et al., 2018). Cardiac remodeling: Exosomes mediate communication between different cell types during cardiac remodeling processes. Fibroblast-derived exosomes carrying miR-21* can induce cardiomyocyte hypertrophy (Bang et al., 2014). Cardiomyocyte-derived exosomes can influence fibroblast activation and extracellular matrix production (Cosme et al., 2017). Vascular tone regulation: Exosomes can modulate vascular tone by transferring vasoactive substances. Platelet-derived exosomes containing thromboxane A2 can induce vasoconstriction (BRILL et al., 2005). Thrombosis: Exosomes can contribute to both pro-thrombotic and anti-thrombotic processes. Platelet-derived exosomes can promote coagulation by exposing phosphatidylserine and tissue factor (Sinauridze et al., 2007). Endothelial cell-derived exosomes carrying tissue factor pathway inhibitor can have anticoagulant effects (Steppich et al., 2005). Cardiac electrophysiology: Exosomes can modulate cardiac electrical activity and arrhythmogenesis. Fibroblast-derived exosomes carrying specific miRNAs can alter cardiomyocyte electrophysiology, potentially contributing to arrhythmias. Endothelial function: Exosomes mediate communication between endothelial cells and other vascular cell types. Exosomes from shear-stressed endothelial cells can transfer atheroprotective molecules to regions of disturbed flow (Hergenreider et al., 2012). Stem cell-mediated repair: Exosomes from various stem cell populations can promote cardiac repair and regeneration. Cardiac stem cell-derived exosomes can stimulate angiogenesis and cardiomyocyte survival after myocardial infarction (Barile et al., 2014). Metabolic regulation: Exosomes can transfer metabolic enzymes and regulators between cells in the cardiovascular system. Adipocyte-derived exosomes carrying specific miRNAs can modulate lipid metabolism in cardiomyocytes (Thomou et al., 2017).

Understanding the diverse roles of exosomes in cardiovascular function has important implications for both basic cardiovascular biology and translational medicine.

Several implications follow. Exosome-based therapies could be developed for cardioprotection, for promoting angiogenesis or for modulating cardiac remodelling, and exosomal cargo could serve as a biomarker for cardiovascular conditions, aiding early diagnosis or risk stratification. Engineered exosomes might deliver therapeutic agents specifically to the heart or vasculature, and studying exosome-mediated communication in the cardiovascular system offers insight into the pathogenesis of cardiac and vascular disorders. Analysis of patient-derived exosomes could inform individualized treatment, exosomes could be incorporated into tissue-engineered constructs to promote vascularization, and targeting exosome-mediated inflammatory pathways may help to reduce vascular inflammation in conditions such as atherosclerosis.

As exosome biology in the cardiovascular system is worked out, it may become possible to develop new diagnostic tools and therapeutic strategies, potentially advancing the management of cardiovascular diseases.

6.5. Regenerative Medicine

The cargo of stem cell-derived exosomes has shown promise in various regenerative applications, offering potential alternatives or complements to cell-based therapies.

Tissue repair: Mesenchymal stem cell (MSC)-derived exosomes have been shown to promote tissue repair in various contexts. For example, Furuta et al. (2016) demonstrated that MSC-derived exosomes promote fracture healing in a mouse model. Exosomes from adipose-derived stem cells can promote cutaneous wound healing by enhancing angiogenesis and collagen synthesis (Hu et al., 2016). Neuroprotection and neuroregeneration: Exosomes from neural stem cells have shown neuroprotective effects. Webb et al. (2018) found that human neural stem cell extracellular vesicles improved tissue and functional recovery in a murine thromboembolic stroke model. MSC-derived exosomes carrying miR-133b have been shown to promote neurite outgrowth and functional recovery after stroke (Xin et al., 2013). Cardiac repair: Exosomes from cardiac progenitor cells and other stem cell sources have shown promise in promoting cardiac repair after injury. Cardiac progenitor cell-derived exosomes can stimulate angiogenesis and cardiomyocyte survival after myocardial infarction (Barile et al., 2014). Bone and cartilage regeneration: MSC-derived exosomes can promote osteogenesis and chondrogenesis.

Exosomes from human-induced pluripotent stem cell-derived MSCs can enhance bone regeneration in critical-sized calvarial defects (Qi et al., 2016). Liver regeneration: Exosomes from various stem cell sources have shown potential in promoting liver regeneration and reducing fibrosis. MSC-derived exosomes can attenuate liver fibrosis by inhibiting the activation of hepatic stellate cells (T. Li et al., 2013). Kidney repair: Stem cell-derived exosomes have shown promise in promoting kidney repair and reducing acute kidney injury. Exosomes from human umbilical cord MSCs can protect against cisplatin-induced nephrotoxicity (Zhou et al., 2013). Lung regeneration: Exosomes from various stem cell sources have shown potential in promoting lung repair and reducing fibrosis. MSC-derived exosomes can attenuate silica-induced pulmonary fibrosis (Phinney et al., 2015). Immunomodulation: Stem cell-derived exosomes can modulate immune responses, potentially benefiting various regenerative processes. MSC-derived exosomes can suppress T cell proliferation and promote regulatory T cell expansion (Phinney & Pittenger, 2017). Angiogenesis: Exosomes from various stem cell types can promote angiogenesis, a decisive process in tissue regeneration. Endothelial progenitor cell-derived exosomes can stimulate angiogenesis in ischemic tissues (Vrijsen et al., 2010). Mitochondrial transfer: Some studies suggest that stem cell-derived exosomes can transfer functional mitochondria to injured cells, promoting cellular energetics and survival (Islam et al., 2012).

The use of exosomes in regenerative medicine offers several potential advantages over cell-based therapies.

Exosomes are generally considered safer than whole cells, carrying lower risks of tumourigenicity or uncontrolled differentiation. They can be produced in large quantities and stored more easily than living cells, and preparations can in principle be standardised more consistently than cell products. They can also be engineered or loaded with defined therapeutic cargoes, may cross barriers such as the blood-brain barrier more readily than whole cells, and are likely to be less immunogenic, which would make allogeneic use possible.

However, several difficulties remain in the development of exosome-based regenerative therapies.

The main obstacles are consistent and reproducible methods for exosome isolation and characterisation; ensuring that therapeutic exosomes reach their intended tissues and cell types; establishing optimal dosing regimens; elucidating the mechanisms by which exosomes promote tissue regeneration; and developing appropriate regulatory frameworks for exosome-based therapeutics.

6.6. Metabolic Disorders

Exosomes play significant roles in metabolic regulation and have been implicated in various metabolic disorders.

Diabetes: Pancreatic β-cell-derived exosomes can transfer microRNAs that regulate insulin secretion and β-cell proliferation (Guay et al., 2015). Adipocyte-derived exosomes in obese individuals can contribute to insulin resistance by transferring specific miRNAs to other metabolic tissues (Deng et al., 2009). Obesity: Adipose tissue-derived exosomes can influence whole-body metabolism by transferring adipokines and miRNAs to distant organs (Thomou et al., 2017). Exosomes from obese adipose tissue can promote macrophage activation and inflammation, contributing to metabolic dysfunction (Deng et al., 2009). Non-alcoholic fatty liver disease (NAFLD): Hepatocyte-derived exosomes in NAFLD can carry proteins and miRNAs that influence lipid metabolism in recipient cells (Hirsova et al., 2016). Exosomes from lipotoxic hepatocytes can activate inflammatory pathways in macrophages, contributing to liver inflammation (Tomita et al., 2017). Metabolic syndrome: Exosomes can mediate cross-talk between different metabolic tissues (e.g., adipose tissue, liver, and muscle), influencing the development of metabolic syndrome (Deng et al., 2009). Platelet-derived exosomes in metabolic syndrome can contribute to vascular inflammation and atherosclerosis (Agouni et al., 2008). Mitochondrial disorders: Exosomes may play a role in the intercellular transfer of mitochondrial DNA and proteins, potentially influencing mitochondrial function in recipient cells (Phinney et al., 2015). Lipid metabolism disorders: Exosomes can transfer enzymes and regulatory factors involved in lipid metabolism between cells, influencing lipid homeostasis (Record et al., 2014).

Understanding the roles of exosomes in metabolic disorders has important implications.

Exosomal cargo could serve as a biomarker for the early detection and monitoring of metabolic disease. Modulating exosome production or uptake may prove to be a therapeutic strategy in its own right, and engineered exosomes could deliver therapeutic agents to specific metabolic tissues. Studying exosome-mediated communication between metabolic tissues also offers insight into how these disorders develop.

6.7. Infectious Diseases

Exosomes play complex roles in host-pathogen interactions and the progression of infectious diseases.

Viral infections: Exosomes can facilitate viral spread by transferring viral components between cells (Nolte-'t Hoen et al., 2016). Host cell-derived exosomes can also transfer antiviral factors, contributing to innate immune responses (J. Li et al., 2013). Bacterial infections: Exosomes can transfer bacterial antigens to immune cells, modulating host immune responses (Bhatnagar & Schorey, 2007). Some bacteria can hijack host exosome pathways to promote their survival and spread (Schorey et al., 2015). Parasitic infections: Parasite-derived exosomes can modulate host immune responses and influence disease progression (Coakley et al., 2015). Host-derived exosomes can also play roles in anti-parasitic immune responses (Silverman et al., 2010). Prion diseases: Exosomes have been implicated in the spread of prion proteins between cells, contributing to disease progression (Vella et al., 2007).

Exosomes in infectious diseases have several potential applications.

Exosomal content could serve as a biomarker for various infections. Exosome-based vaccines could enhance immune responses against pathogens. Therapeutics is another. Targeting exosome-mediated pathogen spread could be a strategy for treating certain infections.

6.8. Pregnancy and Reproduction

Exosomes play important roles in various aspects of reproduction and pregnancy.

Embryo implantation: Endometrial epithelial cell-derived exosomes can modulate trophoblast function and promote embryo implantation (Ng et al., 2013). Placental function: Placental exosomes contribute to maternal-fetal communication and can influence maternal immune responses (Salomon et al., 2014). Fetal development: Exosomes in amniotic fluid may play roles in fetal development and maturation (Keller et al., 2007). Pregnancy complications: Altered exosome profiles have been associated with various pregnancy complications, such as preeclampsia and gestational diabetes (Salomon et al., 2017). Male fertility: Exosomes in seminal fluid can influence sperm function and female reproductive tract responses (Vojtech et al., 2014).

Understanding exosome functions in reproduction could lead to:

new diagnostic tools for pregnancy complications, improved assisted reproductive technologies, and new approaches to managing pregnancy-related disorders.

7. Exosomes as Biomarkers

Exosomes have become promising sources of biomarkers for various diseases due to their unique properties:

7.1. Liquid Biopsy Applications

Exosomes can be isolated from various bodily fluids, offering potential for non-invasive diagnostics.

Cancer detection and monitoring: Tumour-derived exosomes can carry specific proteins, nucleic acids, and lipids that reflect the status of the tumour (Melo et al., 2015). Neurodegenerative diseases: Exosomal biomarkers for diseases like Alzheimer's and Parkinson's could enable earlier diagnosis and monitoring of disease progression (Shi et al., 2014). Cardiovascular diseases: Exosomal markers could help in risk stratification and monitoring of heart diseases (Emanueli et al., 2015).

7.2. Disease-Specific Exosomal Signatures

Different diseases can produce distinct exosomal signatures.

Protein markers are one class: specific proteins are enriched in disease-associated exosomes. Nucleic acid profiles are a second, with distinct RNA or DNA signatures in exosomes from diseased cells. Lipid composition is a third, since exosomal lipid profiles are altered in certain pathological states.

7.3. Challenges and Opportunities in Exosome-Based Diagnostics

While promising, exosome-based diagnostics face several challenges.

Standardisation of isolation methods; sensitivity and specificity of detection; and distinguishing disease-specific exosomes from background exosomes.

Overcoming these challenges could lead to powerful new diagnostic tools for early disease detection and personalised medicine approaches.

This overview covers the major functional implications of exosomal cargo transfer across various physiological and pathological contexts. Exosome research is advancing quickly, and new findings keep widening our understanding of their roles in health and disease.

8. Therapeutic Applications of Exosomes

The unique properties of exosomes make them attractive candidates for various therapeutic applications. Their ability to carry diverse cargoes, cross biological barriers, and target specific cell types has led to extensive research into their potential as drug delivery vehicles and therapeutic agents.

8.1 Drug Delivery Vehicles

Exosomes offer several advantages as drug delivery vehicles.

They are natural carriers. As endogenous vesicles, exosomes are less likely to trigger immune responses compared to synthetic nanoparticles (Kamerkar et al., 2017). Exosomes can cross biological barriers such as the blood-brain barrier, making them potential carriers for CNS-targeted therapies (Alvarez-Erviti et al., 2011). They also allow cellular targeting. The surface proteins of exosomes can be engineered to enhance their targeting to specific cell types or tissues (Tian et al., 2014). A third advantage is cargo protection. The lipid bilayer of exosomes protects their cargo from degradation in the extracellular environment (Kamerkar et al., 2017). Fourth is versatile cargo loading. Exosomes can be loaded with various therapeutic molecules, including small molecule drugs, proteins, and nucleic acids (Batrakova & Kim, 2015).

Examples of exosome-based drug delivery systems under investigation include:

siRNA delivery: Alvarez-Erviti et al. (2011) used engineered, targeted exosomes to deliver siRNA to the mouse brain, knocking down BACE1, a therapeutic target in Alzheimer's disease. Kim et al. (2016) showed that exosomes loaded with paclitaxel delivered the drug to tumour cells and reduced tumour growth in mice, and Haney et al. (2015) used exosomes to deliver catalase to the brain as a potential treatment for Parkinson's disease, demonstrating neuroprotective effects.

8.2 Gene Therapy

Exosomes have shown promise as vectors for gene therapy.

mRNA and miRNA delivery: Exosomes can deliver functional mRNAs and miRNAs to recipient cells, potentially modulating gene expression (Valadi et al., 2007). Although less common, exosomes have also been explored for the delivery of therapeutic DNA (Lamichhane et al., 2015). A further application is CRISPR-Cas9 delivery. Recent studies have investigated the use of exosomes to deliver CRISPR-Cas9 components for gene editing applications (Lin et al., 2018).

8.3 Immunotherapy

Exosomes have potential applications in cancer immunotherapy and treatment of autoimmune diseases.

Dendritic cell-derived exosomes loaded with tumour antigens have been investigated as cancer vaccines in clinical trials (Besse et al., 2016). Exosomes from mesenchymal stem cells have shown immunomodulatory effects and have been proposed for autoimmune and inflammatory conditions (Phinney & Pittenger, 2017), and exosomes bearing chimeric antigen receptors have been explored as an alternative to CAR-T cell therapy, potentially with reduced side effects (Tang et al., 2015).

8.4 Regenerative Medicine Applications

Stem cell-derived exosomes have shown promise in various regenerative medicine applications.

Cardiac repair is one application. Exosomes from cardiac progenitor cells have demonstrated cardioprotective effects in models of myocardial infarction (Barile et al., 2014). Neuroprotection is another. Neural stem cell-derived exosomes have shown potential in promoting functional recovery after stroke and traumatic brain injury (Xin et al., 2013; Y. Zhang et al., 2015). Exosomes from mesenchymal stem cells have been shown to promote skin wound healing by enhancing angiogenesis and cell proliferation (Zhang et al., 2015a).

8.5 Challenges in Exosome-Based Therapeutics

Despite their potential, several challenges need to be addressed for the successful clinical translation of exosome-based therapies.

Clinical-grade exosomes must be produced at scale (Whitford & Guterstam, 2019) and isolated and characterised under standardised protocols that ensure consistent quality (Théry et al., 2018). Therapeutic cargoes must be loaded efficiently without compromising vesicle integrity (Sutaria et al., 2017), and the targeting of exosomes to the desired tissue, together with their in vivo biodistribution, must be better understood (Wiklander et al., 2015). Formulations and storage conditions have to maintain integrity and function over time (Charoenviriyakul et al., 2018); the complex and variable nature of these products raises regulatory questions (Lener et al., 2015); and the safety of exosome-based therapies depends on minimising unintended effects on non-target tissues (Zhu et al., 2017).

The development of exosome-based therapies represents an active area of investigation in nanomedicine, with the potential to advance the treatment of various diseases.

9. Emerging Technologies in Exosome Research

Exosome research is moving fast, with new methods appearing to address the challenges of isolation, characterisation, and manipulation of these complex vesicles.

9.1. Isolation and Purification Techniques

Improving the efficiency and specificity of exosome isolation is key for both research and clinical applications.

Microfluidic devices: Lab-on-a-chip devices for rapid and efficient exosome isolation (Liu et al., 2017). Examples include the microfluidic serum microvesicle chip of Chen et al. (2010) and the immunoaffinity-based ExoChip device (Kanwar et al., 2014). Acoustic separation: Using acoustic waves to separate exosomes based on size and density (Wu et al., 2017). Nanowire-based capture: Ciliated nanowire-on-micropillar structures have been developed for efficient exosome isolation. These microfluidic devices utilize porous silicon nanowires etched onto micropillar sidewalls to selectively trap exosome-like lipid vesicles while filtering out proteins and cell debris. The captured vesicles can be recovered intact by dissolving the porous nanowires in PBS buffer (Wang et al., 2013). Affinity-based techniques: Using antibodies or aptamers to capture specific exosome subpopulations (Balaj et al., 2015; Zhang & Yue, 2019). Size exclusion chromatography: Improved column designs for higher purity and yield of isolated exosomes (Böing et al., 2014). Tangential flow filtration: Scalable method for isolating exosomes from large volumes of biofluids (Heinemann et al., 2014).

9.2. Single-vesicle Analysis

Technologies for analysing individual exosomes provide insights into exosome heterogeneity.

Nanoparticle tracking analysis (NTA): Advanced NTA systems with fluorescence capabilities for analysing specific exosome subpopulations (Dragovic et al., 2011). Flow cytometry: High-resolution flow cytometry techniques for single-exosome analysis (Nolte-'t Hoen et al., 2012). Atomic force microscopy (AFM): AFM techniques for visualizing and analysing individual exosomes at high resolution (Sharma et al., 2010). Single-vesicle sequencing: Methods for sequencing the RNA content of individual exosomes (He et al., 2024). Super-resolution microscopy: Techniques like STORM and PALM for visualizing exosome structure and cargo distribution at nanoscale resolution (Nizamudeen et al., 2018).

9.3. Engineered Exosomes

Developing methods to modify exosomes for enhanced therapeutic potential.

Surface engineering: Techniques for modifying exosome surface proteins to enhance targeting or reduce immunogenicity (Kooijmans et al., 2016). Cargo loading: Advanced methods for loading therapeutic cargo into exosomes, such as electroporation, sonication, and chemical transfection (Sutaria et al., 2017). Hybrid exosomes: Creating hybrid vesicles by fusing exosomes with synthetic liposomes to combine the benefits of both systems (Sato et al., 2016). Exosome mimetics: Synthetic nanoparticles designed to mimic the properties of natural exosomes (Jang et al., 2013).

9.4. Exosome-mimetic Nanovesicles

Synthetic vesicles that mimic the properties of natural exosomes.

Cell-derived nanovesicles: Produced by extruding cells through filters to create vesicles with similar membrane composition to natural exosomes (Jang et al., 2013). Liposome-based mimetics: Synthetic liposomes engineered to display exosome-like surface proteins and carry specific cargoes (García-Manrique et al., 2018). Polymer-based mimetics: Biodegradable polymer nanoparticles have been engineered to mimic exosome properties, such as size, surface functionality, and the ability to deliver therapeutic agents. These biomimetic nanoparticles can serve as efficient drug delivery systems, combining the advantages of synthetic materials with the biological functions of natural exosomes (Wang et al., 2022).

9.5. Machine Learning and Artificial Intelligence in Exosome Research

Applying advanced computational methods to exosome research.

Exosome cargo prediction: Machine learning algorithms for predicting exosomal RNA cargo based on sequence features (Santangelo et al., 2016). Image analysis: AI-powered analysis of electron microscopy and super-resolution microscopy images of exosomes (Ning et al., 2018). Biomarker discovery: Machine learning approaches for identifying exosomal biomarker signatures in complex datasets (Melo et al., 2015). Therapeutic design: AI-assisted design of engineered exosomes for targeted drug delivery (Liang et al., 2021). Exosome-cell interaction prediction: Computational models for predicting exosome-cell interactions

based on surface protein compositions (Hoshino et al., 2015).

These emerging technologies are driving rapid advancements in exosome research, enabling more precise characterisation, manipulation, and application of these complex vesicles.

10. Future Directions and Challenges

As the field of exosome research continues to advance, several key areas of focus and challenges emerge:

10.1. Standardization of Exosome Research

Developing standardised protocols and guidelines is essential for ensuring reproducibility and comparability of results across different studies.

Establishing gold standard methods for exosome isolation from different biological fluids. Defining minimal criteria for exosome characterisation and reporting. Terminology for extracellular vesicles also needs refining and standardising related to extracellular vesicles. Developing universally accepted reference materials for exosome research.

10.2. In Vivo Tracking of Exosomes

Improving our ability to track exosomes in vivo is essential for understanding their biodistribution and functional effects.

Advanced imaging methods are needed for non-invasive, real-time tracking of exosomes in living organisms, together with stable, specific labels that allow long-term tracking without altering vesicle behaviour and tools for quantifying exosome distribution and uptake across tissues.

10.3. Exosome-Mediated Interorgan Communication

Elucidating the roles of exosomes in long-distance signalling between different organs.

Three questions stand out: which exosome populations are characteristic of particular organs and where they travel; how exosomes released by one organ influence the function of distant ones; and what part exosome traffic plays in maintaining physiological balance.

10.4. Exosomes in Personalized Medicine

Evaluating exosomes for diagnostic and therapeutic applications.

This would involve analysing individual patients' exosome signatures for diagnosis, creating patient-specific engineered exosomes for treatment, and using exosome analysis as a companion diagnostic to predict and monitor treatment response.

10.5. Ethical Considerations in Exosome Research and Applications

Addressing ethical issues related to exosome research and clinical applications.

Personal information must be protected in exosome-based liquid biopsies, and appropriate consent procedures are needed for the collection and use of exosomes in research and clinical settings. Disparities in access to exosome-based diagnostics and therapies have to be addressed, and regulatory guidelines for these products remain to be established.

The interdisciplinary nature of exosome research necessitates collaboration between experts in various fields, including cell biology, biochemistry, biophysics, bioengineering, and clinical medicine.

11. Conclusion

The study of exosomes has substantially changed our understanding of intercellular communication and opened up new avenues for diagnostic and therapeutic applications. The diverse array of proteins, lipids, and nucleic acids carried by exosomes enables them to modulate a wide range of physiological and pathological processes. From cancer progression and immune regulation to neurodegenerative diseases and regenerative medicine, exosomes play important roles in various biological contexts.

Key points to consider:

Exosomes are complex, heterogeneous vesicles with cell type-specific characteristics and functions. The cargo of exosomes is selectively packaged through various mechanisms, reflecting the state of the parent cell. Exosomes can transfer functional biomolecules between cells, influencing recipient cell behaviour and function. The potential of exosomes as biomarkers and therapeutic agents is being actively explored across multiple medical fields. Emerging technologies are continually enhancing our ability to isolate, characterize, and manipulate exosomes. Standardisation efforts and addressing technical challenges are important for the advancement of exosome research and clinical applications. Ethical considerations and regulatory frameworks need to be developed to guide the responsible use of exosome-based technologies.

Future research directions may include:

Elucidating the mechanisms of cargo sorting with greater precision, potentially allowing for the engineering of exosomes with specific cargo profiles. Investigating the role of

exosomal cargo in long-distance signalling within organisms, particularly in the context of systemic responses to localised stimuli. Developing exosome-based liquid biopsy techniques for early disease detection and monitoring. Exploring the therapeutic potential of engineered exosomes in various diseases, from cancer to neurodegenerative disorders. Unraveling the complex roles of exosomes in maintaining physiological homeostasis and their dysregulation in disease states. Advancing technologies for large-scale production and characterisation of clinical-grade exosomes for therapeutic applications. Investigating the potential of exosomes in personalised medicine approaches, including tailored diagnostics and therapies.

The field of exosome research stands at an active area of investigation, with the potential to transform our understanding of cellular communication and advance approaches to disease diagnosis and treatment. As we continue to unravel the complexities of exosomal cargo and its functions, we move closer to realizing the full potential of these tiny vesicles in both understanding fundamental biological processes and developing novel therapeutic strategies.

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5.4. METHODS OF OBTAINING AND PURIFYING EXOSOMES

1. Introduction

Exosomes, small extracellular vesicles (EVs) ranging from 30 to 150 nm in diameter, have attracted attention as key players in intercellular communication and potential biomarkers for various diseases (Théry et al., 2018). These nanoscale vesicles, secreted by virtually all cell types, carry a diverse cargo of proteins, lipids, and nucleic acids, reflecting the physiological or pathological state of their cell of origin (Raposo & Stoorvogel, 2013). The burgeoning interest in exosomes stems from their potential applications in diagnostics, therapeutics, and as vehicles for drug delivery (Kalluri & LeBleu, 2020). Figure 5.4.1 compares these methods on purity, yield, throughput and preservation of vesicle function. Size alone does not establish endosomal origin; EV or small EV is the operational term when that origin has not been demonstrated.

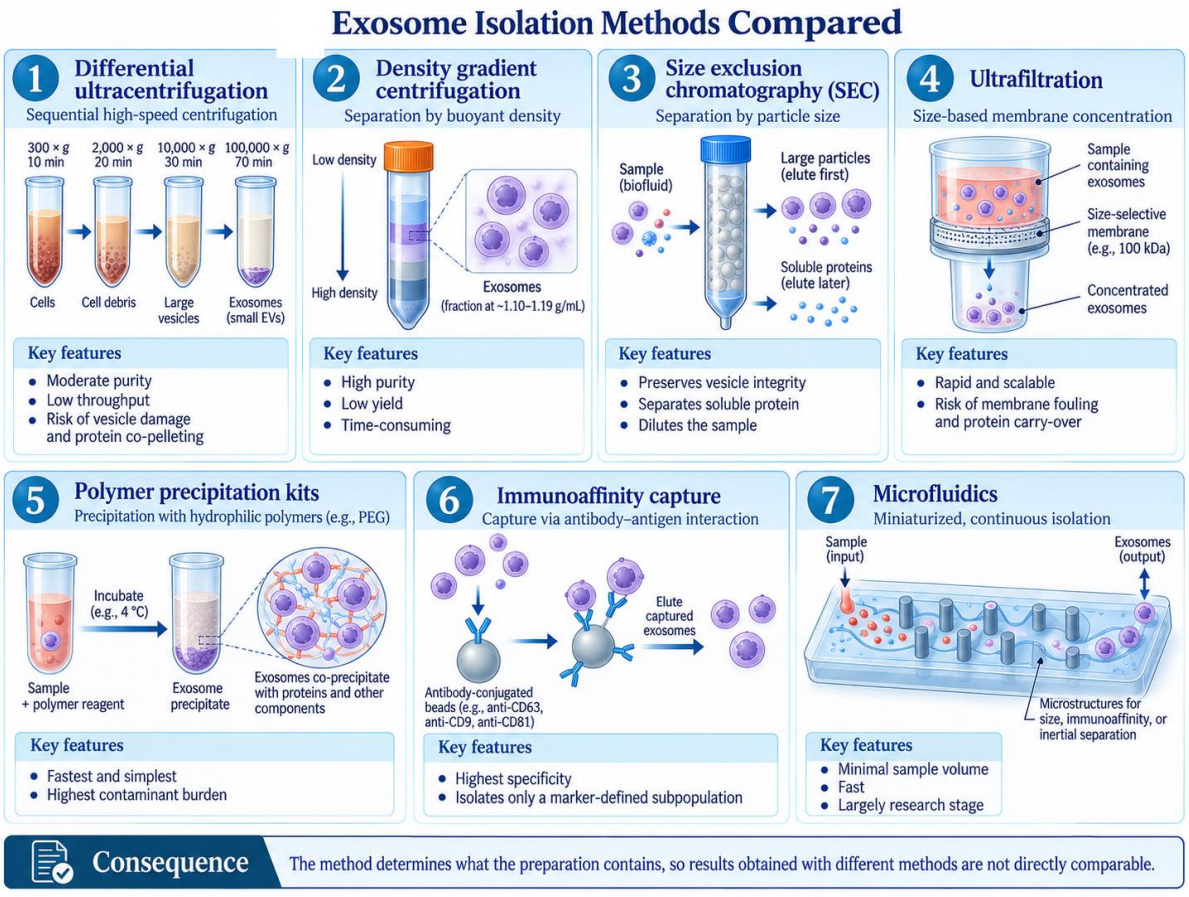


Figure 5.4.1. Exosome isolation methods compared. The method determines what the preparation contains, so results obtained with different methods are not directly comparable.

How exosomes are isolated and purified has turned out to matter as much as what is done with them afterwards. The choice of isolation method can significantly impact the purity, yield, and integrity of the isolated exosomes, thereby influencing downstream analyses and potential clinical applications (Witwer et al., 2013). The sections below set out the principal isolation and purification techniques, their operating principles, and what each gains and gives up; Li et al. (2025) review the same ground for MSC-derived vesicles intended for neurological indications and reach similar conclusions about where the remaining bottlenecks lie. See Figure 5.4.2.

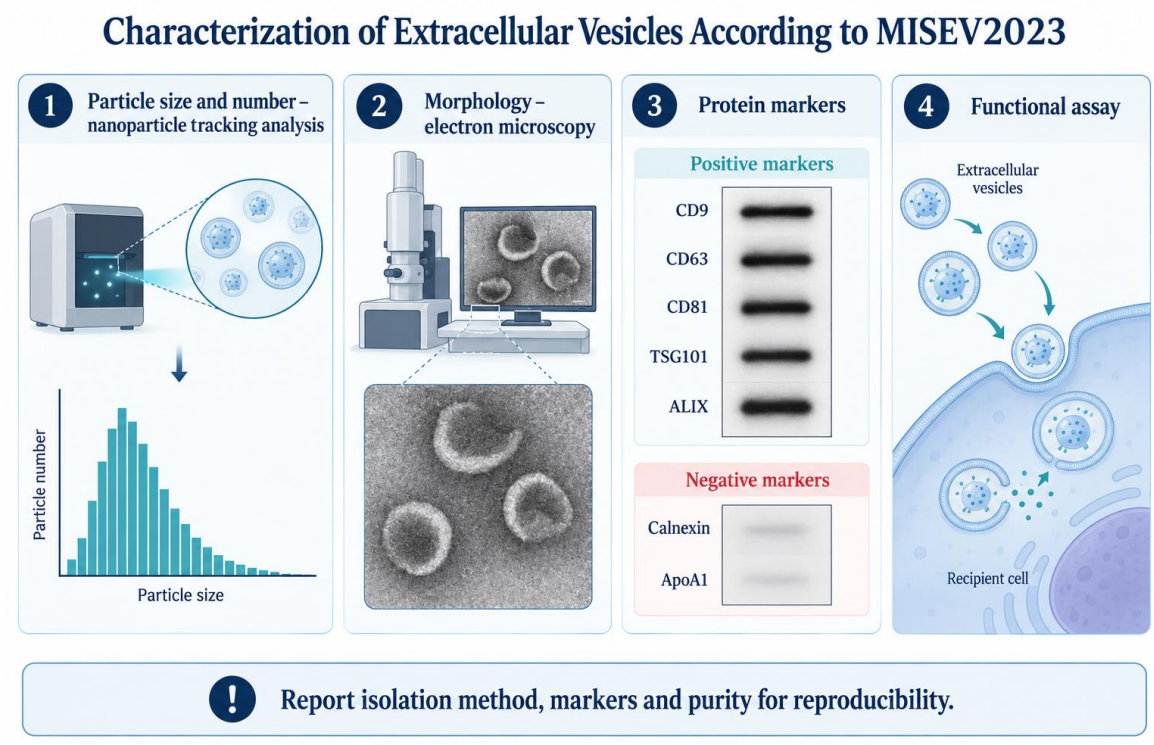


Figure 5.4.2. Conceptual overview of extracellular-vesicle characterisation according to MISEV2023: particle size and number, morphology, positive and negative protein markers, and functional assays. Microscopy and protein-marker depictions illustrate characterisation methods and are not presented as experimental results from a reported study. Marker panels support characterisation but do not by themselves establish endosomal origin.

The methods described below are best understood as points on a trade-off between purity, yield, throughput and preservation of vesicle function, since no single technique optimises all four. Differential ultracentrifugation gives moderate purity and low throughput with substantial equipment requirements and a risk of vesicle damage and protein co-pelleting; density gradient centrifugation improves purity at a further cost in time and yield. Size exclusion chromatography preserves vesicle structure and separates vesicles from soluble protein efficiently, but dilutes the sample and requires a concentration step. Ultrafiltration and precipitation-based kits are rapid and scalable but carry a higher burden of co-isolated contaminants. Immunoaffinity capture provides the highest specificity for a defined marker while selecting, by definition, only a subpopulation. Microfluidic approaches are fast and require minimal sample but remain largely at the research stage. The practical consequence is that the isolation method determines what a preparation contains, and therefore that results obtained with different methods are not directly comparable; MISEV2023 accordingly requires that methods be reported in terms of what they enrich rather than what they are assumed to purify.

2. Traditional Methods of Exosome Isolation

2.1. Differential Ultracentrifugation

Differential ultracentrifugation has long been considered the gold standard for exosome isolation due to its ability to handle large sample volumes and its historical precedence in exosome research (Li et al., 2021). This method involves a series of centrifugation steps at increasing speeds to sequentially remove cells, cellular debris, and larger vesicles, ultimately pelleting exosomes at forces typically exceeding 100,000 × g (Théry et al., 2006).

The process typically follows these steps.

Low-speed centrifugation (300–500 × g) to remove cells and large debris; medium-speed centrifugation (2,000–10,000 × g) to remove smaller debris and larger vesicles; and high-speed ultracentrifugation (100,000–200,000 × g) to pellet exosomes.

While widely used, ultracentrifugation has several limitations. It can be time-consuming, often requiring several hours to complete the isolation process. Additionally, the high forces involved may lead to vesicle aggregation or damage, potentially affecting downstream analyses (Livshits et al., 2015). The method also lacks specificity,

often co-isolating protein aggregates and other non-exosomal particles of similar size or density (Konoshenko et al., 2018).

To enhance purity, many protocols incorporate a washing step, where the exosome pellet is resuspended in phosphate-buffered saline (PBS) and subjected to a second round of ultracentrifugation. This step helps to remove contaminating proteins but may also result in a loss of exosome yield (Théry et al., 2006).

2.2. Density Gradient Ultracentrifugation

To address some of the limitations of differential ultracentrifugation, density gradient ultracentrifugation has been employed as a refinement to the basic ultracentrifugation protocol. This method separates particles based on their buoyant density, allowing for better separation of exosomes from contaminants (Théry et al., 2006).

Two main types of density gradients are commonly used.

Sucrose gradients are one option. Exosomes float at densities of 1.13-1.19 g/mL in sucrose (Théry et al., 2006). Iodixanol gradients are another. These provide better separation of vesicles from viral particles and protein aggregates (Cantin et al., 2008).

The process involves layering the sample on top of a preformed density gradient, followed by ultracentrifugation. Exosomes migrate to their equilibrium density and can be collected from the corresponding gradient fraction. While this method provides higher purity compared to differential ultracentrifugation, it is more time-consuming and may result in lower yields (Li et al., 2017).

3. Size-Based Isolation Methods

3.1. Ultrafiltration

Ultrafiltration is a size-based separation technique that uses membranes with defined molecular weight cut-offs to concentrate and purify exosomes. This method offers several advantages over ultracentrifugation, including shorter processing times, the ability to handle large sample volumes, and reduced vesicle aggregation (Liangsupree et al., 2021).

The process typically involves:

initial filtration through a 0.22 μm filter to remove larger particles; concentration of the sample using a 100 kDa molecular weight cut-off filter; and washing of the retained fraction to remove smaller contaminants.

Ultrafiltration can be performed using centrifugal filter devices or tangential flow filtration systems. While this method is faster and gentler than ultracentrifugation, it may still lead to vesicle deformation due to the pressure applied during filtration (Busatto et al., 2018).

3.2. Size Exclusion Chromatography

Size exclusion chromatography (SEC) has gained popularity as an effective method for exosome isolation, offering advantages in terms of simplicity, scalability, and preservation of vesicle integrity (Guan et al., 2020). SEC separates particles based on their hydrodynamic radius as they pass through a column packed with porous beads.

The principle behind SEC is that larger particles (such as exosomes) elute earlier as they cannot enter the pores of the beads, while smaller particles (like proteins) are retained longer in the column (Böing et al., 2014). This method allows for the collection of relatively pure exosome fractions with minimal impact on their biological activity.

Advantages of SEC include:

preservation of vesicle structure and function; effective separation of exosomes from soluble proteins; and compatibility with a wide range of starting materials (e.g., plasma, urine, cell culture media)

However, SEC may result in sample dilution and may not completely separate exosomes from lipoprotein particles of similar size (Simonsen, 2017).

4. Precipitation-Based Methods

4.1. Polymer-Based Precipitation

Polymer-based precipitation methods have gained popularity due to their simplicity and ability to rapidly isolate exosomes from various biological fluids. These methods typically use polymers such as polyethylene glycol (PEG) or proprietary reagents to induce exosome precipitation (Jang et al., 2023).

The general process involves:

addition of the precipitation reagent to the sample; incubation (usually overnight at 4 °C); and low-speed centrifugation to collect the precipitated exosomes.

Commercial kits based on this principle, such as ExoQuick (System Biosciences) and Total Exosome Isolation reagent (Invitrogen), have made exosome isolation more accessible to researchers without access to ultracentrifugation equipment (Rider et al., 2016).

While these methods are quick and easy to perform, they often co-precipitate non-exosomal contaminants, potentially affecting downstream analyses. Additionally, the precipitating polymers may interfere with some applications,

necessitating additional purification steps (Liangsupree et al., 2021).

4.2. Salting-Out Procedures

Salting-out procedures represent another precipitation-based approach for exosome isolation. These methods exploit the principle that increasing the concentration of kosmotropic salts (such as sodium chloride or ammonium sulfate) can induce the precipitation of proteins and lipid-containing particles, including exosomes (Brownlee et al., 2014).

The process typically involves:

addition of a saturated salt solution to the sample; incubation to allow for precipitation; and low-speed centrifugation to collect the precipitated material.

While this method is simple and cost-effective, it lacks specificity and may co-precipitate a significant amount of non-exosomal material. As such, it is often used as an initial concentration step followed by other purification methods (Liangsupree et al., 2021).

5. Immunoaffinity-Based Methods

Immunoaffinity-based methods leverage the presence of specific proteins on the exosome surface to isolate these vesicles with high specificity. This approach typically uses antibodies against common exosome markers (e.g., CD63, CD9, CD81) immobilized on various supports such as magnetic beads, chromatography matrices, or microfluidic devices (Greening et al., 2015).

The general process involves:

incubation of the sample with the antibody-coated support; washing to remove unbound material; and elution of the captured exosomes.

Advantages of immunoaffinity-based methods include:

high specificity for exosomes; the ability to isolate subpopulations of exosomes based on specific surface markers; and compatibility with small sample volumes.

However, these methods may be biased towards certain exosome subpopulations and may not capture the full diversity of exosomes present in a sample. Additionally, the elution conditions required to release the bound exosomes may affect their integrity or functionality (Li et al., 2017).

6. Microfluidics-Based Techniques

The field of microfluidics has opened up new possibilities for exosome isolation, offering the potential for rapid, efficient, and highly specific separation of these vesicles. Microfluidic devices can incorporate various principles such as immunoaffinity, size-based separation, or deterministic lateral displacement to isolate exosomes (Liga et al., 2015).

Some notable microfluidic approaches include:

The ExoChip uses antibody-coated microchannels to capture exosomes, followed by on-chip quantification using fluorescent carbocyanine dye (Kanwar et al., 2014). The acoustic nanofilter uses ultrasound waves to separate particles based on size, allowing for continuous and contactless exosome isolation (Wu et al., 2017). The nanowire-on-micropillar trap combines size-based trapping with immunoaffinity capture to isolate specific exosome populations (Wang et al., 2013).

Microfluidic techniques offer several advantages: they process samples quickly, they work with small sample volumes, and they can be integrated with downstream analysis platforms.

However, many of these devices are still in the research and development phase and may not be suitable for large-scale exosome isolation (Gholizadeh et al., 2017).

7. Comparative Analysis of Isolation Methods

The choice of exosome isolation method can significantly impact the yield, purity, and integrity of the isolated vesicles. Several studies have compared different isolation techniques to evaluate their performance and suitability for various applications.

Shu et al. (2020) compared ultracentrifugation, size exclusion chromatography, and a combination of ultrafiltration and size exclusion chromatography for isolating melanoma-derived exosomes. They found that the combined ultrafiltration-SEC approach yielded the highest quantity of exosomes with lower levels of protein contaminants.

Baranyai et al. (2015) evaluated ultracentrifugation and size exclusion chromatography for isolating exosomes from blood plasma. Their results indicated that while both methods isolated vesicles in the appropriate size range, SEC provided a higher yield of vesicles with less contamination by plasma proteins.

A detailed study by Patel et al. (2019) compared five different isolation methods: differential ultracentrifugation, density gradient ultracentrifugation, size exclusion chromatography, ultrafiltration, and a commercial precipitation kit. They assessed the methods based on particle yield, size distribution, protein markers, and RNA yield. Their findings highlighted that each method has its strengths and

weaknesses, emphasizing the importance of selecting the appropriate isolation technique based on the downstream application.

8. Impact of Isolation Methods on Exosome Characteristics

The choice of isolation method can have profound effects on the characteristics of the isolated exosomes, potentially influencing the results of downstream analyses and applications. Several studies have investigated these effects:

8.1. Vesicle Integrity

Ultracentrifugation, particularly at high speeds, has been associated with potential damage to exosome structure. Domenis et al. (2017) characterised the proinflammatory profile of synovial fluid-derived exosomes, a preparation obtained by ultracentrifugation, a method that has been associated with vesicle aggregation and co-isolation of contaminants.

8.2. Protein Composition

Different isolation methods may selectively enrich for certain exosome subpopulations or co-isolate non-exosomal proteins. A study by Brennan et al. (2020) found significant differences in the proteomic profiles of exosomes isolated by ultracentrifugation, size exclusion chromatography, and precipitation methods.

8.3. RNA Content

The RNA profile of isolated exosomes can vary depending on the isolation method. Van Deun et al. (2014) reported that density gradient ultracentrifugation yielded exosomal RNA with less contamination from non-exosomal RNA compared to other methods.

8.4. Functional Properties

The biological activity of isolated exosomes may be affected by the isolation procedure. Takov et al. (2019) observed differences in the cardioprotective effects of exosomes isolated by ultracentrifugation versus size exclusion chromatography.

The isolation method therefore needs careful thought when designing experiments and interpreting results in exosome research.

9. Emerging Trends and Future Directions

As the field of exosome research continues to evolve, several trends are shaping how exosomes are isolated and purified:

9.1. Standardization Efforts

There is a growing recognition of the need for standardised protocols in exosome isolation to improve reproducibility and comparability across studies. Initiatives like the EV-TRACK consortium aim to increase transparency and standardisation in extracellular vesicle research (Van Deun et al., 2017).

9.2. Combinatorial Approaches

Researchers are increasingly exploring combinations of different isolation methods to leverage their complementary strengths. For example, coupling ultrafiltration with size exclusion chromatography has shown promise in improving both yield and purity (Shu et al., 2020).

9.3. Microfluidic Advancements

The development of more sophisticated microfluidic devices is enabling new possibilities for rapid, high-throughput exosome isolation. Integration of these devices with downstream analysis platforms could improve exosome-based diagnostics (Liu et al., 2018).

9.4. Machine Learning Applications

Machine learning algorithms are being employed to optimise exosome isolation parameters and predict the most suitable method for specific sample types or downstream applications.

9.5. Novel Affinity Ligands

Research into new affinity ligands, such as aptamers and peptides, is expanding the toolkit for specific exosome capture beyond traditional antibody-based approaches (Gao et al., 2018).

9.6. In vivo Exosome Isolation

Emerging techniques aim to isolate exosomes directly from the bloodstream, potentially allowing for real-time monitoring of exosome dynamics in live organisms (Piao et al., 2018).

10. Conclusion

Isolation methods trade recovery against specificity and may alter vesicle composition or function. Method selection therefore depends on the sample and the downstream measurement, rather than on a single preferred technique.

No single isolation method is likely to prove universally superior. Instead, researchers will need to navigate a complex landscape of isolation techniques, often combining multiple approaches to achieve their desired outcomes. The

ongoing development of novel technologies, particularly in microfluidics and affinity-based capture, promises to further expand the toolkit available for exosome isolation.

Ultimately, progress in exosome isolation methods will be essential for realizing the full potential of these vesicles in basic research, diagnostics, and therapeutic applications. As we gain a deeper understanding of exosome heterogeneity and function, the ability to isolate specific subpopulations of exosomes with high purity and intact biological activity will become increasingly important.

Further exosome research requires improved and standardised isolation, characterisation, and reporting methods. Better analytical reproducibility is necessary both for mechanistic studies and for comparing candidate diagnostic or therapeutic products across laboratories.

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5.5. MECHANISM OF ACTION IN MS TREATMENT

Multiple Sclerosis (MS) is a complex, chronic autoimmune disorder characterised by inflammation, demyelination, and neurodegeneration in the central nervous system (CNS). It affects millions of people worldwide, causing a wide range of debilitating symptoms that significantly impact quality of life (Reich et al., 2018). Despite advances in MS therapies, there remains a need for more effective treatments that can not only manage symptoms but also address the underlying pathological processes and promote repair. The proposed mechanisms, and their evidence status, are summarized in Figure 5.5.1.

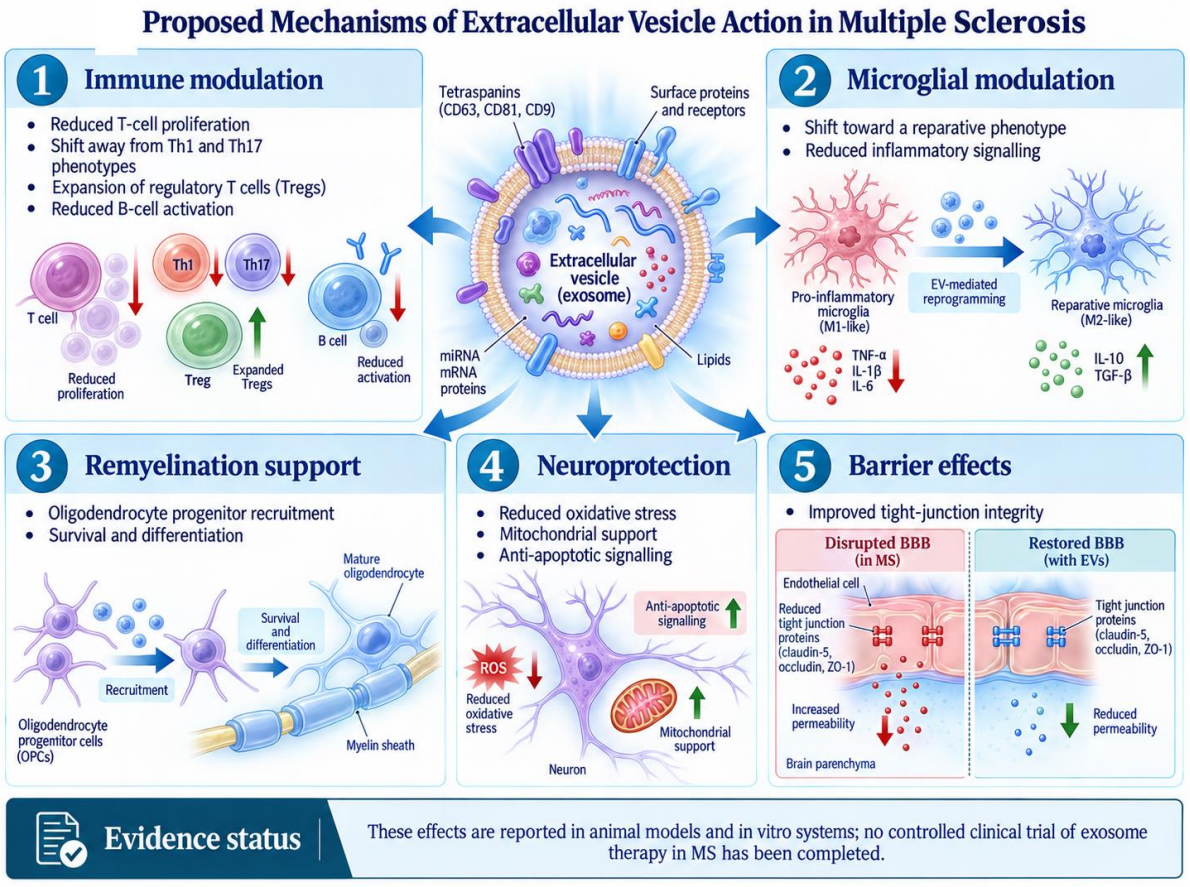


Figure 5.5.1. Proposed mechanisms of extracellular vesicle action in multiple sclerosis. These effects are reported in animal models and in vitro systems; no controlled clinical trial of exosome therapy in MS has been completed.

EVs are studied in MS as mediators of cell communication, candidate biomarkers, and delivery vehicles (Théry et al., 2002). The mechanisms below concern inflammatory signalling, cargo transfer, and repair-related pathways; their relevance to treatment requires separate clinical testing.

1. Exosomes: Nature's Cellular Messengers

1.1. Structure and Composition of Exosomes

Exosomes are small, lipid bilayer-enclosed vesicles secreted by various cell types, including mesenchymal stem cells (MSCs), neurons, astrocytes, and immune cells. Their structure and composition are critical to their function.

Their membranes are enriched in cholesterol, sphingomyelin, and ceramide, which contribute to their stability and ability to fuse with target cells (Skotland et al., 2017). Exosomes display various surface proteins, including tetraspanins (CD9, CD63, CD81), adhesion molecules, and receptors, which play roles in cellular targeting and uptake (Théry et al., 2018). Inside, they carry a diverse payload of proteins, lipids, and nucleic acids, including mRNAs, microRNAs (miRNAs), and other non-coding RNAs. This cargo reflects the cell of origin and can significantly influence recipient cell behaviour (Valadi et al., 2007).

1.2. Biogenesis and Secretion

Understanding the biogenesis of exosomes is important for harnessing their therapeutic potential.

Exosomes originate from the endosomal system, specifically from intraluminal vesicles (ILVs) within multivesicular bodies (MVBs) (Hessvik & Llorente, 2018). ESCRT-dependent and ESCRT-independent mechanisms are both involved. The formation of ILVs involves both Endosomal Sorting Complex Required for Transport (ESCRT)-dependent and independent mechanisms, influencing exosome composition and cargo sorting (Colombo et al., 2014). The release of exosomes is regulated by various Rab GTPases, including Rab27a, Rab27b, and Rab35, which control different steps of exosome secretion (Ostrowski et al., 2010). Exosome secretion can be modulated by various cellular stresses, including oxidative stress, hypoxia, and inflammation, which are relevant in the context of MS (Kucharzewska et al., 2013).

1.3. Uptake Mechanisms

The therapeutic efficacy of exosomes in MS depends on their ability to be internalized by target cells.

Exosomes can be taken up by various endocytic pathways, including clathrin-mediated endocytosis, caveolin-dependent endocytosis, and macropinocytosis (Mulcahy et al., 2014). Membrane fusion is one route. In some cases, exosomes can directly fuse with the plasma membrane of recipient cells, releasing their contents into the cytoplasm (Parolini et al., 2009). Receptor-mediated uptake is another. Specific receptor-ligand interactions can facilitate the targeted uptake of exosomes by particular cell types, which is important for developing targeted therapies in MS (Hoshino et al., 2015).

2. Cell Communication in MS: The Exosome Paradigm

2.1. Exosome-Mediated Signaling in the CNS

Exosomes play critical roles in cell-to-cell communication within the CNS, which is particularly relevant in the context of MS.

Frühbeis et al. (2013) demonstrated that oligodendrocyte-derived exosomes can be internalized by neurons, influencing their metabolism and enhancing their stress tolerance. This communication pathway may be key for maintaining axonal integrity in MS. On astrocyte-oligodendrocyte interactions, Ji et al. (2022) reported that a herbal formulation promoted remyelination in part by regulating miR-219 and miR-338 in exosomes, which in turn promoted oligodendrocyte precursor cell (OPC) differentiation. This finding suggests a potential mechanism for enhancing remyelination in MS. Prada et al. (2018) revealed that glia transfer miRNAs to neurons via extracellular vesicles, a mechanism underlying inflammation-induced synaptic alterations.

2.2. MicroRNA Transfer in MS Pathology and Treatment

Exosomal microRNAs have developed into key players in MS pathophysiology and potential therapeutic targets.

Regulatory miRNAs are one group. Pusic et al. (2014) identified specific miRNAs in dendritic cell-derived exosomes that can enhance myelination and reduce oxidative stress, suggesting their potential in MS therapy. Ebrahimkhani et al. (2017) found distinct miRNA profiles in serum exosomes of MS patients, with some miRNAs correlating with disease activity and progression. Zhang et al. (2017) showed that exosomes derived from mesenchymal stromal cells promote axonal growth of cortical neurons, one of several vesicle-mediated effects of potential relevance to repair.

2.3. Protein and Lipid Transfer in MS

Beyond nucleic acids, exosomal transfer of proteins and lipids plays essential roles in MS pathology and potential treatment.

Lombardi et al. (2019) showed that extracellular vesicles released by microglia can have both detrimental and protective actions on myelin lesions, with astrocytes contributing to remyelination failure. Neuroprotective factors are one class of cargo. Xin et al. (2012) found that exosome-mediated transfer of miR-133b from mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Lipid-mediated signalling is another. Fitzner et al. (2011) demonstrated that oligodendrocyte-derived exosomes are selectively taken up by microglia through macropinocytosis, which may influence microglial activation.

2.4. Exosomes in Neuro-Immune Communication

The role of exosomes in facilitating communication between the nervous and immune systems is particularly relevant in MS.

T Cell Regulation: Yu et al. (2013) showed that exosomes derived from gene-modified dendritic cells expressing membrane-associated TGF-β1 (mTGF-β1-EXOs) can suppress the activation of autoreactive T cells and reduce the severity of experimental autoimmune encephalomyelitis (EAE). This suppression occurs through the enhancement of regulatory T cells (Tregs) and the inhibition of Th17 responses,

suggesting a potential therapeutic approach for mitigating autoimmune responses in MS. B cell modulation is one mechanism. Bai et al. (2017) demonstrated that MSC-derived exosomes reduced intraocular inflammation in experimental autoimmune uveitis, an organ-specific autoimmune model. Toyama et al. (2018) found that microRNA-mediated therapy that limits blood-brain barrier disruption improved vascular cognitive impairment, suggesting that barrier-directed approaches merit study in MS.

3. Reduction of Inflammation: Exosomes as Immunomodulators

3.1. Immunomodulatory Properties of MSC-Derived Exosomes

Mesenchymal stem cell-derived exosomes (MSC-Exos) have been reported to act in modulating the immune response in MS.

T cell suppression is one mechanism. Riazifar et al. (2019) showed that exosomes from interferon-γ-stimulated MSCs reduced neuroinflammation and demyelination in EAE and increased regulatory T cells in the CNS. On cytokine modulation, Bai et al. (2017) showed that MSC-derived exosomes reduced inflammatory cell infiltration in experimental autoimmune uveitis; comparable cytokine data in EAE would need separate confirmation. Chiesa et al. (2011) found that MSCs impair in vivo T-cell priming by dendritic cells, pointing to antigen presentation as one target of MSC-based approaches.

3.2. Exosome-Mediated Regulation of Macrophage and Microglial Polarization

Macrophages and microglia play central roles in the pathology of multiple sclerosis (MS), and exosomes have demonstrated the ability to influence their phenotypes.

M2 Polarisation: Zhao et al. (2018) reported that adipose-derived stem cell exosomes promote macrophage polarisation toward an anti-inflammatory M2 phenotype. This shift could create a more favourable environment for tissue repair in MS by reducing inflammation and supporting regenerative processes. Microglial Modulation: Liu et al. (2018) demonstrated that salidroside (SLDS) treatment promotes microglial polarisation from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype. This transformation is associated with enhanced phagocytic activity and decreased pro-inflammatory cytokine release, suggesting a neuroprotective effect. However, this modulation was achieved through pharmacological intervention rather than exosome-based mechanisms. Role of Microglial Exosomes: Udeochu et al. (2018) showed that microglia-derived exosomes contribute to inflammatory resolution in activated and aged microglia. Their findings indicate that exosome release from microglia helps regulate neuroinflammation and may enhance their neuroprotective properties. However, their study did not provide direct evidence that regulatory T cell-derived exosomes modulate microglial phenotype, as previously suggested. Resolution of Inflammation: Guo et al. (2019) showed that intranasally delivered MSC-derived exosomes loaded with PTEN siRNA supported repair after complete spinal cord injury, illustrating how engineered vesicles can be directed at CNS targets.

Exosomes may therefore help to shape immune responses in MS, but further research is needed to determine the specific role of regulatory T cell-derived exosomes in microglial modulation.

3.3. Exosome-Mediated Modulation of the Blood-Brain Barrier

The integrity of the blood-brain barrier (BBB) is often compromised in MS, and exosomes have shown potential in addressing this issue.

EV interactions with the blood-brain barrier depend on the source, preparation and experimental model. MSC-derived exosomes have demonstrated the ability to cross the BBB and potentially reduce neuroinflammation by delivering bioactive molecules to CNS cells (Dehghani et al., 2025). Neuroprotection is another. Xin et al. (2017) found that secondary release of exosomes from astrocytes contributes to neural plasticity and functional recovery in rats treated after stroke with exosomes from miR-133b-overexpressing MSCs.

3.4. Regulation of Pro-inflammatory Cytokine Production

Exosomes have demonstrated the ability to modulate the production and signalling of pro-inflammatory cytokines implicated in MS pathology.

Suppression of the NF-κB pathway is one mechanism. Yu et al. (2013) demonstrated that mTGF-β1-EXOs can downregulate NF-κB activation, a key regulator of inflammatory gene expression. This suppression leads to a decrease in pro-inflammatory cytokine production,

including TNF-α, IL-1β, and IL-6, contributing to the reduction of neuroinflammation in EAE models. Li et al. (2019) demonstrated that exosomes derived from mesenchymal stem cells (MSCs) can effectively attenuate inflammation in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. MSC-derived exosomes promoted the polarisation of microglia towards the anti-inflammatory M2 phenotype, leading to increased production of IL-10, an anti-inflammatory cytokine. Elevated IL-10 levels subsequently contributed to the suppression of pro-inflammatory immune cell activity, thus reducing CNS inflammation and demyelination. Casella et al. (2018) demonstrated that extracellular vesicles engineered to contain IL-4 modulated neuroinflammation in a mouse model of MS, shifting myeloid cells towards an anti-inflammatory phenotype.

3.5. Antioxidant Effects of Exosomes

Oxidative stress is closely linked to inflammation in MS, and exosomes have shown antioxidant properties.

Jarmalavičiūtė et al. (2015) found that exosomes from dental pulp stem cells rescued human dopaminergic neurons from 6-hydroxydopamine-induced apoptosis, indicating protection against oxidative injury. Activation of the Nrf2 pathway is one mechanism. Bodega et al. (2019) reviewed evidence that microvesicles can act as both scavengers and producers of reactive oxygen species, a dual role relevant to oxidative stress in MS. Support of mitochondrial function is another. Phinney et al. (2015) demonstrated that MSC-Exos can transfer functional mitochondria to macrophages, enhancing their bioenergetics and potentially improving their capacity to resolve inflammation.

4. Promotion of Remyelination: Exosomes as Regenerative Agents

4.1. Stimulation of Oligodendrocyte Precursor Cell Proliferation and Differentiation

Exosomes have shown activity in preclinical models in promoting the proliferation and differentiation of oligodendrocyte precursor cells (OPCs), which is important for remyelination in MS.

miRNA-Mediated OPC Differentiation: Pusic & Kraig (2014) demonstrated that serum exosomes from young rats could promote OPC differentiation and enhance myelination in vitro through the transfer of specific miRNAs. Whether vesicles deliver growth factors such as PDGF or FGF-2 in amounts sufficient to stimulate OPC proliferation in demyelinating conditions has not been established. Current evidence suggests that exosomes can influence OPC differentiation through epigenetic regulation, although direct evidence in MS models is still emerging. Epigenetic mechanisms, including histone modifications and microRNA-mediated gene regulation, are implicated in OPC differentiation and myelination. Future studies focusing specifically on the epigenetic impact of exosomes on OPCs may provide clearer insights into their potential role in promoting remyelination.

4.2. Modulation of the Extracellular Environment

Exosomes can influence the extracellular environment to create conditions more conducive to remyelination.

Extracellular matrix remodelling is one contribution. It has been proposed that MSC-derived exosomes modify the expression of extracellular matrix components and thereby create a more permissive environment for OPC migration and differentiation, but direct evidence is lacking. López-Verrilli et al. (2013) showed that Schwann cell-derived exosomes enhance axonal regeneration in the peripheral nervous system, raising the question of whether comparable vesicles influence OPC behaviour in the CNS. Findings on vesicle-associated miRNAs should not be generalized to enzymatic degradation of extracellular-matrix inhibitors without direct experimental evidence.

4.3. Neuroprotection and Axonal Support

Exosomes can promote remyelination indirectly by supporting neuronal health and axonal integrity.

Delivery of neurotrophic factors is one mechanism. Xin et al. (2012) demonstrated that exosomes transfer miR-133b from mesenchymal stromal cells to neural cells, contributing to neurite outgrowth. Hayakawa et al. (2016) showed that astrocytes release functional mitochondria in extracellular particles that are taken up by neurons after stroke, a mechanism that could in principle support the metabolic needs of repair. Frühbeis et al. (2013) found that oligodendrocyte-derived exosomes can be internalized by neurons, suggesting a potential mechanism for coordinating axon-myelin interactions during remyelination.

4.4. Immunomodulation to Support Remyelination

Exosomes can promote remyelination by modulating the immune environment.

Promotion of the M2 microglial phenotype is one route. Vesicles released by anti-inflammatory microglia have been proposed to promote OPC differentiation and to reinforce

that phenotype, but this remains to be demonstrated directly. While Yu et al. (2013) did not directly investigate the role of Treg-derived exosomes in remyelination, their findings suggest that TGF-β1-enriched exosomes can modulate immune responses by enhancing Treg activity and suppressing inflammatory pathways. Future studies are needed to explore whether Treg-derived exosomes specifically influence remyelination processes in MS through direct effects on oligodendrocytes and their microenvironment. Li et al. (2019) demonstrated that MSC-derived exosomes attenuated inflammation and demyelination in EAE rats by regulating microglial polarisation, which was accompanied by lower levels of pro-inflammatory cytokines. On B cell responses, Bai et al. (2017) showed that MSC-derived exosomes reduced inflammation in experimental autoimmune uveitis; whether they influence B cell responses relevant to demyelination in MS remains untested.

4.5. Exosome-mediated Delivery of Remyelination-Promoting Factors

Exosomes have shown measurable effects in experimental systems as delivery vehicles for various factors that can enhance remyelination.

Growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), and epidermal growth factor (EGF) promote OPC proliferation and differentiation, and vesicle-mediated delivery of such factors has been proposed as a more targeted and sustained alternative to conventional delivery; direct evidence in demyelination models is still required. microRNA Transfer: Vesicle-associated miRNAs have been investigated as mediators of myelin-related effects in preclinical systems (Pusic & Kraig, 2014). Mechanistic results depend on the cell source and preparation and require direct functional validation. Protein delivery is one mechanism. Frühbeis et al. (2013) showed that oligodendrocytes release exosomes carrying myelin proteins such as proteolipid protein (PLP), which are taken up by neurons. These proteins, when delivered to demyelinated axons, may serve as a template or stimulus for new myelin formation. Enzyme delivery is another. Pusic et al. (2016) showed that environmental enrichment stimulates immune cells to secrete exosomes that promote CNS myelination and may regulate inflammation. Whether vesicles can also deliver enzymes that degrade inhibitory extracellular-matrix components such as chondroitin sulfate proteoglycans remains to be shown. Stevanato et al. (2016) characterised the content, stoichiometry, and transfer of miRNA from exosomes derived from a human neural stem cell line, providing a basis for studying vesicle-mediated signalling in repair.

4.6. Exosome-mediated Regulation of Oligodendrocyte Metabolism

Recent research has highlighted the role of exosomes in regulating oligodendrocyte metabolism, which is key for efficient remyelination.

Fitzner et al. (2011) demonstrated that oligodendrocyte-derived exosomes are selectively taken up by microglia through macropinocytosis; whether comparable vesicles transfer myelin lipids to OPCs and support their differentiation remains to be shown. Otero-Ortega et al. (2017) showed that administration of MSC-derived extracellular vesicles promoted white matter repair in an experimental model of subcortical stroke. Phinney et al. (2015) demonstrated that MSC-derived exosomes can transfer functional mitochondria to recipient cells. In the context of remyelination, this mitochondrial transfer could support the high energy demands of oligodendrocytes during myelin production. Whether vesicles can deliver glucose transporters or other metabolic machinery to oligodendrocytes, and thereby support the energy demands of remyelination, has not been established.

4.7. Exosome-mediated Modulation of the Extracellular Matrix

The extracellular matrix (ECM) is essential to regulating remyelination, and exosomes have been shown to modulate ECM composition and signalling.

Extracellular vesicles have been proposed to carry matrix metalloproteinases (MMPs) capable of remodelling the extracellular matrix, which could in principle create a more permissive environment for OPC migration and differentiation. Regulation of fibronectin is one mechanism. Stoffels et al. (2013) showed that fibronectin aggregation in MS lesions impairs remyelination; whether extracellular vesicles influence this aggregation remains to be clarified. No direct evidence from Yu et al. (2013) supports a role for exosomes in laminin signalling. However, other studies indicate that exosomes may interact with extracellular matrix components, including laminin, which is essential for oligodendrocyte survival and myelination. Further research is required to clarify whether exosomal transfer of laminin receptors or their regulatory molecules enhances oligodendrocyte precursor cell (OPC) differentiation and remyelination. Regulation of hyaluronan is another. Back et al.

(2005) demonstrated that hyaluronan accumulation can inhibit OPC maturation and remyelination.

4.8. Exosome-mediated Promotion of Oligodendrocyte Survival

Enhancing oligodendrocyte survival is central for successful remyelination, and exosomes have shown potential in this area.

Apoptosis-related mechanisms are one group. Tati et al. (2024) investigated bone-marrow-MSC-derived EVs in an in vitro corneal epithelial repair model. Their findings concern a non-MS experimental system and do not establish neuroprotection or clinical efficacy in MS. Jarmalavičiūtė et al. (2015) showed that exosomes can transfer antioxidant enzymes like catalase and superoxide dismutase to recipient cells, potentially protecting oligodendrocytes from oxidative stress-induced damage. Modulation of endoplasmic reticulum stress is another. Momen-Heravi and Bala (2018) found that extracellular vesicles from oral squamous carcinoma carry an oncogenic miRNA profile and reprogram monocytes through NF-κB signalling, illustrating how vesicles can reshape recipient-cell signalling. Basso and Bonetto (2016) reviewed extracellular vesicles as a form of communication in the brain, including their influence on recipient-cell homeostatic processes such as autophagy, which is important for oligodendrocyte health and myelin production.

4.9. Exosome-mediated Axonal Support during Remyelination

Supporting axonal health is important for successful remyelination, and exosomes have shown potential in providing this support.

Delivery of neurotrophins is one mechanism. Xin et al. (2012) showed that exosomes transfer miR-133b from mesenchymal stromal cells to neural cells, contributing to neurite outgrowth. Hayakawa et al. (2016) demonstrated that astrocytes release functional mitochondria in extracellular particles that are taken up by neurons after stroke, a mechanism that could support metabolic needs during repair. The vesicle-uptake mechanism introduced above illustrates a possible communication pathway. Its contribution to functional remyelination requires direct testing. Regulation of axonal transport is a fourth possibility, since axonal transport is key for maintaining axonal integrity during remyelination, although vesicle-mediated effects on transport remain to be demonstrated.

4.10. Exosome-mediated Regulation of Remyelination Timing

The timing of remyelination is essential for its success, and exosomes have been implicated in regulating this process.

Zhang et al. (2017) showed that exosomes derived from mesenchymal stromal cells promote axonal growth of cortical neurons. Drago et al. (2017) found that ATP modifies the proteome of extracellular vesicles released by microglia and influences their action on astrocytes, showing that the local environment shapes vesicle content and effects. Age matters as well: Pusic and Kraig (2014) showed that serum exosomes from young animals could enhance remyelination in older recipients, suggesting a potential approach to overcoming age-related decline in remyelination efficiency.

5. Exosome Engineering for Enhanced Remyelination

5.1. Surface Modification

Exosomes could be engineered to express specific surface proteins, allowing for targeted delivery to particular cell types in the CNS. This approach could be used to enhance the delivery of remyelination-promoting factors specifically to OPCs or oligodendrocytes. Alvarez-Erviti et al. (2011) demonstrated that exosomes could be engineered to express specific surface proteins, allowing for targeted delivery to particular cell types in the CNS. See Figure 5.5.2.

Antigen-directed immune modulation has also been tested with vesicles from sources other than MSCs. Li et al. (2025) modified anti-inflammatory macrophage-derived exosomes with MOG peptide. Subcutaneous administration induced antigen-specific tolerance and protected against EAE in mice. The source-cell and antigen dependence of the effect is relevant to product design; protection in this induced model does not establish treatment efficacy in human MS.

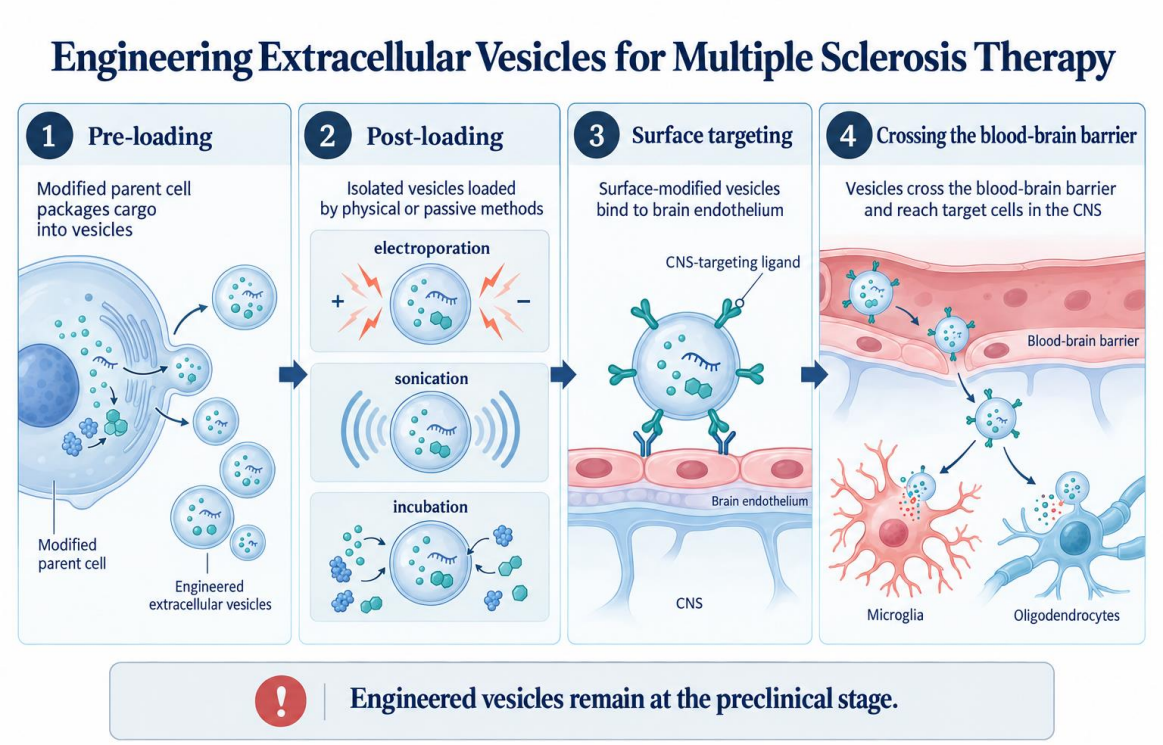


Figure 5.5.2. Strategies for engineering extracellular vesicles for multiple sclerosis therapy. These approaches remain at the preclinical stage.

5.2. Cargo Loading

Methods for loading exosomes with therapeutic proteins have been developed, opening up possibilities for delivering specific remyelination-promoting factors directly to target cells in the CNS. Kojima et al. (2018) developed genetically encoded devices that boost exosome production and load therapeutic cargo, and showed that exosomes produced by implanted cells could deliver that cargo within the brain.

5.3. Hybrid Exosomes

Hybrid exosomes created by fusing exosomes with liposomes allow for the incorporation of hydrophobic drugs. This approach could be used to deliver lipophilic remyelination-promoting agents more effectively. Sato et al. (2016) created hybrid exosomes by fusing them with liposomes, allowing for the incorporation of hydrophobic drugs.

5.4. Exosome Mimetics

Cell-derived nanovesicles that mimic the properties of natural exosomes but can be produced in larger quantities have been developed. These exosome mimetics could be engineered to carry specific remyelination-promoting cargoes. Jang et al. (2013) developed cell-derived nanovesicles that mimic the properties of natural exosomes but can be produced in larger quantities.

5.5. Stimuli-Responsive Exosomes

Exosomes that could release their cargo in response to specific stimuli, such as pH changes or enzymatic activity, have been developed. This approach could allow for more controlled release of remyelination-promoting factors in the context of MS lesions. Tian et al. (2018) developed surface-functionalized exosomes targeted to ischemic brain tissue, illustrating how vesicle surfaces can be engineered to direct cargo to a lesion site.

6. Challenges and Future Directions in Exosome-mediated Remyelination

6.1. Exosome Heterogeneity

The heterogeneity of exosome populations, even those derived from a single cell type, presents a challenge. Théry et al. (2018) highlighted the heterogeneity of exosome populations, even those derived from a single cell type. Understanding and controlling this heterogeneity will be central for developing more targeted and effective exosome-based therapies for remyelination.

6.2. Scalability

Producing therapeutic quantities of exosomes remains a challenge. Watson et al. (2016) reviewed various approaches to large-scale exosome production, but further advancements are needed to make exosome-based therapies more feasible for clinical use.

6.3. Targeting and Biodistribution

While exosomes can cross the blood-brain barrier, ensuring their efficient targeting to sites of demyelination remains a challenge. Hoshino et al. (2015) demonstrated that exosomal integrins play a role in target cell specificity, suggesting a potential avenue for improving targeting.

6.4. Long-term Effects

The long-term effects of exosome-based therapies on remyelination and overall CNS function need to be thoroughly investigated. Longitudinal studies in animal models and carefully designed clinical trials will be important in this regard.

6.5. Combination Therapies

The therapeutic efficacy of exosomes in neurodegenerative diseases, including MS, may be enhanced through combination strategies. While Y. Zhu et al. (2017) focused on osteoarthritis models, their findings suggest that exosomes can potentiate regenerative responses when combined with other therapeutic modalities. Future research should explore how exosomes might interact with neuroprotective agents, small molecules, or remyelination-enhancing drugs to develop a more integrated treatment approach for MS.

6.6. Personalized Exosome Therapies

Given the heterogeneity of MS, developing personalised exosome-based therapies that take into account individual patient characteristics and disease subtypes could enhance treatment efficacy. This approach could involve tailoring exosome content or surface modifications based on patient-specific factors.

6.7. Exosome-based Biomarkers

In addition to their therapeutic potential, exosomes may serve as valuable biomarkers for monitoring remyelination processes and treatment responses in MS patients. Selmaj et al. (2017) reviewed the role of exosomes in CNS inflammation and MS, including evidence that exosomal miRNA profiles change with disease activity. Developing standardised methods for exosome isolation and analysis from patient samples will be important for realizing this potential.

6.8. Standardization of Exosome Isolation and Characterization

One of the major challenges in exosome research is the lack of standardised protocols for isolation and characterisation. Witwer et al. (2013) highlighted the need for consensus on best practices in the field. Recent advancements, such as the development of microfluidic devices for exosome isolation (Liu et al., 2017), offer promising solutions but require further validation and standardisation for clinical applications.

6.9. Exosome Loading Efficiency

While exosomes have properties that may support their use as delivery vehicles for therapeutic molecules, improving loading efficiency remains a challenge. Vader et al. (2016) reviewed various methods for exosome loading, including electroporation, sonication, and chemical transfection. However, each method has limitations in terms of efficiency and potential damage to exosome integrity. Developing more efficient and gentle loading techniques is key for maximizing the therapeutic potential of engineered exosomes.

6.10. Exosome-Mediated Off-Target Effects

EV preparations can interact with multiple cell types, raising the possibility of off-target effects. Biodistribution depends on administration route and cellular source (Wiklander et al., 2015). Product characterisation and direct testing are therefore necessary to evaluate safety and efficacy before therapeutic use in MS.

6.11. Immunogenicity of Therapeutic Exosomes

While exosomes are generally considered less immunogenic than whole-cell therapies, potential immune responses should still be considered, particularly with repeated administration. X. Zhu et al. (2017) assessed the toxicity and immunogenicity of extracellular vesicles (EVs) in a preclinical model and found that while EVs derived from HEK293T cells exhibited minimal toxicity, they still had the potential to trigger immune activation in certain conditions. Exosome-based therapies therefore need further refinement, potentially through surface modifications or the use of autologous sources, to minimise the risk of adverse immune reactions.

6.12. Exosome-Mediated Transfer of Pathogenic Factors

While exosomes have shown remyelination-related effects in preclinical systems, there is also evidence that they can transfer pathogenic factors. For instance, Sáenz-Cuesta et al. (2014) reviewed evidence that vesicles from people with MS can carry inflammatory mediators. Ensuring that therapeutic exosomes do not inadvertently transfer harmful molecules will be essential for their safe use in MS treatment.

6.13. Optimizing Exosome Dose and Treatment Regimen

Determining the optimal dose and treatment schedule for exosome-based therapies in MS remains a challenge. Cosenza et al. (2018) found that MSC-derived exosomes were more immunosuppressive than microparticles in inflammatory arthritis, showing that vesicle type and preparation influence therapeutic effects. Conducting systematic dose-response studies and long-term treatment trials will be essential for optimising exosome-based therapies for MS.

6.14. Combining Exosomes with Other MS Therapies

Given the complexity of multiple sclerosis (MS), a heterogeneous therapeutic approach is often necessary. While the potential of exosome-based therapies in MS is still under investigation, previous research on other degenerative diseases, such as osteoarthritis (OA), has demonstrated that exosomes derived from mesenchymal stem cells (MSCs) can enhance tissue repair and modulate immune responses (Y. Zhu et al., 2017). Understanding how exosomes interact with established MS therapies, such as disease-modifying treatments or immunosuppressive agents, remains an open area of research. Future studies should evaluate whether combining exosome-based approaches with conventional MS therapies could lead to synergistic effects in neuroprotection and remyelination.

6.15. Exosome-Based Biomarkers for Personalized Treatment

While exosomes warrant further investigation as therapeutic agents, they also have potential as biomarkers for disease progression and treatment response. Selmaj et al. (2017) reviewed evidence that exosomal miRNA profiles change with MS disease activity. Developing exosome-based biomarker panels could enable more personalised treatment approaches, allowing clinicians to monitor remyelination processes and adjust therapies accordingly.

6.16. Scalability and Cost-Effectiveness

For exosome-based therapies to become widely available for MS treatment, scalable and cost-effective production methods need to be developed. Watson et al. (2016) reviewed various approaches to large-scale exosome production, but further advancements are needed to make exosome-based therapies economically viable for long-term MS treatment.

6.17. Regulatory Challenges

As a relatively new therapeutic modality, exosome-based treatments face unique regulatory challenges. Börger et al. (2017) discussed the regulatory considerations for exosome-based therapies, and argued for standardised characterisation methods and potency assays. Engaging with regulatory agencies early in the development process will be central for smoothing the path to clinical translation.

6.18. Enhancing Exosome Targeting to the CNS

CNS **delivery depends on EV source,** engineering, administration route and barrier state. Alvarez-Erviti et al. (2011) demonstrated delivery of siRNA to the mouse brain using targeted engineered v**esi**cles. This preclinical result does not establish reliable blood-brain barrier passage for all EV preparations or efficacy in MS.

6.19. Understanding Exosome-Mediated Intercellular Communication in MS

While we know that exosomes play important roles in cell-to-cell communication, the specific mechanisms by which they influence remyelination processes in MS are not fully understood. Croese and Furlan (2018) reviewed the role of extracellular vesicles in MS pathogenesis and repair. Further research into the molecular mechanisms of exosome-mediated signalling in the context of MS could reveal new therapeutic targets and strategies.

6.20. Exosome Engineering for Enhanced Therapeutic Effects

Recent advances in bioengineering have opened up new possibilities for enhancing the therapeutic properties of exosomes. Luan et al. (2017) reviewed the engineering of exosomes as refined biological nanoplatforms for drug delivery. Applying similar approaches to create exosomes with enhanced remyelination-promoting properties could lead to more effective therapies for MS.

6.21. Long-Term Safety and Efficacy Studies

While short-term studies have shown early findings for exosome-based therapies in MS models, long-term safety and efficacy data are still lacking. Conducting detailed long-term studies in both animal models and human patients will be essential for establishing the viability of exosome-based treatments for a chronic condition like MS.

6.22. Patient-Derived Exosomes for Autologous Therapy

Kordelas et al. (2014) described donor-MSC-derived EV administration in a compassionate-use case of refractory graft-versus-host disease. This single case in another condition cannot establish an autologous EV strategy, comparative safety or therapeutic efficacy in MS.

6.23. Exosome-Mediated Remyelination in Progressive MS

While much of the current research focuses on relapsing-remitting MS, understanding and enhancing the potential of exosomes in promoting remyelination in progressive forms of MS is important. Welton et al. (2017) used cerebrospinal fluid extracellular vesicle enrichment for protein biomarker discovery in MS. Extending this research to develop targeted therapies for progressive MS could address a significant unmet medical need.

6.24. Exosomes and Remyelination in Aging

As the efficacy of remyelination processes tends to decrease with age, understanding how exosome-based therapies can be optimised for older MS patients is important. Pusic & Kraig (2014) showed that exosomes from young rats could enhance remyelination in older animals. Further research into age-related changes in exosome function and how to counteract these changes could lead to more effective treatments for MS across all age groups.

7. Ethical Considerations in Exosome-Based MS Therapies

7.1. Source of Exosomes

Donor-derived EV research requires explicit consent for collection and intended uses, protection of donor information and transparent handling of commercial interests. Autologous origin does not itself guarantee product quality or safety.

7.2. Genetic Modification Concerns

Engineering EV-producing cells introduces additional questions about cargo composition, off-target effects and product consistency. Research protocols should specify these risks and the tests used to assess them.

7.3. Patient Consent and Education

Informed consent should distinguish research participation from established treatment. Information should cover uncertain benefit, known and unknown risks, alternatives, monitoring and the right to withdraw.

8. Economic Impact of Exosome Therapies in MS

8.1. Cost-Benefit Analysis

A cost-effectiveness assessment of EV therapy for MS requires credible estimates of clinical benefit, harms, production costs and longer-term resource use. Without demonstrated patient benefit, economic advantage remains unestablished.

8.2. Healthcare System Integration

Production, storage, transport, quality control and clinical monitoring are relevant cost inputs. Potential future reductions in care costs should be evaluated as explicit model assumptions, not as observed benefits of EV therapy.

8.3. Insurance Coverage Considerations

Coverage and reimbursement depend on jurisdiction and evidence requirements. Any proposed outcomes-based payment model would require validated outcomes and reliable follow-up; it does not substitute for establishing efficacy and safety.

9. Global Perspective on Exosome Research in MS

9.1. International Collaborations

International research programs require compatible protocols, transparent data-sharing arrangements and consistent reporting. Harmonization can improve comparability, but cannot compensate for inadequate study design.

9.2. Regulatory Differences Across Countries

Regulatory requirements differ between jurisdictions. Product developers should obtain current jurisdiction-specific guidance. Research-reporting recommendations and regulatory authorization are distinct.

9.3. Access to Exosome Therapies in Developing Countries

Equitable access is a consideration for future development. Assessments should include affordability, infrastructure,

trial participation and capacity for follow-up. These are planning priorities, not evidence that therapeutic EV use is ready for routine care.

10. Conclusion

The experiments described above support several testable roles for EVs. Their main findings and limitations are:

Exosomes, particularly those derived from mesenchymal stem cells (MSCs), have demonstrated reported immunomodulatory and neuroprotective effects in MS models, especially in experimental autoimmune encephalomyelitis (EAE) animal studies. These nanovesicles suppress the proliferation of activated peripheral blood mononuclear cells (PBMCs) and modulate the balance between pro-inflammatory and anti-inflammatory cytokines, potentially mitigating the autoimmune response characteristic of MS. Exosomes have also shown the ability to promote remyelination, reduce neuroinflammation, and improve functional outcomes in various preclinical MS models. The therapeutic effects of exosomes are mediated through multiple pathways, including the transfer of microRNAs, proteins, and other bioactive molecules that influence gene expression and cellular function in target tissues. EV products avoid the administration of viable cells, but safety, storage requirements and targeting depend on the specific product. Comparative superiority requires direct evidence. Exosomes from various stem cell sources, including bone marrow, adipose tissue, and umbilical cord, have been explored, each showing promise in different aspects of MS treatment. Innovative delivery methods complete this picture. Novel approaches such as intranasal administration and nanoparticle-based targeting are being developed to enhance the efficacy of exosome therapies and improve their ability to cross the blood-brain barrier.

Most studies summarized here are preclinical, so their findings should be interpreted as mechanistic or proof-of-concept evidence rather than clinical efficacy. Future research directions should focus on:

Optimising exosome isolation, characterisation, and manufacturing processes for clinical applications. Conducting rigorous clinical trials to assess the safety and efficacy of exosome-based therapies in human MS patients. Investigating the long-term effects and potential side effects of exosome treatments. Testing whether combinations improve outcomes beyond their individual components and assessing additional harms. Developing standardised protocols for exosome production and administration to facilitate regulatory approval and clinical implementation.

EV-based interventions for MS remain investigational. Preclinical findings support further research, while clinical benefit, optimal delivery and long-term safety require direct evaluation.

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5.6. PRECLINICAL STUDIES: ANIMAL MODELS AND IN VITRO STUDIES

Preclinical EV experiments test whether a defined preparation changes inflammation, demyelination, or repair in a specified model. The choice of model and endpoint determines how far each result can inform MS treatment research.

A 2026 systematic review of experimental MS distinguished the effects of bone marrow-derived MSCs from those of their exosomes. The pooled exosome effect on clinical scores was not statistically significant and showed substantial heterogeneity. These animal-model findings require independent validation and cannot establish benefit in patients (Kariminejad-Farsangi et al., 2026).

Exosomes, diminutive extracellular vesicles ranging from 30 to 150 nm in diameter, have attracted considerable interest due to their function in intercellular communication and their therapeutic potential. These nanovesicles, once regarded as cellular debris, are now understood to carry proteins, lipids and nucleic acids between cells. In the context of MS, exosomes derived from various cell types, particularly mesenchymal stem cells (MSCs), have demonstrated promise in modulating immune responses, fostering remyelination, and providing neuroprotection. Size alone does not establish endosomal origin; EV or small EV is the operational term when that origin has not been demonstrated.

The studies below cover EV interventions in EAE, cuprizone-induced demyelination, and related experimental systems. Table 5.6.1 and Figure 5.6.1 summarise the original study set; the additional 2024 findings are discussed separately. Model effects provide a rationale for clinical investigation, rather than evidence of patient benefit.

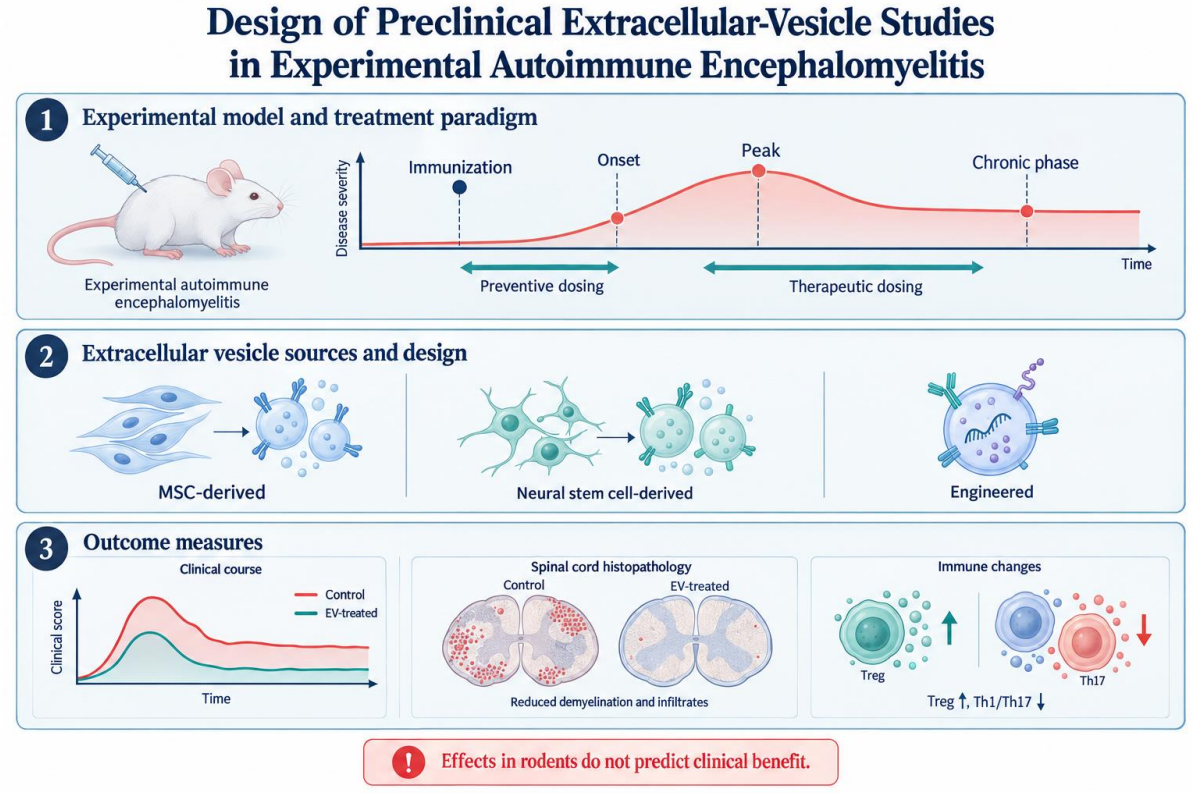


Figure 5.6.1. Design of preclinical extracellular-vesicle studies in experimental autoimmune encephalomyelitis: treatment paradigm, vesicle sources and outcome measures.

Table 5.6.1. Primary preclinical studies and a systematic-review synthesis of extracellular-vesicle interventions in models of MS and related demyelination. The synthesis entry summarises preclinical studies; no entry establishes clinical efficacy in patients.

Study	Model	EV source	Route	Cargo / variant	Main reported findings
Farinazzo et al. (2018)	Chronic EAE, mouse	Adipose stromal cell nanovesicles	Intravenous	Native	Effects on clinical course seen with early but not advanced-stage intervention
Rajan et al. (2016)	EAE, mouse	Periodontal ligament stem cell secretome / EMVs	Intravenous, after symptom onset	Native	Immune modulation and reduced severity; EMVs from MS donors also active
Fathollahi et al. (2021)	EAE, mouse	MSC small EVs	Intranasal, daily	Native	Reduced clinical score and spinal cord lesions; EVs outperformed parent cells in this model
Jafarinia et al. (2020)	EAE, mouse	Adipose-derived MSC EVs	Intravenous	Native	Reduced T-cell proliferation, leukocyte infiltration and demyelination
Riazifar et al. (2019)	EAE, mouse	MSC exosomes, IFN-γ-stimulated	Intravenous	IFNγ-Exo	Lower clinical scores, less demyelination, increased spinal cord Tregs
Ahmadvand Koohsari et al. (2021)	EAE, mouse	Umbilical cord MSC EVs	Intravenous	Native	Cytokine rebalancing and reduced leukocyte infiltration
Wang et al. (2023)	EAE, mouse	Bone marrow MSC exosomes	Intrathecal	miR-23b-3p	Reduced inflammation via inhibition of microglial pyroptosis
Baharlooi et al. (2021, 2022)	In vitro, PBMCs and CD4+ T cells from RRMS patients	Umbilical cord MSC exosomes	Co-culture	Native	Suppressed proliferation and inflammatory cytokine production
Zhang et al. (2022)	EAE and cuprizone, mouse	Bone marrow MSC exosomes	Intravenous	Native	Promoted OPC differentiation and shifted microglial polarisation
Giunti et al. (2021)	EAE, mouse; activated microglia	MSC small EVs, IFN-γ-primed	Intravenous / co-culture	miR-467f, miR-466q	Attenuated microglial inflammatory signalling
Mattera et al. (2023)	Cuprizone, mouse	Plasma-derived EVs	Intranasal	Transferrin-loaded	Enhanced remyelination versus free transferrin
Xiao et al. (2022)	Cuprizone, mouse	Neural stem cell exosomes, PDGFA-engineered	Intranasal	Montelukast-loaded	OPC-targeted delivery with improved remyelination
Abdallah et al. (2024)	Ethidium bromide demyelination, canine	Adipose-derived MSC exosomes	Intrathecal	Native	Remyelination and reduced neuroinflammation; parent cells performed better in this comparison

Study	Model	EV source	Route	Cargo / variant	Main reported findings
Xun et al. (2022)	Meta-analysis of 12 rodent EAE studies	MSC-EVs (various)	Various	Various	Pooled improvement in clinical score, with substantial methodological heterogeneity

Each study is described in detail in the sections that follow.

1. Preclinical Studies: Animal Models and In Vitro Studies

1.1. Experimental Autoimmune Encephalomyelitis (EAE) Model Studies

1.1.1. Farinazzo et al. (2018)

Farinazzo and colleagues conducted a dual-phase investigation utilising an experimental autoimmune encephalomyelitis (EAE) mouse model to assess the therapeutic potential of nanovesicles derived from adipose mesenchymal stem cell (ASC-NVs) in multiple sclerosis (MS).

Study 1: Early-Stage Intervention

The model was a chronic EAE mouse model and the study phase was the preclinical (early) stage of EAE. ASC-derived nanovesicles were administered intravenously prior to symptom onset (3, 8, and 13 days post-immunization). The aim was to evaluate ASC-NVs' efficacy in preventing disease development, focusing on their impact on inflammation, T cell activation, and spinal cord demyelination. Prophylactic administration of ASC-NVs significantly mitigated EAE severity, evidenced by reduced clinical scores, diminished spinal cord inflammation, and decreased demyelination. The treatment also inhibited T cell adhesion and CNS infiltration, indicating potent immunomodulatory effects when administered before disease onset.

Study 2: Advanced-Stage Intervention

The model was a chronic EAE mouse model and the study phase was the post-clinical (advanced) stage of EAE. ASC-NVs were administered after symptom onset (12, 16, and 20 days post-immunization) to assess their therapeutic potential during the chronic EAE phase. The focus was on ASC-NVs' ability to ameliorate established diseases by reducing ongoing inflammation and demyelination. Unlike the early-stage intervention, ASC-NVs administered after disease onset did not significantly alter the EAE clinical course. The treatment showed limited effect on T cell activation and failed to reduce spinal cord inflammation or demyelination, highlighting the critical importance of timing in ASC-NV therapeutic efficacy for EAE.

Timing therefore appears decisive in autoimmune disease of this kind. The study suggests that while ASC-NVs effectively prevent disease progression when administered early, their efficacy is markedly reduced in established disease stages.

1.1.2. Li et al. (2019)

Li and collaborators investigated the therapeutic effects of bone marrow mesenchymal stem cell-derived exosomes (BMSC-exosomes) on experimental autoimmune encephalomyelitis (EAE), a rat model commonly employed to study multiple sclerosis (MS). The study was structured in two phases to evaluate the impact of early and late-stage intervention with BMSC-exosomes.

Study 1.

The study phase was the early stage of EAE. BMSC-derived exosomes were administered to EAE rats immediately following disease induction at a 100 μg dose. This early intervention aimed to assess exosomes' potential to mitigate the initial inflammatory response and impede disease progression. Early administration of BMSC-exosomes resulted in significant reduction of inflammation and demyelination in the rats, which suggests that early treatment may limit disease severity.

Study 2.

The study phase was the advanced stage of EAE. BMSC-exosomes were administered at an increased dose of 400 μg. This phase aimed to evaluate exosome efficacy in a more established disease context. The higher dose of BMSC-exosomes led to more robust suppression of the inflammatory response and further reduction in clinical symptoms, demonstrating that even in later disease stages, exosome therapy could offer significant therapeutic benefits.

These findings emphasize the importance of both dosage and timing in the therapeutic application of BMSC-exosomes for treating autoimmune disorders such as MS.

1.1.3. Zhang et al. (2017)

Zhang and colleagues conducted a study to evaluate the effects of exosomes derived from human bone marrow mesenchymal stem cells (hMSCs) on functional recovery following traumatic brain injury (TBI) in rats. The investigation focused on comparing the therapeutic outcomes of exosomes generated from hMSCs cultured under two distinct conditions: traditional 2-dimensional (2D) cultures and 3-dimensional (3D) collagen scaffolds.

Traumatic Brain Injury (TBI) rat model. Systemic administration of exosomes derived from hMSCs cultured under 2D and 3D conditions. Exosomes were injected via the tail vein 24 hours post-TBI. Functional recovery was assessed using a modified Morris water maze test, neurological severity score, and footfault test. Histological and immunohistochemical analyses were performed to evaluate lesion volume, neurovascular remodeling, and neuroinflammation. In the reported experimental setting, EV preparations derived from hMSCs cultured in three-dimensional conditions were associated with greater activity on selected spatial-learning and inflammatory readouts than their comparators. These data do not establish superior efficacy in patients. Both 2D and 3D exosome treatments significantly increased the number of newborn endothelial cells and mature neurons, indicating enhanced angiogenesis and neurogenesis. The 3D-derived exosomes exhibited superior outcomes in promoting neurogenesis. Exosome treatment markedly reduced neuroinflammation, as evidenced by decreased activation of astrocytes and microglia.

The study concludes that exosomes derived from hMSCs, especially those generated in 3D cultures, offer a promising cell-free therapeutic approach for TBI, promoting neurovascular remodeling and functional recovery while mitigating neuroinflammation.

1.1.4. Rajan et al. (2016)

Rajan and colleagues investigated the therapeutic potential of secretome derived from human periodontal ligament stem cells (hPDLSCs) in treating experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS). The study specifically compared the effects of secretome and exosomes/microvesicles (EMVs) derived from hPDLSCs of both relapsing-remitting MS (RR-MS) patients and healthy donors.

Experimental Autoimmune Encephalomyelitis (EAE) mouse model. Mice were treated with hPDLSCs-conditioned medium (CM) or purified EMVs obtained from either RR-MS patients or healthy donors. Treatments were administered intravenously after symptom onset, approximately 14 days post-immunization with myelin oligodendrocyte glycoprotein (MOG). The study evaluated clinical disease scores, body weight, histological analysis of spinal cord and spleen tissues, cytokine levels, and expression of pro-inflammatory and anti-inflammatory markers. Mice treated with hPDLSCs-CM or EMVs from both MS patients and healthy donors exhibited significant improvement in clinical disease scores and body weight compared to untreated EAE mice. These treatments also enhanced spine density and remyelination in the spinal cord. The secretome and EMVs from hPDLSCs demonstrated potent anti-inflammatory and immunosuppressive effects by reducing pro-inflammatory cytokines (IL-17, IFN-γ, TNF-α, IL-6, and IL-1β) while increasing the anti-inflammatory cytokine IL-10. The treatments also attenuated spinal cord apoptosis by downregulating STAT1, p53, cleaved caspase 3, and Bax. Histological analysis revealed reduced demyelination and axonal loss in treated mice's spinal cords. Immunohistochemical evaluations showed decreased pro-inflammatory CD4+ T cell infiltration and increased IL-10 expression in the spinal cord and spleen.

The implication is that hPDLSC-derived secretome and EMVs, particularly those from MS patients, hold significant promise as a cell-free therapeutic strategy for treating MS by modulating immune responses and promoting tissue repair.

1.1.5. Fathollahi et al. (2021)

Fathollahi and colleagues evaluated the therapeutic effects of intranasally administered small extracellular vesicles (SEVs) derived from mesenchymal stem cells (MSCs) on experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS). The study aimed to assess SEVs' potential to modulate immune responses and reduce disease severity.

The model was Experimental Autoimmune Encephalomyelitis (EAE) in C57BL/6 mice. Mice were divided into three groups: one receiving daily intranasal MSC-SEV administration, another receiving intranasal MSC administration, and a control group receiving PBS. SEV treatment was administered daily from day 15 to 27 post-EAE induction, while MSCs were administered on days 15 and 24. The study evaluated clinical scores, histological analysis of CNS lesions, T cell proliferation, regulatory T cell frequency, transcription factor expression, and splenocyte cytokine levels. Both MSC-SEVs and MSC treatments significantly reduced clinical scores compared to the control

group, with MSC-SEVs demonstrating a more pronounced effect in alleviating disease symptoms. The SEV-treated group exhibited a significant reduction in spinal cord demyelinated lesions compared to control and MSC-treated groups. CD4+CD25+Foxp3+ regulatory T cell frequency increased in both MSC-SEVs and MSC-treated groups. FoxP3 gene expression increased, while Tbx21, Gata3, and Rorc expression decreased. TGF-β levels were higher in the SEV-treated group, while IL-10 levels increased only in the MSC-treated group.

In this EAE model, intranasal MSC-SEV administration produced larger reductions in clinical scores and spinal cord demyelination than the MSC comparator under the reported experimental conditions. This is preclinical evidence and does not establish superiority, safety, or therapeutic efficacy in people with MS.

1.1.6. Baharlooi et al. (2022)

Baharlooi and colleagues explored the immunomodulatory effects of human umbilical cord mesenchymal stem cells (UC-MSCs) and their derived exosomes on CD4+CD25− conventional T cells isolated from relapsing-remitting multiple sclerosis (RRMS) patients and healthy controls. This study aimed to compare the therapeutic potential of UC-MSCs and their exosomes in modulating immune responses, essential for developing effective MS treatments.

The model was In vitro co-culture of CD4+CD25− conventional T cells from RRMS patients and healthy controls. UC-MSCs were isolated from human umbilical cord tissues, and exosomes were derived via ultracentrifugation. The anti-inflammatory effects of UC-MSCs and their exosomes were assessed on T cells by measuring cytokine production (IFN-γ, IL-10, and IL-17) and T cell proliferation using flow cytometry. The study focused on T cell proliferation suppression and cytokine production modulation, evaluating UC-MSCs and their exosomes' potential to regulate immune responses in MS patients and healthy individuals. Both UC-MSCs and their exosomes significantly inhibited CD4+CD25− T cell proliferation in MS patients and healthy controls. Notably, exosomes exhibited a higher suppression index than UC-MSCs under these in vitro conditions; this comparison does not establish clinical superiority. Treatment with UC-MSCs and their exosomes resulted in a significant decrease in pro-inflammatory cytokines (IFN-γ and IL-17) and an increase in the anti-inflammatory cytokine IL-10. This effect was observed in both MS patients and healthy controls.

The study concludes that UC-MSC-derived exosomes may serve as a promising cell-free therapeutic option for treating MS by effectively modulating immune responses and reducing inflammation, potentially offering an alternative to direct MSC therapy.

1.1.7. Baharlooi et al. (2021)

Baharlooi and colleagues investigated the suppressive effects of umbilical cord mesenchymal stem cells (UC-MSCs) and their released exosomes on peripheral blood mononuclear cell (PBMC) proliferation in relapsing-remitting multiple sclerosis (RRMS) patients and healthy controls. The study aimed to evaluate the potential of UC-MSCs and their exosomes as a therapeutic approach for MS by examining their immunomodulatory effects.

The model was an in vitro co-culture system using PBMCs from RRMS patients and healthy controls. UC-MSCs were isolated from human umbilical cords, and exosomes were derived through ultracentrifugation. CFSE-labeled PBMCs were co-cultured with either UC-MSCs or their exosomes. PBMC proliferation was measured using flow cytometry after 72 hours. The study assessed PBMC proliferation suppression by both UC-MSCs and their exosomes, focusing on the difference in suppression efficiency between the two. The potential for allostimulation by UC-MSCs and their exosomes was also evaluated. Both UC-MSCs and their exosomes significantly suppressed PBMC proliferation from RRMS patients and healthy controls. Exosomes demonstrated a higher suppression index compared to UC-MSCs, indicating a stronger immunosuppressive effect. Neither UC-MSCs nor their exosomes induced significant proliferation stimulation in non-activated PBMCs, suggesting the treatments did not elicit an alloreactive immune response. The study observed that the suppressive capacity of UC-MSCs and their exosomes decreased with higher cell passages, emphasizing the importance of using early-passage cells for therapeutic applications.

These in vitro findings support further study of UC-MSC-derived exosomes as immunomodulatory candidates. They do not establish a cell-free treatment for MS, comparative safety, or clinical efficacy.

1.1.8. Jafarinia et al. (2020)

Jafarinia and colleagues conducted a comparative study to assess the therapeutic effects of human adipose-derived mesenchymal stem cells (hADSCs) and their extracellular vesicles (hADSC-EVs) on experimental autoimmune

encephalomyelitis (EAE), a mouse model for multiple sclerosis (MS). Given the concerns in cell therapy related to immune rejection, genetic instability, and potential malignancies, the focus has shifted toward extracellular vesicles (EVs) secreted by mesenchymal stem cells as a safer alternative.

The model was EAE induced in C57BL/6 mice. Mice were divided into three groups: a control group treated with saline, a group treated with autologous adipose-derived MSCs, and a group treated with MSC-derived EVs. Treatments were administered intravenously, and the mice were monitored over a period of 30 days. Clinical scores were recorded every other day. On Day 30, mice were sacrificed for further analysis, including splenocyte isolation for proliferation assays and flow cytometry to determine regulatory T cell (Treg) frequency. Spinal cords were analysed for leukocyte infiltration, demyelination, and expression of myelin-related proteins. Both hADSC and hADSC-EV treatments significantly reduced clinical scores and T cell proliferation compared to the control group. The frequency of CD4+ CD25+ Foxp3+ Treg cells was significantly higher in the hADSC-treated group. Both treatments led to a significant decrease in inflammation and demyelination in the spinal cords of treated mice, though no significant differences were observed in the expression of myelin basic protein (MBP) and oligodendrocyte transcription factor 2 (OLIG2) between the groups.

This suggests that intravenous administration of hADSC-EVs may effectively attenuate EAE by reducing T cell proliferation, clinical severity, leukocyte infiltration, and demyelination, offering a potential therapeutic approach for chronic MS.

1.1.9. Riazifar et al. (2019)

Riazifar and colleagues explored the therapeutic potential of exosomes derived from mesenchymal stem cells (MSCs) in treating autoimmune and neurodegenerative disorders, with a focus on multiple sclerosis (MS). Using an experimental autoimmune encephalomyelitis (EAE) mouse model, the study reported therapeutic effects of MSC-derived exosomes, particularly those stimulated by interferon-gamma (IFNγ-Exo), in this animal model; such findings are preclinical and do not establish efficacy in patients.

EAE mouse model. Intravenous administration of exosomes, with a focus on those derived from MSCs stimulated by interferon-gamma (IFNγ-Exo). The outcomes assessed were the mean clinical score in EAE mice, demyelination and neuroinflammation levels, and the regulatory T cell population in the spinal cords. IFNγ-Exo treatment resulted in significant reduction of the mean clinical score in EAE mice. The treatment decreased demyelination and neuroinflammation in the CNS. An increase in the population of CD4+CD25+FOXP3+ regulatory T cells within the spinal cords was observed. IFNγ-Exo suppressed the proliferation of activated peripheral blood mononuclear cells (PBMCs) and reduced levels of pro-inflammatory cytokines while increasing immunosuppressive cytokines like indoleamine 2,3-dioxygenase.

The study's findings suggest that MSC-derived exosomes, particularly IFNγ-Exo, could serve as a promising cell-free therapeutic approach for treating MS and other autoimmune diseases by modulating the immune system and promoting a tolerogenic immune response.

1.1.10. Ahmadvand Koohsari et al. (2021)

Ahmadvand Koohsari and colleagues conducted a study to assess the therapeutic effects of extracellular vesicles (EVs) derived from human umbilical cord mesenchymal stem cells (hUCSC-EVs) on experimental autoimmune encephalomyelitis (EAE), an established mouse model of multiple sclerosis (MS). The study aimed to explore how hUCSC-EVs influence clinical outcomes and the balance between pro-inflammatory and anti-inflammatory cytokines.

The model was EAE in C57BL/6 mice. EAE was induced in mice, followed by intravenous treatment with hUCSC-EVs or a vehicle. The clinical score and body weight of the mice were recorded daily. The study evaluated maximum mean clinical score (MMCS), cytokine levels (both pro-inflammatory and anti-inflammatory), body weight, leukocyte infiltration, and the percentage of GFAP and MBP-positive cells in the spinal cord. Mice treated with hUCSC-EVs exhibited a significantly lower maximum mean clinical score (MMCS) and improved body weight compared to the control group. The treatment was associated with a reduction in pro-inflammatory cytokines (such as IL-17a, TNF-α, and IFN-γ) and an increase in anti-inflammatory cytokines (IL-4 and IL-10). Additionally, there was a notable increase in the frequency of regulatory T cells (Tregs) in the spleen of treated mice. The treatment with hUCSC-EVs led to a significant decrease in leukocyte infiltration in the spinal cord. However, there was no significant difference in the percentage of GFAP and MBP-positive cells between the experimental groups.

The study concludes that intravenous administration of hUCSC-EVs can alleviate induced EAE by modulating the balance of pro-inflammatory and anti-inflammatory

cytokines, reducing leukocyte infiltration, and promoting a regulatory immune response. On this evidence, hUCSC-EVs produce effects qualitatively similar to those reported for EVs from other mesenchymal stem cell sources, such as adipose tissue or bone marrow, although no direct head-to-head comparison was made.

1.1.11. Wang et al. (2023)

Wang and colleagues conducted a study to explore the therapeutic potential of exosomal miR-23b-3p derived from bone marrow mesenchymal stem cells (BMSCs) in treating experimental autoimmune encephalomyelitis (EAE), a widely used mouse model of multiple sclerosis (MS). The study focused on understanding how these exosomes could alleviate EAE by targeting microglial pyroptosis, an inflammatory form of programmed cell death.

The model was EAE in C57BL/6 mice. Exosomes containing miR-23b-3p were isolated from BMSCs and administered intrathecally to EAE-induced mice. The study included control groups treated with either phosphate-buffered saline (PBS) or exosomes without the miR-23b-3p mimic. The outcomes assessed were clinical scores, levels of inflammatory cytokines, microglial pyroptosis, and expression of the NEK7/NLRP3 inflammasome pathway in the spinal cord. Exosomal miR-23b-3p significantly reduced clinical scores in EAE mice, indicating an alleviation of disease severity. The treatment inhibited microglial pyroptosis, as evidenced by decreased levels of caspase-1, ASC, and NLRP3 proteins in the spinal cord. There was a marked decrease in pro-inflammatory cytokines (IL-6, IL-1β, IL-18, and TNF-α) and an increase in the anti-inflammatory cytokine IL-10 in treated mice. The study demonstrated that miR-23b-3p directly targets NEK7, leading to reduced activation of the NLRP3 inflammasome, which is central in the pathogenesis of MS.

This EAE study supports miR-23b-3p-containing BMSC exosomes as a mechanistic candidate for further investigation. Clinical relevance, dosing, safety, and efficacy in MS remain to be established.

1.1.12. Taghizadeh et al. (2022)

Taghizadeh and colleagues explored the therapeutic potential of anti-Caspr-conjugated gold nanoparticles (GNPs) as a novel treatment strategy for multiple sclerosis (MS), using the experimental autoimmune encephalomyelitis (EAE) mouse model. The study aimed to compare the effectiveness of these GNPs, combined with polyethylene glycol (PEG) and exosomes, against a standard MS treatment (Glatiramer acetate) in modulating immune responses and reducing disease severity.

The model was EAE in female C57BL/6 mice. Mice were treated with anti-Caspr-conjugated GNPs combined with PEG and exosomes, and their effects were compared with those of Glatiramer acetate, a commonly used MS treatment. Treatments were administered over 25 days post-EAE induction. The outcomes assessed were clinical scores, body weight changes, histopathological examination of spinal cords (for demyelination and immune cell infiltration), regulatory T cell populations, and the expression of inflammatory cytokines (IFN-γ, IL-17) and microRNA-326. The mice treated with anti-Caspr-conjugated GNPs showed significant improvement in clinical scores and body weight compared to the untreated EAE control group. Histological analysis revealed reduced demyelination and decreased infiltration of inflammatory cells in the spinal cords of treated mice. Treatment with GNPs led to an increase in regulatory T cells (Tregs) and a decrease in pro-inflammatory cytokines IFN-γ and IL-17, as well as a reduction in the expression of miR-326, which is associated with Th17 differentiation and MS severity.

The reported EAE findings support further preclinical investigation of anti-Caspr-conjugated GNPs combined with PEG and exosomes. They do not establish this platform as a therapeutic approach for patients with MS.

1.2. Cuprizone-Induced Demyelination Model Studies

1.2.1. Zhang et al. (2022)

Zhang and colleagues investigated the therapeutic potential of exosomes derived from bone marrow mesenchymal stromal cells (MSC-Exo) in promoting remyelination and reducing neuroinflammation in the demyelinating central nervous system (CNS), using two animal models: the experimental autoimmune encephalomyelitis (EAE) model, which mimics multiple sclerosis (MS), and the cuprizone (CPZ) diet model, which induces toxic demyelination.

The models used were EAE and CPZ diet-induced demyelination in C57BL/6 mice. MSC-Exo were isolated from rhesus monkey bone marrow mesenchymal stromal cells and administered intravenously. For the EAE model, treatments began 10 days post-immunization, and for the CPZ model, treatments began on the day of CPZ diet withdrawal. The outcomes assessed were neurological and cognitive function, oligodendrocyte progenitor cell (OPC) differentiation, remyelination, neuroinflammation, and

activation of the TLR2/IRAK1/NF-κB signalling pathway in the CNS. MSC-Exo treatment significantly increased the number of newly generated and mature oligodendrocytes (OLs) and the level of myelin basic protein (MBP) in the spinal cord of EAE mice. In the CPZ model, MSC-Exo also promoted remyelination in the corpus callosum. The treatment reduced neuroinflammation by increasing the polarisation of microglia to the anti-inflammatory M2 phenotype while reducing the pro-inflammatory M1 phenotype. Additionally, MSC-Exo decreased the levels of pro-inflammatory cytokines (IL-1β, TNF-α) and increased anti-inflammatory cytokines (IL-10, TGF-β). MSC-Exo inhibited the TLR2/IRAK1/NF-κB signalling pathway, which is known to impede remyelination and promote neuroinflammation, thereby contributing to functional recovery in both models.

This study provides evidence that MSC-Exo not only enhances remyelination by promoting OPC differentiation but also modulates neuroinflammation by shifting microglial polarisation. MSC-Exo therefore merits study as a cell-free therapeutic approach for treating demyelinating diseases like MS.

1.2.2. Giunti et al. (2021)

Giunti and colleagues investigated the role of microRNAs (miRNAs) shuttled by mesenchymal stem cell-derived small extracellular vesicles (s-EVs) in modulating neuroinflammation, with a focus on their effects on microglia, the resident immune cells in the brain. The study aimed to identify specific miRNAs that could downregulate the pro-inflammatory phenotype of activated microglia and elucidate their mechanism of action in models of neuroinflammation, including experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS).

The models used were EAE in C57BL/6 mice and lipopolysaccharide (LPS)-activated N9 microglial cells. s-EVs were isolated from murine mesenchymal stem cells (MSCs) pre-activated with IFN-γ, which were then administered to EAE mice or co-cultured with activated microglia. The study focused on miR-467f and miR-466q, which were identified as key miRNAs in modulating the inflammatory response. The outcomes assessed were the effects of these miRNAs on the expression of pro-inflammatory cytokines (TNF-α, IL-1β) and neuroprotective markers (Cx3cr1, Cd206, Nr4a2) in microglia, as well as their impact on the MAPK signalling pathway, particularly the p38 MAPK pathway. s-EVs derived from IFN-γ-primed MSCs significantly reduced the expression of pro-inflammatory cytokines in activated microglia, while enhancing the expression of neuroprotective markers. This effect was associated with the downregulation of the p38 MAPK pathway, a key signalling pathway in neuroinflammation. In EAE mice, treatment with s-EVs led to a significant decrease in the expression of neuroinflammation markers in the spinal cord, although it did not alter the overall disease course.

This study provides evidence that MSC-derived s-EVs, particularly those enriched with specific miRNAs, could serve as an investigational therapeutic tool for controlling neuroinflammation in diseases like MS by modulating microglial activity and inflammatory pathways.

1.2.3. Mattera et al. (2023)

Mattera and colleagues investigated the potential of transferrin-loaded extracellular vesicles (EVTf) to promote remyelination in the central nervous system (CNS), using the cuprizone (CPZ) mouse model of demyelination. The study aimed to explore whether EVs could serve as efficient nanocarriers to deliver transferrin (Tf) directly to the CNS, thereby enhancing the differentiation of oligodendrocyte progenitor cells (OPCs) and accelerating remyelination in demyelinated tissues.

The model was Cuprizone (CPZ) diet-induced demyelination in C57BL/6j mice. EVs were isolated from mouse plasma and loaded with transferrin (Tf). These EVTf were administered intranasally (IN) to CPZ-induced demyelinated mice. The effects of the treatment were compared to those of free Tf and vehicle control. The outcomes assessed were remyelination in the corpus callosum by immunohistochemistry and electron microscopy, focusing on markers such as myelin basic protein (MBP) and oligodendrocyte differentiation markers (PDGFRα). The internalization of EVTf by OPCs and their biodistribution in the CNS were also evaluated. EVTf treatment significantly increased remyelination in the corpus callosum, as evidenced by higher MBP levels and a greater number of myelinated axons compared to controls. Notably, the remyelination effect was more pronounced with EVTf compared to free Tf, indicating that EVs effectively protect and deliver their cargo to the target cells. The study demonstrated that EVTf promoted the differentiation of OPCs into mature oligodendrocytes, contributing to the observed remyelination. This effect was confirmed through increased expression of mature oligodendrocyte markers and a decrease in OPC markers. EVTf were found to be internalized by

OPCs via clathrin-caveolae and lipid raft endocytosis pathways, and their cargo was delivered to lysosomes and recycling endosomes, facilitating the maturation of OPCs.

The study concludes that transferrin-loaded EVs are an investigational strategy for enhancing remyelination in demyelinating diseases like multiple sclerosis, providing a novel and effective method for targeting and delivering therapeutic agents to CNS.

1.2.4. Xiao et al. (2022)

Xiao and colleagues explored the therapeutic potential of targeted extracellular vesicles (ExoPs) loaded with montelukast (Mon) for treating demyelinating diseases, using the cuprizone-induced demyelination mouse model. The study aimed to develop a targeted drug delivery system that enhances the differentiation of oligodendrocyte progenitor cells (OPCs) and promotes remyelination in the central nervous system (CNS).

The model was Cuprizone (CPZ)-induced demyelination in C57BL/6 mice. Exosomes were isolated from neural stem cells (NSCs) and genetically engineered to overexpress PDGFA (ExoPs), which enhances their targeting to OPCs. Montelukast was loaded into these ExoPs (ExoPs-Mon) and administered intranasally to the CPZ mice. The treatment effects were compared with ExoPs without Mon and free Mon administered via the same route. The outcomes assessed were remyelination in the corpus callosum by immunohistochemistry, electron microscopy and quantification of myelinated axons. Additionally, the study examined the accumulation and differentiation of OPCs, expression of neuroinflammatory markers, and the overall neuroprotective effects of the treatments. ExoPs-Mon treatment significantly improved remyelination in the corpus callosum, as indicated by increased myelin thickness and a higher percentage of myelinated axons compared to other treatment groups. ExoPs-Mon promoted the differentiation of OPCs into mature oligodendrocytes, reducing the accumulation of undifferentiated OPCs at the lesion site. This effect was greater than that observed with either ExoPs alone or free Mon. ExoPs-Mon treatment reduced neuroinflammation and axonal damage, as evidenced by lower expression levels of pro-inflammatory cytokines and fewer markers of axonal injury.

The study suggests that ExoPs-Mon is an investigational therapeutic approach for enhancing remyelination and neuroprotection in demyelinating diseases by improving drug delivery to OPCs and modulating the lesion microenvironment.

1.3. Other Relevant Studies

1.3.1. Tang et al. (2022)

Tang and colleagues investigated the potential of extracellular vesicles (EVs) as a delivery system for the natural compound neferine, focusing on its effects on neurodegenerative disease proteins and motor deficits in an Alzheimer's disease (AD) mouse model. The study aimed to enhance delivery of neferine to the brain and assess its therapeutic efficacy in reducing protein aggregation and improving motor function in APP/PS1 transgenic mice.

The model was the APP/PS1 double transgenic mouse model of Alzheimer's disease. Neferine was encapsulated within EVs isolated from neuron-2a cells and administered intravenously to APP/PS1 mice. The effects were compared to those of free neferine administered at the same dose. The outcomes assessed were motor coordination using a rotarod test, the level of β-amyloid (Aβ) deposition in the brain, and the expression of neurodegenerative proteins such as huntingtin (HTT74), tau (P301L), and alpha-synuclein (A53T). Mice treated with EV-encapsulated neferine showed significant improvements in motor coordination compared to those treated with free neferine, as measured by increased latency to fall in the rotarod test. EV-neferine significantly reduced the deposition of fibrillated Aβ in the brains of APP/PS1 mice, with a greater effect than free neferine. This was confirmed through Western blot and dot blot analyses. EV-neferine treatment also reduced the levels of mutant neurodegenerative proteins (HTT74, P301L tau, and A53T alpha-synuclein) in neuronal cell models, indicating enhanced autophagy and protein clearance.

The study concludes that EV-encapsulated neferine offers an investigational approach to treating Alzheimer's disease by effectively crossing the blood-brain barrier (BBB) and mitigating neurodegenerative processes in the brain.

1.3.2. Choi et al. (2022)

Choi and colleagues reviewed the current strategies for the targeted delivery of exosomes to the brain, focusing on the advantages and challenges associated with each method. The study treats exosomes as natural nanoparticles able to cross the blood-brain barrier (BBB) and delivering therapeutic agents to the central nervous system (CNS).

The review discussed several strategies for enhancing exosome delivery to the brain.

Receptor-mediated transcytosis is the most widely used strategy for crossing the BBB. Therapeutics can achieve

brain delivery by expressing ligands that bind to receptors like transferrin receptor (TfR), low-density lipoprotein receptor (LDLR), and insulin receptor (INSR). Cell-penetrating peptides are a second: short peptides that can translocate macromolecules across cell membranes without receptor interaction. Neurotropic virus-derived peptides, such as those from the rabies virus glycoprotein (RVG), have been utilised to induce brain-targeting of exosomes.

Results and Challenges.

Exosomes naturally exhibit low efficiency in crossing the BBB. The review emphasizes the need for further research to understand the mechanisms underlying BBB crossing and to improve exosome engineering for targeted delivery. The authors explore various strategies for modifying the surface of exosomes, such as chemical modifications or genetic engineering, to enhance their brain targetability. While preclinical studies show early findings, the review reinforces the need for well-designed clinical trials to evaluate the safety and efficacy of exosome-based therapies for CNS diseases.

The review identifies delivery strategies and methodological obstacles in exosome-based CNS research. Its conclusions concern experimental development, rather than established treatment efficacy.

1.3.3. Xun et al. (2022)

Xun and colleagues conducted a thorough meta-analysis to evaluate the therapeutic efficacy of mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) in preclinical rodent models of multiple sclerosis (MS). The study aimed to consolidate existing evidence on the role of MSC-EVs in improving clinical outcomes and to identify knowledge gaps that need to be addressed before clinical trials can be initiated.

The meta-analysis focused on preclinical rodent models of MS, specifically using the experimental autoimmune encephalomyelitis (EAE) model. The authors systematically reviewed and analysed data from 12 studies that met the inclusion criteria. These studies included various types of MSC-EVs, differing in source, dose, and administration route. The primary outcome assessed was the clinical score of the rodents, a standard measure of disease severity in MS models. The pooled analysis revealed that MSC-EVs significantly improved the clinical scores in EAE models, with a substantial therapeutic effect. Significant heterogeneity was observed across studies, with factors such as animal species, MSC source, and gender influencing the outcomes. The analysis identified several critical gaps, including variability in dosing units, timing, and delivery methods of MSC-EVs, which must be standardised before progressing to clinical trials.

1.3.4. Abdallah et al. (2024)

Abdallah and colleagues conducted a study to compare the therapeutic effects of mesenchymal stem cells (MSCs) and stem cell-derived exosomes on experimentally induced multiple sclerosis (MS) in a canine model. The study aimed to evaluate the potential of these treatments in promoting remyelination and reducing neuroinflammation in a large animal model, which more closely mimics human MS than rodent models.

The model was ethidium bromide-induced demyelination in the spinal cord of Mongrel dogs. Dogs were divided into three groups: a control group treated with saline, a group treated with autologous adipose-derived MSCs, and a group treated with exosomes derived from MSCs. Treatments were administered intrathecally, and the dogs were monitored over a period of 28 days. The outcomes assessed were clinical status using the Olby scale for gait evaluation, magnetic resonance imaging (MRI) for spinal cord lesions, cerebrospinal fluid (CSF) analysis, and histopathological evaluation, including electron microscopy to assess the degree of remyelination and axonal integrity. Both the MSC and exosome-treated groups showed significant improvement in clinical scores compared to the control group, with the MSC group displaying the most notable improvement. MRI scans revealed a faster resolution of hyperintense lesions in the treated groups, particularly in the MSC-treated dogs, indicating effective remyelination. Histological and electron microscopy analysis confirmed extensive remyelination in both treated groups, with a more pronounced effect in the MSC group. Both treatments demonstrated neuroprotective effects, with the MSC group showing greater efficacy in restoring normal spinal cord architecture and promoting axonal recovery.

The comparison supports further preclinical assessment of MSCs and their EV preparations. Experimental remyelination and inflammatory readouts cannot establish efficacy or long-term safety in people with MS.

Z. Shi et al. (2024) investigated MSC-EVs carrying miR-181a-5p in EAE mice and stimulated microglial cultures. Intrathecal EV administration reduced experimental disease severity and demyelination. Mechanistic experiments implicated targeting of USP15 and regulation of the RelA/NEK7 axis, with reduced microglial inflammation and pyroptosis. These results add a defined cargo-related

hypothesis for testing rather than evidence of clinical remyelination.

Read across rather than in sequence, this body of work divides along four axes, and the differences between studies matter more than their individual conclusions. The first is the source cell. Bone marrow and adipose-derived MSCs dominate, umbilical cord MSCs appear in a smaller group of studies, and neural stem cells, dendritic cells and plasma are each represented by single reports; because vesicle cargo reflects the parent cell and its culture conditions, results obtained with one source cannot be assumed to transfer to another. The second is whether the vesicles were modified. Native preparations and primed or engineered preparations (IFN-γ stimulation, miR-23b-3p, transferrin or montelukast loading, PDGFA surface engineering) are frequently discussed as though they were one intervention, yet an engineered vesicle is a different product with a different rationale, and the comparison that would justify engineering, namely engineered against native vesicles from the same batch, is rarely performed.

The third axis is the model and the timing of intervention. Most studies use EAE, which is driven by peripheral autoimmunity and responds readily to immunomodulation; cuprizone and ethidium bromide models test remyelination with little adaptive immune involvement. Effects in one model therefore answer a different question from effects in the other, and the distinction matters for MS, where inflammation and failed repair coexist. Timing compounds this: Farinazzo and colleagues observed effects with early intervention but not at an advanced stage, which is precisely the situation in which patients usually present. The fourth axis is route and dose. Intravenous administration predominates, with intranasal delivery used in most remyelination studies and intrathecal delivery in only a few; doses are reported in incompatible units, as particle counts, protein mass or donor-cell equivalents, so no dose-response relationship can be constructed across the literature. This alone prevents the field from converting these studies into a rational first-in-human dose.

Two further limits apply to the endpoints. Clinical score in EAE, the most commonly reported outcome, is a composite of motor function in an animal whose disease course bears little resemblance to progressive MS, and histological remyelination does not establish functional recovery. The meta-analysis by Xun and colleagues (2022) pooled twelve rodent studies and reported an overall improvement in clinical score, but the heterogeneity it documented, in source, preparation, dose, route and outcome definition, is the finding of greatest practical importance: it indicates that the pooled estimate describes a family of loosely related experiments rather than one reproducible intervention. A more recent meta-analysis of bone-marrow-derived MSCs and their exosomes in experimental MS reported improvements in neuro-behavioural scores together with the same wide dispersion between studies, and again could not isolate the variables responsible (Kariminejad-Farsangi et al., 2026). Taken together, the preclinical literature establishes plausibility and identifies mechanisms worth testing; it does not yet identify a defined product, a dose or a patient population.

A 2025 cuprizone study provides a more recent remyelination-focused example. Mirab et al. treated chronically demyelinated mice with human umbilical-cord MSC-derived exosomes and reported improved motor performance, increased corpus-callosum remyelination, reduced astroglial and microglial responses, and higher expression of oligodendrocyte-lineage genes. These results remain preclinical and do not establish efficacy or dosing in people with MS (Mirab et al., 2025).

2. Critical Evaluation of Current Limitations and Challenges

2.1. Methodological Heterogeneity

One of the most significant challenges facing the field of exosome-based therapies for multiple sclerosis is the lack of standardisation in exosome isolation, characterisation, and administration protocols. This variability makes direct comparisons between studies difficult and hinders the development of standardised therapeutic approaches. For instance, the methods used for exosome isolation range from ultracentrifugation (as seen in Farinazzo et al., 2018) to more advanced techniques like tangential flow filtration, each with its own advantages and potential biases in exosome population selection. This diversity in methodology not only affects the composition and purity of the isolated exosomes but also impacts their biological activity and therapeutic potential. Standardisation of these protocols is important for reproducibility and for advancing exosome-based therapies towards clinical application.

2.2. Limited Long-term Data

Most studies focus on short-term outcomes, typically ranging from a few weeks to a couple of months post-treatment. This leaves critical questions about the long-term safety and efficacy of exosome therapies unanswered. For example, while studies like Li et al. (2019) show promising

short-term results, the potential for long-term side effects or the durability of therapeutic effects remains largely unexplored. Long-term studies are essential to understand the sustained impact of exosome therapies on disease progression, potential immune reactions to repeated administrations, and any unforeseen consequences of altering cellular communication pathways over extended periods.

2.3. Scalability Issues

Current exosome production methods, often relying on cell culture-based approaches, may not be suitable for large-scale clinical application. The development of scalable, GMP-compliant production methods is important for translating these therapies to clinical practice. This challenge is particularly evident in studies using more complex engineered exosomes, such as those in Xiao et al. (2022), where the scalability of production becomes even more critical. Large-scale production needs to maintain the quality, purity, and biological activity of exosomes while being cost-effective. This may require significant innovations in bioprocessing technologies and the development of novel bioreactor systems specifically designed for exosome production.

2.4. Incomplete Understanding of Cargo

While studies like Giunti et al. (2021) and Wang et al. (2023) have identified specific miRNAs as therapeutic agents, a full understanding of the full range of bioactive molecules in exosomes and their individual contributions to therapeutic effects is still lacking. This gap in knowledge makes it challenging to optimise exosome content for maximal therapeutic efficacy. Advanced proteomics, metabolomics, and transcriptomics approaches are needed to fully characterize exosome cargo and understand how different components interact to produce therapeutic effects. Moreover, the relationship between cell source, culture conditions, and exosome cargo needs to be elucidated to enable precise engineering of exosome content.

2.5. Potential Off-target Effects

The systemic administration of exosomes, as used in many studies, could lead to off-target effects in other organs. More research is needed to understand and mitigate these potential risks. For instance, while intranasal administration as explored by Fathollahi et al. (2021) might reduce systemic exposure, the long-term effects of repeated CNS-targeted administration need further investigation. Biodistribution studies using advanced imaging techniques are key to track exosome fate in vivo and identify potential sites of accumulation or unintended effects. Additionally, the impact of exosome therapies on normal physiological processes mediated by endogenous exosomes needs to be carefully evaluated.

2.6. Variability in Animal Models

The reliance on different animal models (EAE, cuprizone, etc.) across studies, while providing diverse insights, also complicates the interpretation and comparison of results. For example, while the EAE model used by Farinazzo et al. (2018) mimics the autoimmune aspects of MS, it may not fully capture the progressive neurodegeneration seen in human MS. The cuprizone model used in studies like Mattera et al. (2023) focuses more on demyelination and remyelination processes but lacks the autoimmune component. This diversity in models makes it challenging to translate findings to human MS, which is a heterogeneous disease with various subtypes and stages. Developing broader models that better recapitulate the full spectrum of MS pathology is essential for advancing exosome therapies.

2.7. Donor Variability

The impact of donor characteristics on exosome composition and efficacy is not fully understood. Studies like Rajan et al. (2016), which compared exosomes from MS patients and healthy donors, illustrate how much exosome activity can vary with the source. This variability could have significant implications for the development of standardised therapies. Factors such as donor age, health status, and genetic background may all influence exosome composition and functionality. Understanding and controlling these variables is central for developing consistent and effective exosome-based therapies.

2.8. Regulatory Challenges

The complex and variable nature of exosomes presents unique regulatory challenges that need to be addressed for clinical translation. Unlike traditional small molecule drugs, exosomes contain a multitude of bioactive components, making their characterisation and quality control more complex. The regulatory pathway for exosome-based therapies is still evolving, and clear guidelines for their development and approval are needed. Issues such as defining product identity, ensuring batch-to-batch consistency, and establishing appropriate potency assays need to be addressed. Collaboration between researchers, industry, and regulatory bodies is necessary to develop appropriate frameworks for exosome-based therapeutics.

2.9. Limited Comparative Studies

While some studies, like Abdallah et al. (2024), have compared exosome therapies to cellular therapies, more head-to-head comparisons between exosome-based treatments and current MS therapies are needed to establish their relative efficacy and safety profiles. This lack of comparative data makes it difficult to position exosome therapies within the current treatment landscape for MS. Comprehensive studies comparing exosome therapies to established MS treatments across various disease stages and subtypes are necessary to determine where exosome therapies might fit in the treatment paradigm and which patient populations might benefit most.

2.10. Mechanism of Action Clarity

While several studies have provided insights into exosome mechanisms of action, a clearer understanding of how exosomes interact with various cell types in MS pathology is still lacking. For instance, while Wang et al. (2023) elucidated the role of miR-23b-3p in inhibiting microglial pyroptosis, the full spectrum of exosome interactions with other cell types like oligodendrocytes, astrocytes, and various immune cells remains to be fully characterised. Understanding these complex interactions is important for optimizing exosome therapies and predicting potential side effects. Advanced in vitro models, such as organoids or microfluidic "organ-on-a-chip" systems, could provide valuable insights into these mechanisms.

3. Future Directions and Recommendations

3.1. Standardization Initiatives

There is an urgent need for the development and adoption of standardised protocols for exosome isolation, characterisation, and quantification. This could involve collaborative efforts between research institutions and regulatory bodies to establish guidelines that ensure reproducibility and comparability across studies. For example, the International Society for Extracellular Vesicles (ISEV) could contribute substantially to developing and promoting these standards. Standardised protocols should cover all aspects of exosome research, from isolation techniques to storage conditions, and from characterisation methods to functional assays. This would not only improve the quality and reliability of research but also accelerate the translation of exosome-based therapies to clinical practice.

3.2. Long-term Safety and Efficacy Studies

Future research should prioritise long-term follow-up studies to assess the durability of therapeutic effects and identify any potential late-onset side effects. This could involve extended observational periods in animal models, possibly up to several months post-treatment, and careful monitoring in early-phase clinical trials. Long-term studies should evaluate not only clinical outcomes but also potential immunogenicity, effects on normal physiological processes, and any cumulative effects of repeated administrations. Additionally, these studies should assess the impact of exosome therapies on disease progression and quality of life measures over extended periods.

3.3. Advanced Engineering Approaches

Building on studies like Xiao et al. (2022), future research should focus on developing more sophisticated exosome engineering techniques. This could include the creation of 'designer' exosomes with optimised cargo profiles, enhanced targeting capabilities, and improved stability. Techniques like CRISPR-Cas9 could be explored for precise modification of exosome-producing cells to alter exosome content or surface properties. Another avenue could be the development of hybrid exosomes that combine the beneficial properties of exosomes from different cell sources. Furthermore, exploring methods to load exosomes with specific therapeutic cargoes, such as small molecule drugs or nucleic acids, could expand their therapeutic potential.

3.4. Combination Therapy Exploration

Future studies should investigate the potential synergistic effects of combining exosome therapies with current MS treatments or other emerging therapies. For instance, combining exosomes with immunomodulatory drugs or remyelination-promoting agents could lead to broader treatment strategies. These combination approaches could target multiple aspects of MS pathology simultaneously, potentially leading to more effective disease management. Studies should explore optimal timing and dosing of combination therapies, as well as potential interactions between different treatment modalities.

3.5. Personalized Medicine Approaches

Variability in EV source and preparation supports investigation of patient and product characteristics in future MS studies. Potential biomarkers of treatment response, if validated prospectively, might help design studies of dose,

cargo, and delivery. No personalised EV regimen has yet been established as effective for patients with MS.

3.6. Biomarker Development

The potential of exosomes as biomarkers for MS progression and treatment response should be further explored. This could involve longitudinal studies correlating changes in circulating exosome profiles with disease progression and treatment outcomes. Advanced proteomics and transcriptomics approaches could be used to identify exosomal biomarkers indicative of disease activity, treatment response, or impending relapses. Developing non-invasive methods to monitor these biomarkers could greatly improve disease management and treatment decision-making in MS.

3.7. Scalable Production Methods

Significant effort should be directed towards developing scalable, GMP-compliant methods for exosome production. This might involve exploring bioreactor-based systems for large-scale cell culture or investigating cell-free methods of exosome synthesis. Innovations in bioprocessing technologies, such as microfluidic-based systems or continuous production methods, could help address the challenges of large-scale exosome production. Additionally, developing robust quality control measures and potency assays suitable for industrial-scale production will be key for the clinical translation of exosome therapies.

3.8. Mechanistic Studies

More detailed studies are needed to elucidate the full spectrum of exosome interactions with various cell types involved in MS pathology. This could involve advanced imaging techniques, single-cell analysis, and systems biology approaches to map the complex interactions between exosomes and the CNS microenvironment. In vitro models that better recapitulate the complexity of the CNS, such as 3D organoids or microfluidic "brain-on-a-chip" systems, could provide valuable insights into these mechanisms. Understanding these interactions at a molecular level could inform the development of more targeted and effective exosome therapies.

3.9. Novel Delivery Strategies

Building on the success of intranasal delivery demonstrated by Fathollahi et al. (2021), future research should explore other innovative delivery methods to enhance CNS targeting and reduce systemic exposure. This could include the development of novel formulations or devices for targeted exosome delivery. For example, exosomes could be encapsulated in nanoparticles designed to cross the blood-brain barrier more efficiently, or they could be combined with focused ultrasound techniques to enhance local delivery to specific brain regions. Exploring alternative routes of administration, such as intrathecal or intra-arterial delivery, could also provide new opportunities for targeted therapy.

3.10. Regulatory Pathway Development

Researchers and regulatory agencies should work together to establish clear guidelines for the development and approval of exosome-based therapies. This might involve creating new regulatory frameworks specifically tailored to the unique characteristics of exosome therapeutics. Key areas to address include defining product identity and purity standards, establishing appropriate potency assays, and developing guidelines for preclinical and clinical testing of exosome therapies. Collaborative efforts between academia, industry, and regulatory bodies will be essential in navigating the complex regulatory landscape and accelerating the clinical translation of exosome therapies.

3.11. Cross-Disease Applications

Given the potential broader neuroprotective effects of exosomes suggested by studies like Zhang et al. (2017) and Tang et al. (2022), future research should explore the applicability of exosome therapies to other neurological disorders that share common pathological mechanisms with MS. This could include neurodegenerative diseases like Alzheimer's and Parkinson's disease, as well as other autoimmune disorders of the CNS. Investigating the efficacy of exosome therapies across different neurological conditions could not only expand their therapeutic potential but also provide insights into shared pathological mechanisms and common therapeutic targets.

3.12. Advanced Animal Models

While the current animal models have provided valuable insights, there is a need for the development of more sophisticated models that better recapitulate the complex and heterogeneous nature of human MS. This could involve the creation of humanized mouse models or the increased use of non-human primate models for late-stage preclinical testing. Developing models that capture different aspects of MS pathology, including both the inflammatory and neurodegenerative components, would be particularly valuable. Additionally, exploring the use of patient-derived xenograft models could provide a more personalised approach to preclinical testing of exosome therapies.

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5.7. PRECLINICAL, HUMAN MECHANISTIC, AND BIOMARKER STUDIES OF EVs IN MS

Extracellular vesicles (EVs) participate in intercellular communication and have been investigated in MS biology and biomarker research. Many studies use the term "exosome," although an endosomal origin has not always been established for the isolated particles. Here, "EV" is used when vesicle biogenesis is uncertain, while the terminology of the cited studies is retained when identifying their reported materials. See Figure 5.7.1.

Additional human EV biomarker studies remain exploratory. An EV proteomic study of 80 patients identified profiles associated with treatment response (Torres Iglesias et al., 2024). A prospective pilot study found selected EV-miRNA differences between MS subtypes, but no significant modulation of the tested miRNAs after one year of siponimod in its small paired subgroup (Vrînceanu et al., 2026). Neither study establishes a validated clinical test or efficacy of administering EVs.

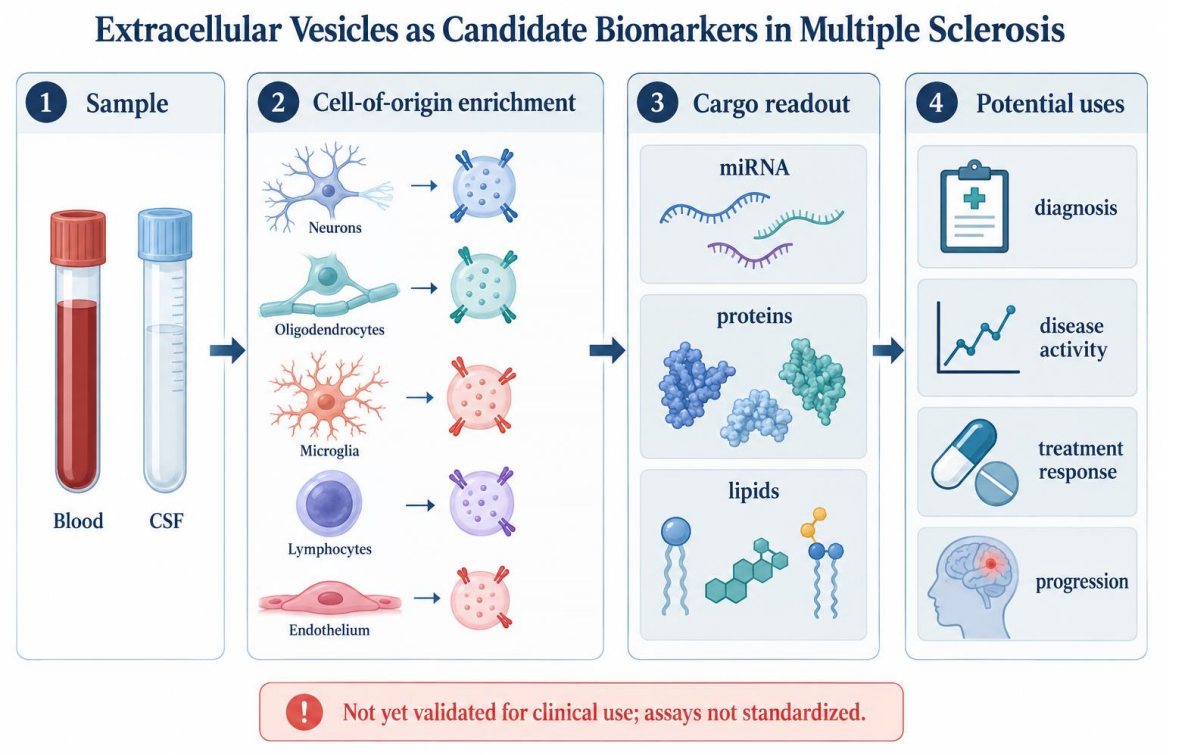


Figure 5.7.1. Extracellular vesicles as candidate biomarkers in multiple sclerosis, from sampling and cell-of-origin enrichment to cargo readout and potential uses. None is yet validated for clinical use.

The possible use of EVs as biomarkers or therapeutic delivery systems remains investigational in MS. Findings from cultured cells, patient-derived specimens, and animal models answer different questions and cannot establish clinical efficacy in patients. Evidence from those settings is therefore discussed separately from interventional human outcomes.

The eight studies below examine patient-derived material, experimental systems, or candidate biomarkers. An additional published treatment case is considered separately. Its uncontrolled design requires a different interpretation from the mechanistic and biomarker findings.

Preclinical and Human Mechanistic Evidence

Study 1: IFNγ-Stimulated Dendritic Cell Exosomes for Remyelination (Pusic et al., 2014)

Rationale:

Pusic et al. (2014) examined whether vesicles from IFNγ-stimulated dendritic cells could support myelin-related processes in preclinical systems.

Methodology:

Dendritic cells were derived from rat bone marrow, not from MS patients. Vesicle preparations were evaluated using brain-slice and animal experiments, including myelination and injury-related readouts.

Key Findings.

The preparation used was vesicles from stimulated dendritic cell cultures, which were characterised experimentally. The preparation promoted oligodendrocyte maturation and myelin-related signals in the experimental systems. The authors investigated miRNAs, including miR-219, associated with oligodendrocyte differentiation. In terms of evidence category, this was preclinical research, not therapeutic administration to patients.

Significance:

These results provide a rationale for further investigation. Clinical remyelination, dose selection, and patient safety remain separate questions.

Study 2: Circulating Exosomes and Regulatory T Cell Suppression (Kimura et al., 2018)

Rationale:

Kimura et al. (2018) examined how circulating vesicles might affect regulatory T-cell induction in MS.

Methodology:

Vesicles isolated from patient and healthy-control plasma were tested in T-cell cultures. The investigators examined let-7i and its relationship to IGF1R and TGFBR1 expression.

Key Findings.

Patient-derived preparations reduced regulatory T-cell induction in culture. Increased let-7i was linked to reduced expression of receptors involved in T-cell differentiation. Inhibition of let-7i counteracted the suppressive effect in culture. This was not evidence from therapeutic EV administration in an EAE trial.

Significance:

The findings concern an immunoregulatory mechanism. They neither validate a treatment-response biomarker nor demonstrate clinical benefit from EV therapy.

Study 3: Microparticles as Biomarkers in MS (Sáenz-Cuesta et al., 2014)

Rationale:

Sáenz-Cuesta et al. (2014) examined circulating microparticles as correlates of MS status and treatment exposure. Microparticles should not be equated automatically with exosomes.

Methodology:

Flow cytometry quantified platelet-, total leukocyte-, and monocyte-derived microparticles in blood samples.

Key Findings.

In untreated MS, Platelet-derived microparticle levels exceeded those in controls. Across clinical subtypes, RRMS showed the highest levels across the measured subtypes; SPMS levels were similar to controls. Treated groups showed increased microparticle counts. These associations do not establish a validated measure of treatment efficacy.

Significance:

The reported circulating microparticle differences may inform biomarker research in MS. Microparticle measurements cannot automatically be attributed specifically to exosomes, and prospective analytical and clinical validation would be required before using such markers to guide care.

Ex Vivo Human and Mechanistic Studies

Study 4: MSC-Derived Exosomes for Immunomodulation (Baharlooi et al., 2021)

Rationale:

Baharlooi et al. (2021) compared umbilical cord MSCs and their vesicles in PBMC proliferation assays. The experiments addressed cellular activity, not comparative patient safety.

Methodology:

Umbilical cord MSCs were cultured, and their exosomes were isolated using ultracentrifugation. The exosomes were tested for their ability to suppress the proliferation of activated peripheral blood mononuclear cells (PBMCs) from MS patients and healthy controls. Flow cytometry was used to assess PBMC proliferation, and suppression indices were calculated to quantify the immunosuppressive effect of MSC-derived exosomes.

Key Findings.

Both preparations suppressed activated PBMC proliferation; the vesicle preparation produced greater suppression under the tested conditions. Suppressive activity diminished with extensive sub-culturing. This does not establish a clinically optimal passage number. Low alloreactivity in the assay does not exclude immune reactions in patients.

Significance:

The study supports further product and mechanism research. It does not show that EV preparations eliminate immune rejection or long-term safety concerns.

Study 5: Serum-Derived Exosomes Expressing Myelin Proteins in MS (Galazka et al., 2018)

Rationale:

The presence of myelin antigens in the peripheral circulation has been hypothesized to contribute to autoimmune

processes in MS. Galazka et al. (2018) investigated whether exosomes isolated from the serum of MS patients contained CNS-derived myelin proteins and if these exosomes could be linked to disease activity. The study focused on the presence of myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) within serum-derived exosomes.

Methodology:

Serum-derived vesicles were isolated and characterised, and myelin-protein content was assessed. The study also examined cerebrospinal fluid preparations. These were specimen-based investigations, not treatment outcomes.

Key Findings.

MBP, PLP, and MOG were detected. The clearest disease-activity association concerned MOG, with higher levels during RRMS relapse. Exosomes carrying MOG were able to stimulate the proliferation of MOG-specific T cells in vitro, indicating that these exosomes might perpetuate autoimmune responses in MS. The study found a strong correlation between exosomal MOG levels and MS disease activity, particularly during relapse phases in RRMS patients.

Significance:

The findings support investigation of vesicle-associated myelin proteins as biomarkers. Detection and experimental immunogenicity do not establish a validated diagnostic test or a causal effect on clinical progression.

Study 6: MSC-Derived Exosomes Modulate Pro-Inflammatory Cytokines in RRMS (Baharlooi et al., 2022)

Rationale:

Pro-inflammatory cytokines play a critical role in the pathogenesis of MS, driving immune-mediated attacks on myelin and contributing to neurodegeneration. Baharlooi et al. (2022) aimed to investigate the effects of MSC-derived exosomes on the production of key pro-inflammatory cytokines in RRMS patients, particularly interferon-gamma (IFN-γ) and interleukin-17 (IL-17), which are known to exacerbate MS pathology.

Methodology:

Exosomes were isolated from MSCs and co-cultured with CD4+CD25− conventional T cells (Tconv) from RRMS patients and healthy controls. The production of cytokines IFN-γ, IL-17, and anti-inflammatory interleukin-10 (IL-10) was measured using flow cytometry.

Key Findings.

IL-17-producing cells decreased in both groups. The reported significant IFN-γ reduction concerned healthy-control cells and should not be generalized to the RRMS group. In addition to suppressing pro-inflammatory cytokines, MSC-derived exosomes increased the production of IL-10, an anti-inflammatory cytokine that helps regulate immune responses and promote tolerance. In the reported cell-culture conditions, the EV preparation showed stronger inhibition of selected inflammatory readouts than the comparator MSC preparation. This is an ex vivo comparison, not evidence of superior clinical efficacy.

Significance:

The study observed changes in cytokine production in patient-derived cells exposed to MSC-derived EV preparations. This ex vivo finding may inform mechanistic research but cannot establish that EV treatment restores immune balance, reduces disease activity, or outperforms MSC administration in people with MS. Comparative activity should be confined to the experimental conditions reported.

Study 7: Treg-Derived Exosomes and Immune Dysregulation in MS (Azimi et al., 2018)

Rationale:

Tregs are critical for maintaining immune tolerance, and their dysfunction is a hallmark of autoimmune diseases like MS. Exosomes derived from Tregs are thought to play a role in regulating immune responses by delivering suppressive signals to autoreactive immune cells. Azimi et al. (2018) explored whether exosomes derived from Tregs in MS patients retain their immunosuppressive function or if defects in these exosomes contribute to the immune dysregulation seen in MS.

Methodology:

Tregs and their exosomes were isolated from MS patients and healthy controls. The effects of these exosomes on the proliferation and apoptosis of conventional T cells (Tconv) were measured to assess their immunosuppressive capacity.

Key Findings.

Exosomes from MS patients' Tregs exhibited significantly reduced capacity to suppress Tconv proliferation and induce apoptosis compared to those from healthy controls. The diminished function of Treg-derived exosomes in MS patients suggests that these vesicles may be defective, contributing to the inadequate control of autoreactive T cells and the perpetuation of autoimmune responses. The study suggests that restoring the suppressive function of Treg-

derived exosomes could be a potential therapeutic strategy for MS, aimed at enhancing immune tolerance and preventing further autoimmune attacks.

Significance:

This study identified an experimental difference in the suppressive activity of Treg-derived extracellular vesicles from people with MS. The finding is mechanistic and hypothesis-generating; it does not establish that manipulating these vesicles will restore immune balance or produce clinical benefit. Future work should first establish reproducibility, molecular mechanisms, product characterisation, and safety before therapeutic efficacy is assessed in controlled clinical studies.

Study 8: EV-Derived miRNA Signatures for RRMS-SPMS Phenotype Differentiation (Wasilewska et al., 2026)

Rationale:

Wasilewska et al. examined whether circulating EV-derived miRNA profiles differ between stable relapsing-remitting MS and secondary progressive MS.

Methodology:

Plasma from 30 participants with stable RRMS, 30 with SPMS, and 30 healthy controls underwent EV isolation and characterisation, RNA sequencing and RT-qPCR, together with protein biomarker measurements and integrative modelling.

Key Findings.

Four EV-derived miRNAs differed between stable RRMS and SPMS. miR-760 showed the strongest individual discriminatory signal, and a combined miRNA-protein model achieved an AUC of 0.942 in the study cohort. The design was exploratory and requires independent validation before clinical use.

Significance:

The study adds human biomarker evidence to the EV literature but does not establish an EV-based diagnostic test or therapeutic effect (Wasilewska et al., 2026).

A separate category of evidence is the treatment report by Mohammad Jafari et al. (2025). One patient received intrathecal and intravenous UC-MSC-derived vesicles together with intravenous laser therapy. The authors reported short-term symptomatic improvement. A single uncontrolled observation, concomitant treatment, and limited follow-up prevent attribution of benefit to the vesicles or estimation of long-term safety. It establishes that a human administration report exists; it does not establish efficacy.

Evidence synthesis

A consistent reading across these studies is that the strongest signals cluster around immunomodulation and remyelination-related biology, but the reported magnitude of effect is inseparable from study design choices. Source-cell selection, route of administration, timing relative to disease stage, and endpoint definition all influence whether a study reads primarily as a proof-of-concept experiment, a mechanistic study, or a translational model. This is why direct side-by-side synthesis is more useful than sequential summary when judging the maturity of the evidence.

The eight studies provide mechanistic and biomarker observations involving EVs and related particles in MS. In vitro and ex vivo findings, together with observations in animal models, support further study of immune modulation and remyelination pathways. They do not establish the safety or efficacy of EV treatment in patients. Standardisation of particle identification and prospective clinical evaluation remain necessary.

Key Insights from the Studies

Experimental myelin effects and patient-sample myelin proteins answer different questions. Neither demonstrates restored myelin in treated MS patients. Kimura et al. (2018) and Baharlooi et al. (2021, 2022) investigated EV-associated immunological mechanisms using patient-derived samples and experimental systems. Changes in cytokines or regulatory T cell function in those settings are not controlled evidence that EV administration suppresses MS activity in patients. Sáenz-Cuesta et al. (2014) studied circulating microparticles and Galazka et al. (2018) reported serum EV-associated myelin proteins. These measurements may inform biomarker development, but analytic specificity, reproducibility, and patient-level predictive value need prospective validation.

Challenges and Future Directions

Therapeutic EV administration in MS remains investigational. The studies discussed above do not provide controlled evidence of patient benefit. Before a treatment claim can be made, product identity, dose, delivery, safety, and clinically meaningful outcomes require prospective evaluation.

Comparable EV studies require consistent isolation, characterisation, quantification, and functional assays. Therapeutic work also needs product-specific dose and safety data, followed by prospective assessment of clinical outcomes. Cargo engineering, combinations with DMTs, and

biomarker-guided selection are research strategies whose value must be tested directly.

Conclusion

These eight studies illustrate potential biomarker applications and experimental immunological or remyelination mechanisms. The reported cellular and animal-model effects are hypothesis-generating and should not be presented as demonstrated clinical treatment effects. Translation to MS care depends on reproducible EV characterisation, manufacturing standards, and prospective assessment of patient outcomes.

These observations remain insufficient for routine clinical use. Translation will require standardised isolation and characterisation, reproducible manufacturing, adequately controlled clinical studies, and long-term assessment of safety and efficacy. Until those requirements are met, the evidence should be described as preclinical or translational rather than therapeutic proof.

EV-based approaches remain an investigation platform for MS. Their use as delivery systems and disease biomarkers warrants further study, but clinical utility, long-term safety, and therapeutic efficacy have not been established by the studies summarized here. Any future role in routine care will depend on controlled clinical validation.

Work published in 2025 carried the EV biomarker question into larger clinical cohorts. Vesicle surface markers measured in blood separated people with relapsing-remitting MS from healthy controls, and particular vesicle characteristics varied with time since the last relapse (Lim Falk et al., 2025). In a separate cohort, microRNA carried by circulating vesicles was proposed as a predictor of response to disease-modifying therapy (Torres-Iglesias et al., 2025). Both are association studies and require external validation before clinical use. A broader 2025 review of extracellular vesicles in neurology likewise emphasized their potential as biomarkers and delivery platforms while identifying analytical standardisation, cell-of-origin attribution and clinical validation as continuing translational constraints (Manolopoulos et al., 2025).

Recent work broadens this biomarker evidence without establishing clinical utility. A 2024 study identified circulating CX3CR1+/UCHL1+ microglial EVs as a candidate blood marker in MS, and a 2025 study characterised L1CAM+ EV profiles during anti-CD20 treatment in relapsing-remitting MS (Duan et al., 2024; Anandan et al., 2025). Contemporary reviews also emphasize that EV nomenclature, separation, cell-of-origin assignment, and functional validation remain major constraints on interpretation (Manna et al., 2024). A 2026 review of exosome-mediated repair and immunoregulation summarizes preclinical evidence for immune modulation and remyelination but does not identify controlled therapeutic trials of exosome administration in MS; clinical efficacy therefore remains unproven (Sayed et al., 2026). A 2024 review that considered MSCs and MSC-derived exosomes together reached a similar translational conclusion: the biological rationale is substantial, but manufacturing, dosing, route, and controlled human evidence remain limiting factors (Hamidi et al., 2024).

Additional recent studies reinforce the distinction between biomarker development and therapeutic proof. Proteomic profiling of cell-of-origin-enriched circulating EVs identified different protein patterns in treatment responders and non-responders, but the findings came from an observational cohort and require independent validation (Torres Iglesias et al., 2024). A 2025 review of brain-derived blood biomarkers highlighted the potential of CNS-derived EVs to complement soluble markers such as NfL and GFAP, while emphasizing unresolved problems in isolation, cell-of-origin assignment and clinical standardisation (Anandan et al., 2025). A 2024 review of exosome biology in MS similarly summarized diagnostic and therapeutic hypotheses but found no basis for treating exosome administration as an established clinical therapy (Hasaniani et al., 2024).

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5.8. ADMINISTRATION ROUTES AND METHODS

Route selection changes both exposure and risk. Intravenous delivery exposes peripheral immune tissues, while uptake by systemic organs can limit CNS delivery. Intrathecal administration places a preparation in the CSF but adds procedural risks and does not guarantee penetration into brain or spinal-cord tissue. Intranasal delivery avoids an invasive procedure, although the delivered dose and relevance of rodent distribution data to humans remain uncertain. Figure 5.8.1 compares these considerations.

An experimental formulation incorporates teriflunomide-loaded ginger-derived vesicles into a thermoresponsive nasal gel (Bhom et al., 2026). Its in vitro characterisation and release studies concern a plant-derived drug-delivery platform, which is distinct from an MSC-EV product. They do not demonstrate clinical nose-to-brain delivery, remyelination or efficacy in people with MS.

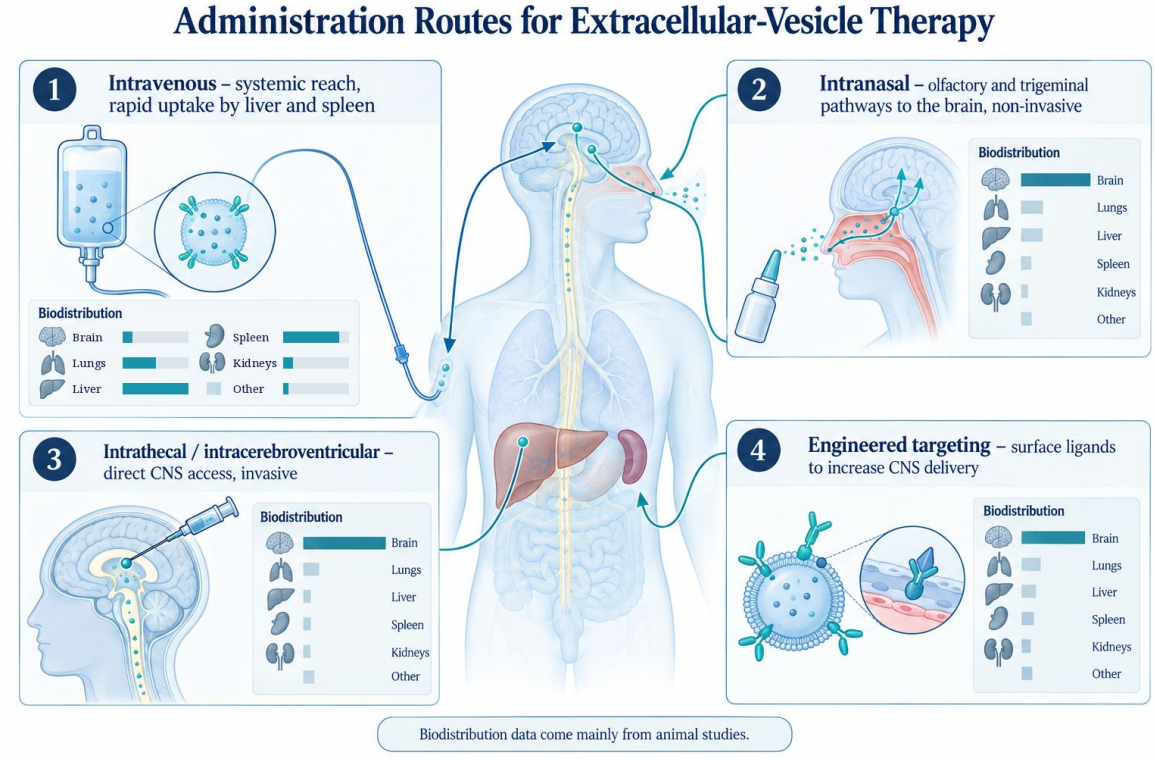


Figure 5.8.1. Administration routes for extracellular-vesicle therapy and their schematic biodistribution. Biodistribution data derive mainly from animal studies.

Localized delivery, whether by hydrogel depot or direct application, offers the highest local concentration and the lowest systemic exposure, and it is the only approach in which sustained release is realistic; it is also the least applicable to a disease whose lesions are multifocal and unpredictable in location. No study has compared these routes head to head using the same vesicle preparation, the same dose and the same endpoint, which means that apparent differences in efficacy between routes in the published literature are confounded by differences in product and model. For clinical translation the practical consequence is that route selection should follow the therapeutic target rather than convenience: peripheral immunomodulation favours the intravenous route, compartmentalised CNS inflammation favours the intrathecal route, and repair-oriented strategies aimed at diffuse white matter favour routes with demonstrated CNS deposition. Each of these choices remains a hypothesis awaiting direct comparison.

The routes below are compared by biodistribution, CNS exposure, procedural risk, and manufacturing requirements. Evidence from cell administration is kept separate from evidence concerning EV preparations.

1. Intravenous Delivery

1.1. Introduction

Intravenous delivery is practical and exposes the peripheral immune compartment. The main questions concern tissue

distribution, effective dose, and uptake by intended target cells.

Intravenous EV administration is an investigational delivery approach in MS. The prospect of modulating more than one disease pathway is a mechanistic rationale, not evidence that the treatment improves clinical outcomes. Dose, product identity, biodistribution, and long-term safety require direct study before clinical benefit can be assessed.

1.2. Rationale for Intravenous Administration of Exosomes in MS

1.2.1. Systemic Distribution and Holistic Approach

Intravenous administration of exosomes offers the advantage of systemic distribution, which is particularly relevant in the context of MS. While MS is primarily considered a CNS disorder, mounting evidence suggests that it also involves significant peripheral immune dysregulation. By delivering exosomes intravenously, we can potentially target both the central and peripheral aspects of MS pathology simultaneously.

The systemic approach afforded by IV administration aligns well with the complex pathophysiology of MS. For instance, recent studies have shown that peripheral immune cells, such as autoreactive T cells and B cells, are key determinants in initiating and perpetuating the inflammatory cascade in MS. Intravenously administered exosomes have the potential to modulate these peripheral immune responses, potentially interrupting the pathogenic cycle at its source.

Moreover, the systemic distribution of exosomes following IV administration may help address the widespread nature of MS pathology. MS lesions can occur throughout the CNS, affecting various regions of the brain and spinal cord. The ability of IV-administered exosomes to reach multiple sites within the CNS could provide a more thorough therapeutic approach compared to localised treatments.

1.2.2. Blood-Brain Barrier Penetration

One of the most significant challenges in developing effective treatments for CNS disorders, including MS, is the blood-brain barrier (BBB). The BBB is a highly selective semipermeable barrier formed by endothelial cells that tightly regulate molecular trafficking between the peripheral circulation and the CNS. While this barrier serves to protect the brain from potentially harmful substances, it also restricts the entry of many therapeutic agents, limiting their efficacy in treating neurological conditions like MS (Ortiz et al., 2014). The BBB not only excludes large molecules but also many small ones, making the delivery of drugs to the CNS a major challenge (Zhao et al., 2015). Interest in exosomes as carriers rests largely on the observation that vesicles of endosomal origin cross this barrier more readily than synthetic particles of comparable size, although the mechanisms, their efficiency and their consistency between preparations are still being worked out (Sun et al., 2025).

In MS, the BBB is often compromised during episodes of active inflammation. This breakdown of the BBB facilitates the infiltration of autoreactive immune cells into the CNS, contributing to the formation of MS lesions and disease progression (Ortiz et al., 2014). Recent studies have highlighted the role of endothelial dysfunction in promoting this BBB disruption, which in turn exacerbates neuroinflammation in MS (Zhao et al., 2015). Despite this, delivering therapeutic agents to the CNS remains challenging due to the dynamic nature of BBB permeability.

Extracellular vesicles are being investigated as carriers for CNS-directed cargo because some EV populations cross or interact with the blood-brain barrier in experimental systems. This property does not by itself establish efficient or selective drug delivery in patients (Matsumoto et al., 2017). Their small size and endogenous origin further enhance their ability to penetrate the BBB, even under inflammatory conditions such as those found in MS (Matsumoto et al., 2017). The exact mechanism by which exosomes cross the BBB is still not fully understood, but several pathways have been proposed. One hypothesis suggests that exosomes utilize transcytosis, a process by which they are taken up by endothelial cells at the BBB and then transported across the barrier into the CNS (Matsumoto et al., 2017). Another theory posits that exosomes may exploit receptor-mediated transport systems to cross the BBB, which would allow them to deliver targeted therapies to specific CNS regions (Matsumoto et al., 2017).

Sutaria et al. (2017) reviewed EV-mediated nucleic-acid delivery and possible applications in CNS disease. BBB permeability, tissue distribution, and target-cell uptake vary with the preparation and experimental setting. Endogenous origin and small size do not establish superior BBB penetration or clinical delivery efficiency.

The ability of exosomes to cross the BBB following intravenous (IV) administration has been demonstrated in several studies. For instance, Alvarez-Erviti et al. (2011)

engineered exosomes expressing a neuron-specific targeting peptide that successfully delivered siRNA to the brain following IV injection. This led to widespread gene knockdown in neurons, demonstrating that exosomes can serve as efficient delivery vehicles for therapeutic agents to treat neurodegenerative conditions (Alvarez-Erviti et al., 2011). Such findings hold significant implications for MS treatment, as IV-administered exosomes could deliver therapeutic agents directly to sites of neuroinflammation and degeneration within the CNS (Osorio-Querejeta et al., 2018). Moreover, recent research has indicated that exosomes may enhance therapeutic outcomes in MS by crossing the compromised BBB more effectively during active inflammation. In MS patients, the increased permeability of the BBB could allow exosomes to penetrate more efficiently into the CNS and deliver therapeutic cargo to affected areas (Osorio-Querejeta et al., 2018). This ability to cross the BBB under both physiological and pathological conditions positions exosomes as a candidate tool in the development of novel treatments for MS and other CNS disorders (Banks et al., 2020). Furthermore, studies have highlighted that extracellular vesicles, including exosomes, can facilitate targeting and crossing of the BBB, offering an investigational strategy for delivering therapeutic agents to the CNS (Saint-Pol et al., 2020).

1.2.3. Non-invasive and Repeatable Administration

Intravenous administration of exosomes offers a significant advantage in terms of its non-invasive nature and suitability for repeated dosing. This is particularly important for chronic conditions like MS that may require long-term treatment. Compared to more invasive routes such as intrathecal administration, which involves direct injection into the cerebrospinal fluid, IV administration is associated with lower risks of complications and is generally better tolerated by patients.

The ability to administer exosome treatments repeatedly without significant risk or discomfort to the patient is central for maintaining therapeutic efficacy over time. This is especially relevant in the context of MS, where the disease course can be unpredictable and may require ongoing management. The ease of IV administration could improve patient compliance and allow for more flexible treatment regimens tailored to individual patient needs.

Moreover, the repeatability of IV exosome administration opens up possibilities for developing pulsed or maintenance therapy protocols. Such approaches could be particularly beneficial in managing relapsing-remitting forms of MS, where regular treatments might help prevent or reduce the frequency and severity of relapses.

1.2.4. Modulation of Peripheral Immune Responses

One of the key advantages of intravenous exosome administration in MS treatment is its potential to modulate peripheral immune responses. MS is characterised by dysregulation of the immune system, with autoreactive T cells and B cells playing central roles in disease pathogenesis. IV-administered exosomes have the unique ability to interact with immune cells in the periphery, potentially modulating their behaviour and mitigating the inflammatory cascade that contributes to MS progression.

Research has shown that exosomes, particularly those derived from mesenchymal stem cells (MSCs), possess potent immunomodulatory properties. They can influence various aspects of immune cell function, including proliferation, differentiation, and cytokine production. For instance, MSC-derived exosomes have been shown to promote the expansion of regulatory T cells (Tregs), which is closely involved in maintaining immune tolerance and suppressing autoimmune responses.

Furthermore, exosomes can carry and transfer specific microRNAs (miRNAs) that regulate gene expression in recipient cells. Some of these miRNAs have been implicated in immune regulation and could be harnessed to modulate the immune response in MS. Whether such signals, given intravenously, can shift the immune repertoire of a patient deeply or durably enough to matter is unknown. An immune reset remains an aspiration here, with no human measurement behind it.

The ability of IV-administered exosomes to modulate peripheral immune responses could also have implications for managing MS-related fatigue and other systemic symptoms. Recent research has suggested that peripheral inflammation contributes to fatigue in MS patients. By modulating systemic inflammatory responses, IV exosome therapy could alleviate these debilitating symptoms, improving overall quality of life for MS patients (Patejdl et al., 2016).

The important role of peripheral immune cells, particularly T cells and B cells, in the pathogenesis of MS is well-documented by Ghasemi, Razavi, and Nikzad (2017). IV-administered exosomes have the potential to target these autoreactive immune cells, thereby mitigating the inflammatory cascade that contributes to MS progression.

1.2.5. Potential for Combination Therapy

Intravenous administration of exosomes presents additional avenues for combination therapy in MS treatment. The ease of IV administration makes it relatively straightforward to integrate exosome therapy with existing MS treatments, potentially enhancing their efficacy or addressing multiple aspects of the disease simultaneously.

For instance, exosomes could be used in conjunction with disease-modifying therapies (DMTs) that are the current mainstay of MS treatment. While DMTs primarily focus on reducing inflammation and preventing relapses, exosomes could complement these effects by promoting neuroprotection and remyelination. This multi-pronged approach could lead to broader disease management and improved patient outcomes.

Moreover, the flexibility of IV administration allows for precise timing of exosome delivery in relation to other treatments. This could be particularly beneficial in developing synergistic treatment protocols that maximize therapeutic efficacy while minimising side effects. For example, exosomes could be administered at specific intervals following DMT infusions to capitalize on the immunomodulatory effects of both treatments.

The potential for combination therapy extends beyond just pharmaceutical interventions. IV exosome administration could also be integrated with non-pharmacological approaches such as physical therapy or cognitive rehabilitation. By promoting neural repair and plasticity, exosomes could enhance the benefits of these interventions, leading to improved functional outcomes for MS patients.

1.3. Evidence from Preclinical Studies

1.3.1. Mesenchymal Stem Cell-Derived Exosomes

Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have been studied extensively in experimental autoimmune encephalomyelitis (EAE), but the evidence remains preclinical. Li et al. (2019) reported that intravenously administered vesicles derived from bone-marrow MSCs reduced inflammatory readouts and demyelination in EAE rats. The treated animals showed lower spinal-cord concentrations of pro-inflammatory mediators including TNF-α, IL-1β, and IL-17, together with increased expression of myelin basic protein. These findings support investigation of immunomodulatory and remyelination-related mechanisms rather than a clinical efficacy claim. A 2024 systematic review and meta-analysis of stem-cell-derived EV and conditioned-medium studies in animal models of MS likewise found overall reductions in disease severity and histological measures of inflammation or demyelination, while identifying substantial methodological heterogeneity and major barriers to translation (Barabadi et al., 2024). The preclinical literature therefore does not yet establish an effective human dose, route, manufacturing specification, or long-term safety profile.

Building on these results, Riazifar et al. (2019) conducted a detailed study examining the immunomodulatory effects of IV-administered MSC-Exos in the EAE model. They found that MSC-Exos treatment led to a significant reduction in the number of infiltrating CD4+ T cells in the CNS, along with a decrease in the production of pro-inflammatory cytokines. Interestingly, they also observed an increase in the population of regulatory T cells (Tregs), suggesting that MSC-Exos can promote immune tolerance.

Extracellular vesicles, including vesicles derived from stem cells, have produced immunomodulatory and tissue-repair signals in experimental neurodegenerative models, including MS-related systems (Riazifar et al., 2017). These observations support study of both central and peripheral mechanisms but do not by themselves demonstrate therapeutic benefit in patients (Wiklander et al., 2015). Reported neuroprotective effects in preclinical models include changes in inflammatory signalling, remyelination-related pathways, and neuronal survival, effects that have been attributed in part to the molecular cargo carried by the vesicles (Yuan et al., 2017).

These studies collectively demonstrate the breadth of proposed biological effects of IV-administered MSC-Exos in MS, highlighting their ability to modulate both central and peripheral aspects of the disease pathology.

1.3.2. Long-term Effects on Chronic Models

While acute models of MS provide valuable insights, it is equally important to assess extracellular vesicle therapy in models that reflect the progressive course of MS. Laso-García et al. (2018) addressed this question in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease, a progressive model of MS, rather than in chronic EAE.

The investigators administered extracellular vesicles derived from human adipose-tissue MSCs intravenously to SJL/J mice once the disease was established. Compared with controls, treated mice showed improved motor deficits, reduced brain atrophy, increased cell proliferation in

the subventricular zone, and fewer inflammatory infiltrates in the spinal cord.

These results come from a single preclinical study in one virus-induced model. They do not establish that effects persist after treatment is stopped, and they cannot be extrapolated directly to people with progressive MS.

With regard to mechanism, vesicle treatment reduced glial fibrillary acidic protein and Iba-1 staining in the brain and increased myelin protein expression, and changes in spinal cord microglial morphology suggested modulation of microglial activation (Laso-García et al., 2018). These findings are consistent with an anti-inflammatory and possibly pro-myelinating effect in this model, but they do not demonstrate remyelination or oligodendrocyte protection in patients.

1.3.3. Engineered Exosomes for Enhanced Efficacy

The field of exosome research has rapidly evolved to include engineered exosomes designed for enhanced therapeutic efficacy. A pioneering study in this area was conducted by Zhuang et al. (2011), who demonstrated the potential of using exosomes as delivery vehicles for therapeutic compounds in neuroinflammatory conditions.

Zhuang and colleagues engineered exosomes to carry curcumin, a natural anti-inflammatory compound. When administered intravenously to mice with EAE, these curcumin-loaded exosomes effectively suppressed brain inflammation and reduce disease severity. The researchers observed a significant reduction in the number of inflammatory lesions in the brain and spinal cord of treated animals, along with improved clinical scores.

This study highlighted the potential of using exosomes as natural nanocarriers for delivering therapeutic agents across the BBB. The ability to load exosomes with specific compounds opens up new possibilities for enhancing the efficacy of exosome therapy in MS treatment.

More recently, Chen et al. (2024) reported on the development of engineered exosomes enriched with select microRNAs (miRNAs) for the treatment of neurological injuries. While their study focused on traumatic brain injury and stroke models, the findings have significant implications for MS research. The researchers found that IV administration of these miRNA-enriched exosomes resulted in enhanced neuroprotection and improved functional recovery compared to unmodified exosomes.

The work of Chen et al. (2024) demonstrates the potential for tailoring exosome cargo to target specific pathways involved in neuroinflammation and neurodegeneration. This approach could be particularly valuable in MS treatment, where multiple pathological processes contribute to disease progression. By engineering exosomes to carry specific miRNAs or other therapeutic molecules, it may be possible to develop more targeted and effective treatments for different aspects of MS pathology.

1.3.4. Exosome-mediated Delivery of Therapeutic Proteins

A further line of exosome research is the use of these vesicles for delivering therapeutic proteins to the CNS. A notable study in this area was conducted by Haney et al. (2015), who investigated the potential of macrophage-derived exosomes to deliver catalase, a potent antioxidant enzyme, across the BBB in a Parkinson's disease model. Aryani and Denecke (2014) emphasize that exosomes can cross the BBB and transport therapeutic proteins, making them ideal for treating neurodegenerative diseases such as Parkinson's and MS.

Yuan and colleagues (2017) showed that macrophage-derived exosomes can act as natural nanocarriers for protein delivery to the inflamed brain after intravenous administration, reducing oxidative stress and neuroinflammation in treated animals. The study demonstrated the ability of IV-administered exosomes to deliver functional proteins across the BBB, a finding with significant implications for MS treatment.

Building on this concept, Haney et al. (2015) used exosomes to deliver the antioxidant enzyme catalase to the brain in a model of Parkinson's disease, illustrating vesicle-mediated protein delivery in neuroinflammatory conditions. They engineered macrophage-derived exosomes to carry a fusion protein consisting of the antioxidant enzyme catalase and a brain-targeting peptide. When administered intravenously in a mouse model of neuroinflammation, these engineered exosomes effectively delivered the therapeutic protein to the brain, resulting in significant neuroprotection and reduction of inflammatory markers.

The implications of these findings for MS treatment are profound. Oxidative stress and neuroinflammation are key components of MS pathology, contributing to both demyelination and axonal damage. The ability to deliver antioxidant enzymes or other neuroprotective proteins directly to the CNS using IV-administered exosomes could provide

a powerful new tool in the fight against MS-related neurodegeneration.

Moreover, the flexibility of exosome engineering allows for the potential delivery of a wide range of therapeutic proteins. For instance, growth factors such as brain-derived neurotrophic factor (BDNF) or insulin-like growth factor 1 (IGF-1), which have shown promise in promoting remyelination and neuroprotection in MS models, could be delivered via exosomes. This approach could overcome the limitations of direct protein administration, such as short half-life and poor BBB penetration, potentially enhancing the efficacy of these therapeutic strategies.

1.3.5. Exosome-mediated Immunomodulation

While the ability of exosomes to deliver therapeutic cargo is impressive, their inherent immunomodulatory properties also matter for their therapeutic potential for MS. A notable study by Casella et al. (2018) demonstrated the immunomodulatory effects of extracellular vesicles engineered to contain IL-4 in the EAE model. Casella and colleagues found that these IL-4-containing vesicles, injected into the cisterna magna, could effectively suppress ongoing EAE. The treatment resulted in a significant reduction in the number of inflammatory infiltrates in the CNS, along with a decrease in the production of pro-inflammatory cytokines. Importantly, they observed an increase in the population of regulatory T cells (Tregs) and a shift towards an anti-inflammatory M2 phenotype in macrophages.

Exosomes may therefore reprogram the immune response in MS, promoting a more tolerogenic environment. The ability to modulate both adaptive and innate immune responses through IV exosome administration could provide a broader approach to managing MS-related inflammation compared to current therapies that often target specific immune cell subsets or pathways.

Hu et al. (2024) reviewed mechanisms through which MSCs can influence Th17/Treg balance. This mechanistic review does not provide a pooled estimate of repeated EV administration in EAE or establish durable clinical benefit in progressive MS.

In addition, Dendrou, Fugger, and Friese (2015) discuss in detail the role of immune cells, particularly T cells and B cells, in MS immunopathogenesis. The authors highlight how inflammation in MS is driven by dysregulated immune cells targeting the CNS, which results in neuroinflammation and demyelination. This account suggests where exosomes might act therapeutically that can modulate both peripheral and central immune responses by suppressing pathogenic immune cells, promoting a more regulatory immune environment.

The immunomodulatory potential of exosomes in neuroinflammatory disorders is further supported by the work of Gupta and Pulliam (2014). In their review, they highlight that exosomes can significantly contribute to the development of several neuropathologies by packaging and spreading toxic forms of aggregated proteins, such as those responsible for Parkinson's disease, Alzheimer's disease, and Creutzfeldt-Jakob disease. Moreover, they note that exosomes secreted by resident brain cells in response to pathogenic stimuli can influence bystander cells through the transfer of dysregulated miRNAs, suppressing essential gene expression in recipient cells. This points to the dual nature of exosomes in neuroinflammation – while they can contribute to pathogenesis, their ability to modulate immune responses also presents opportunities for therapeutic interventions in conditions like MS.

1.4. Pharmacokinetics and Biodistribution

Peripheral targeting may be relevant even when CNS entry is limited. Turano et al. (2024) tracked labelled adipose-MSC EVs after intravenous administration in EAE mice. EV-associated label was detected in draining lymph nodes, with preferential uptake by macrophages and additional uptake by dendritic cells and CD4+ T cells. Distribution was assessed at a specified time point using a labelled preparation, so these findings should not be treated as a universal human biodistribution profile.

1.4.1. Circulation Time and Clearance

The circulation time of IV-administered exosomes is a critical factor in determining their therapeutic window and potential for reaching target tissues. Takahashi et al. (2013) visualized and tracked exosomes from murine melanoma cells in mice after intravenous injection, using a luciferase-based reporter. They found that the exosomes were cleared from the circulation very rapidly, with a blood half-life of roughly 2 minutes and only minimal signal remaining 4 hours after injection.

This relatively short circulation time presents both challenges and opportunities for MS treatment. On one hand, it suggests that frequent dosing may be necessary to maintain therapeutic levels of exosomes in the circulation. On the other hand, rapid clearance could reduce the risk of systemic side effects associated with long-term persistence of exosomes in the bloodstream.

More recently, Charoenviriyakul et al. (2017) compared exosomes from different mouse cell lines and showed that yield, physicochemical properties, and pharmacokinetics differ by cell type. They found that surface modification of exosomes, such as PEGylation, could significantly extend their circulation time. This approach could be applied to enhance the therapeutic efficacy of exosomes in MS treatment by prolonging their availability for uptake by target tissues.

1.4.2. Tissue Distribution

The tissue distribution of IV-administered exosomes is a critical consideration for MS treatment, as it determines the potential for both on-target and off-target effects. Wiklander et al. (2015) used bioluminescence imaging to track the biodistribution of IV-administered exosomes in mice. They observed rapid accumulation in the liver and spleen within 30 minutes of injection, with lower levels detected in other organs including the brain.

While the accumulation of exosomes in filtering organs like the liver and spleen could limit their availability for CNS targeting, it's important to note that MS is not solely a CNS disorder. The modulation of peripheral immune responses in these organs could also contribute to the therapeutic effects of exosomes in MS.

Interestingly, Kodali et al. (2019) recently reported on the development of a novel exosome formulation with enhanced brain targeting capabilities. By modifying the surface of exosomes with a brain-penetrating peptide, they achieved significantly higher brain accumulation following IV administration compared to unmodified exosomes. This approach could enhance the CNS-specific effects of exosome therapy in MS.

1.4.3. CNS Penetration

The ability of IV-administered exosomes to cross the BBB and penetrate the CNS is particularly relevant for MS treatment. While early studies demonstrated the potential for exosomes to cross the BBB, recent research has provided more detailed insights into this process.

Banks et al. (2020) conducted a wide-ranging study on the BBB penetration of IV-administered exosomes under various conditions. They found that inflammation, a key feature of MS pathology, enhanced the ability of exosomes to cross the BBB. This finding suggests that the BBB disruption often observed in active MS lesions could facilitate the entry of therapeutic exosomes into the CNS.

1.5. Challenges and Considerations

1.5.1. Optimizing CNS Targeting

Despite the ability of exosomes to cross the BBB, a significant portion of IV-administered exosomes still accumulates in peripheral organs. Enhancing CNS targeting remains a key challenge in optimising exosome therapy for MS. This issue has been a focal point in many recent studies. For example, research by Osorio-Querejeta et al. (2018) highlighted the importance of targeted delivery to achieve effective therapeutic outcomes in demyelinating diseases, emphasizing the natural capacity of exosomes to interact with CNS cells. Similarly, Jia et al. (2018) developed exosomes decorated with a neuropilin-1-targeting peptide and loaded with cargo for simultaneous imaging and therapy of glioma. These modified exosomes showed significantly higher brain accumulation compared to unmodified ones, offering an investigational strategy to enhance CNS-specific effects while reducing off-target effects in peripheral organs (Osorio-Querejeta et al., 2018).

1.5.2. Dosing and Treatment Regimens

Establishing optimal dosing regimens for exosome therapy in multiple sclerosis (MS) remains a significant challenge, requiring careful consideration of exosome concentration, administration frequency, and treatment duration. A thorough review of 64 preclinical studies by Gupta and colleagues (2021) revealed substantial variability in dosing strategies, with repeated administrations (weekly or biweekly) consistently outperforming single-dose regimens in terms of therapeutic efficacy, including reduced inflammation and enhanced remyelination.

For example, in neuroinflammation models, biweekly injections of mesenchymal stem cell–derived extracellular vesicles (MSC-EVs) over four weeks produced significantly greater clinical improvements and histological recovery compared to single administration, highlighting the advantages of sustained dosing.

The implication is that moderate, repeated exosome dosing schedules, such as weekly or biweekly treatments over several weeks, are more effective than single high-dose treatments, and may offer durable immunomodulatory benefits particularly relevant for chronic or progressive MS.

Furthermore, Osorio-Querejeta et al. (2018) pointed out that repeated and well-timed dosing could be critical to maximize both the therapeutic effects and safety of exosome-based therapies in chronic diseases like MS.

1.5.3. Standardization and Quality Control

Ensuring consistent production and characterisation of therapeutic exosomes is important for clinical translation. The heterogeneity of exosome populations and variability in isolation methods are significant hurdles to overcome. Recent efforts, such as the guidelines established by Théry et al. (2018), aim to provide a standard framework for studying extracellular vesicles. These protocols address the need for uniformity in exosome isolation and characterisation, which is essential for developing reliable exosome-based therapies (Osorio-Querejeta et al., 2018).

1.5.4. Immune Responses and Long-term Safety

While exosomes generally exhibit low immunogenicity, the potential for adverse immune reactions, particularly with repeated dosing, is an area that requires further investigation. Zhu et al. (2017) conducted comprehensive toxicity and immunogenicity studies and reported minimal effects in mice after sustained dosing of extracellular vesicles. Their findings suggested minimal adverse immune responses, though subtle changes in the immune profile were observed over time. As noted by Osorio-Querejeta et al. (2018), these long-term studies are key to fully understanding the immunological consequences of exosome therapy, especially in the context of chronic administration for MS patients.

1.6. Future Directions and Emerging Approaches

1.6.1. Engineered Exosomes with Enhanced Therapeutic Properties

The development of exosomes with enhanced therapeutic properties through genetic engineering or cargo loading is an active area of research. For example, Kojima et al. (2018) reported genetically encoded devices that boost exosome production and load therapeutic cargo, which implanted cells used to deliver that cargo within the brain. Whether such engineered vesicles can be directed against autoreactive, MOG-specific T cells in EAE has not yet been shown.

This approach of engineering exosomes to target specific pathogenic cell populations or molecular pathways has investigational potential for developing more targeted and effective MS therapies.

1.6.2. Combination Therapies and Synergistic Approaches

The exploration of the synergistic effects of intravenous (IV) exosome administration combined with other treatments for multiple sclerosis (MS) is a burgeoning area of research. Exosomes, which are nanoscale extracellular vesicles secreted by various cell types, have garnered attention for their potential therapeutic applications due to their ability to facilitate intercellular communication and modulate immune responses. In the context of MS, a disease characterised by neuroinflammation and demyelination, the combination of exosome therapy with established treatments may enhance therapeutic efficacy and improve patient outcomes.

Exosomes derived from mesenchymal stem cells (MSCs) have been shown to possess significant anti-inflammatory properties, which can be particularly beneficial in the treatment of MS. For instance, studies have demonstrated that MSC-derived exosomes can suppress the proliferation of activated peripheral blood mononuclear cells (PBMCs) in MS, indicating their potential to modulate immune responses in this autoimmune disorder (Baharlooi et al., 2020). Furthermore, exosomes can deliver bioactive molecules, including microRNAs and proteins, that can influence the behaviour of target cells, thereby promoting neuroprotection and remyelination (Komlakh et al., 2022; Pusic et al., 2014). The ability of exosomes to cross the blood-brain barrier (BBB) enhances their therapeutic potential, making them suitable candidates for treating central nervous system (CNS) diseases like MS (Fan et al., 2022).

Combining IV exosome administration with established MS treatments, such as monoclonal antibodies or disease-modifying therapies (DMTs), could yield synergistic effects. For example, alemtuzumab, an anti-CD52 monoclonal antibody, has shown substantial efficacy in treating relapsing forms of MS (Perumal et al., 2012). The immunomodulatory effects of alemtuzumab could be complemented by the anti-inflammatory actions of MSC-derived exosomes, potentially leading to improved clinical outcomes. This combination approach may help mitigate the adverse effects associated with high-dose monoclonal antibody therapies, such as secondary autoimmune disorders (Perumal et al., 2012).

Moreover, the use of exosomes in conjunction with other immunomodulatory agents, such as fingolimod, could enhance therapeutic efficacy. Fingolimod, an oral DMT, acts by sequestering lymphocytes in lymph nodes, thereby reducing their migration to the CNS (Portaccio, 2011); in experimental autoimmune encephalomyelitis, its efficacy also depends on modulation of astrocyte sphingosine 1-phosphate receptor 1 (Choi et al., 2011). The addition of

exosomes could, in principle, further modulate the immune response by delivering anti-inflammatory signals directly to the CNS; this combination has not yet been tested in clinical studies.

The timing and route of administration of exosomes are critical factors that can influence their therapeutic efficacy. Research indicates that the route of administration (IV versus intraperitoneal) can lead to different biodistributions of exosomes, affecting their therapeutic outcomes (Mycko & Baranzini, 2020). Therefore, optimising the administration protocol for exosomes in conjunction with other MS treatments is essential for maximizing their synergistic effects.

In addition to their immunomodulatory properties, exosomes can also play a role in neuroprotection and remyelination. Studies have shown that exosomes can promote the differentiation of oligodendrocyte precursor cells into mature oligodendrocytes, which are essential for myelination in the CNS (Pusic et al., 2014). This property could be particularly beneficial when combined with therapies aimed at enhancing remyelination, such as clemastine, which has been shown to promote white matter integrity in MS models. The combination of exosomes with such therapies may lead to enhanced remyelination and functional recovery in MS patients. Clinical interpretation should also consider the later disability-worsening signal in a small advanced-progressive-MS cohort; clemastine is not an established MS repair therapy (Kocot et al., 2025).

Furthermore, the potential for exosomes to serve as delivery vehicles for therapeutic agents adds another layer of complexity to their use in MS treatment. Exosomes can encapsulate various therapeutic molecules, including small interfering RNAs (siRNAs) and anti-inflammatory cytokines, allowing for targeted delivery to the CNS (Fan et al., 2022). This capability could be harnessed to enhance the effectiveness of existing DMTs by co-delivering exosomes with these agents, thereby improving their bioavailability and therapeutic outcomes.

The safety profile of exosome therapy remains uncertain. Findings from preclinical studies and clinical extracellular-vesicle studies in other diseases cannot be assumed to apply to MS. When exosome administration is studied with other treatments, adverse events, product interactions, and immunological effects require prospective monitoring.

Combining intravenous exosome administration with established MS treatments is a hypothesis for future research rather than an established therapeutic strategy. Controlled studies would need to define dosing, interactions, safety, and efficacy before any clinical role could be assessed.

1.6.3. Novel Imaging Techniques for Tracking Exosome Biodistribution

Advancing imaging technologies to track the biodistribution and target engagement of intravenously administered exosomes could provide insights for optimising treatment protocols. Busato et al. (2016) demonstrated SPION-labeled exosomes for non-invasive tracking with magnetic resonance imaging. This is a technical proof of concept; whether it can monitor exosome biodistribution or guide dosing in people with MS remains to be established.

1.7. Conclusion

Intravenous exosome administration remains investigational in MS. Experimental studies suggest that exosomes can carry immunomodulatory or repair-related cargo, but their ability to improve clinical outcomes in patients has not been established.

Significant questions remain open in translating these findings to clinical applications. Product characterisation, CNS targeting, standardised manufacturing, dose selection, and regulatory oversight require further work.

Advancing the field will require controlled clinical trials and long-term safety follow-up before any role for intravenous exosome therapy in routine MS care can be assessed.

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2. Intrathecal Delivery

2.1. Introduction

Intrathecal delivery places the preparation in the CSF, bypassing the vascular barrier at the injection site. Distribution within CNS tissue, clearance, and procedure-related harm still require evaluation.

In the context of MS treatment, the intrathecal route of administration offers a unique advantage by bypassing the blood-brain barrier (BBB), allowing for precise and targeted delivery of therapeutic agents directly to the CNS. This approach has the potential to advance MS treatment by addressing one of the most significant challenges in CNS drug delivery: achieving therapeutic concentrations of drugs within the brain and spinal cord while minimising systemic exposure and associated side effects.

The intrathecal route has been explored for various therapeutic agents in MS and related conditions, with early findings. For instance, studies have shown that intrathecal administration of baclofen can effectively reduce spasticity in MS patients, significantly improving their comfort and quality of life. Similarly, intrathecal delivery of triamcinolone acetonide has demonstrated benefits in progressive MS patients, particularly those with predominant spinal symptoms, without contributing to neuronal atrophy or disease progression. These findings indicate the potential of intrathecal administration as a viable and effective route for delivering therapeutic agents in MS.

A higher CSF exposure cannot be assumed to improve efficacy or reduce toxicity. These outcomes depend on the preparation, dose, target cells, and clinical setting.

2.2. Rationale for Intrathecal Exosome Administration in MS

2.2.1. Direct CNS Targeting

One of the most significant advantages of intrathecal administration is its ability to deliver therapeutic agents directly into the cerebrospinal fluid (CSF), effectively bypassing the blood-brain barrier (BBB) and enabling more efficient targeting of the CNS. This direct delivery approach is particularly relevant in the context of MS, where the primary site of pathology is within the CNS.

The BBB, while central for protecting the brain from potentially harmful substances, also presents a substantial obstacle for many therapeutic agents. Traditional systemic administration often requires high doses to achieve therapeutic concentrations in the CNS, leading to potential systemic side effects. Intrathecal delivery circumvents this issue by allowing for the administration of lower doses directly into the CSF, potentially enhancing efficacy while minimising systemic exposure.

Recent studies have examined this approach in various neurological conditions. For instance, Chung et al. (2016) investigated the biodistribution of idursulfase after intrathecal administration in a model of Hunter syndrome, showing that this route allowed for direct penetration into the CNS. Similarly, Mueller et al. (2013) showed in mice that intraventricular methotrexate administration affected cuprizone-induced demyelination, indicating that agents delivered directly into the cerebrospinal fluid can reach CNS targets. Intrathecal delivery may therefore achieve therapeutic effects at lower doses compared to systemic administration.

Moreover, the direct CNS targeting afforded by intrathecal administration aligns well with the growing understanding of MS as a disease with significant compartmentalised inflammation within the CNS. This localised inflammation, particularly in progressive forms of MS, can be challenging to target with systemic therapies. Intrathecal delivery of exosomes offers the potential to directly modulate this compartmentalised inflammation, potentially leading to more effective disease management.

2.2.2. Overcoming BBB Limitations

The blood-brain barrier (BBB) represents a significant challenge in the treatment of CNS disorders, including MS. This highly selective semipermeable border of endothelial cells prevents many therapeutic agents from reaching the CNS in sufficient concentrations, limiting their efficacy. Intrathecal administration of exosomes circumvents this barrier, allowing for the delivery of therapeutic cargo directly to the site of action.

The ability to bypass the BBB is particularly important for large molecules, biologics, and cell-derived therapies like exosomes, which may have limited BBB penetration when administered systemically. Exosomes, with their complex cargo of proteins, lipids, and nucleic acids, can potentially deliver a wide range of therapeutic molecules directly to the CNS when administered intrathecally. This approach could be especially beneficial for delivering neuroprotective or remyelinating factors that may otherwise struggle to cross the BBB in sufficient quantities.

Furthermore, recent research has indicated that the BBB becomes compromised in MS, particularly during active inflammation. While this increased permeability might theoretically enhance the CNS penetration of systemically administered therapies, it also raises concerns about the potential influx of harmful substances into the CNS. Intrathecal administration provides a controlled method of delivering therapeutic agents directly to the CNS, bypassing the potentially compromised BBB and ensuring more predictable drug concentrations in the target tissues.

The ability to overcome BBB limitations through intrathecal administration could expand the range of therapeutic options available for MS treatment. Molecules that have shown promise in preclinical studies but failed in clinical trials due to poor BBB penetration could be revisited using this delivery approach. For instance, certain neurotrophic factors or remyelinating agents that have limited BBB permeability could be delivered via exosomes intrathecally, opening new avenues for neuroprotection and repair in MS.

2.2.3. Targeting Intrathecal Inflammation

Progressive forms of MS are characterised by compartmentalised inflammation within the CNS, which can be particularly challenging to target with systemic therapies. This localised inflammation, often manifesting as meningeal inflammation and cortical pathology, is thought to drive disease progression and cortical demyelination. Intrathecal administration of exosomes offers the potential to directly modulate this localised inflammation, addressing a key aspect of MS pathology that has proven difficult to target with conventional treatments.

Compartmentalized inflammation behind an intact blood-brain barrier is one reason why intrathecal administration has been explored in progressive MS. Work in this area suggests that novel therapeutic agents delivered intrathecally may be necessary for managing progressive MS, particularly in targeting the compartmentalised inflammatory processes that drive disease progression.

The potential of intrathecal therapies to target this compartmentalised inflammation has been demonstrated in several studies. For instance, Bergman et al. (2020) reported the two-year extension of an intrathecal treatment trial of rituximab, an anti-CD20 monoclonal antibody, in progressive MS. Their findings indicated the potential of this approach in resolving

meningeal inflammation, a key feature of progressive MS that is difficult to target with systemic therapies.

Exosomes, with their diverse cargo and immunomodulatory properties, could offer a varied approach to targeting intrathecal inflammation when administered via the intrathecal route. They could deliver anti-inflammatory molecules directly to the site of inflammation, modulate the activity of resident immune cells in the CNS, and potentially promote a more favourable inflammatory milieu conducive to repair and remyelination.

Moreover, the ability to engineer exosomes to carry specific therapeutic cargoes opens up possibilities for tailored approaches to targeting intrathecal inflammation. For example, exosomes could be loaded with anti-inflammatory cytokines, neuroprotective factors, or microRNAs that modulate inflammatory pathways, providing a multipronged approach to addressing the complex inflammatory environment in progressive MS.

2.2.4. Potential for Enhanced Efficacy

By delivering exosomes directly to the CNS, intrathecal administration may allow for lower doses of therapeutic agents to achieve the desired effects, potentially reducing the risk of systemic side effects while enhancing efficacy. This targeted approach could be particularly beneficial in addressing specific aspects of MS pathology, such as promoting remyelination or modulating localised immune responses.

The potential for enhanced efficacy through intrathecal administration has been demonstrated in various studies using different therapeutic agents. For instance, Hoffmann et al. (2006) found that repeat intrathecal triamcinolone acetonide application was beneficial for progressive MS patients without contributing to neuronal atrophy or disease progression. This suggests that intrathecal delivery can achieve therapeutic effects at doses that minimise systemic exposure and associated risks.

In the context of exosome therapy, the potential for enhanced efficacy is of particular interest. Exosomes derived from mesenchymal stem cells (MSCs) have shown promise in promoting neuroprotection, immunomodulation, and potentially remyelination in preclinical models of MS. When delivered intrathecally, these exosomes could exert their therapeutic effects more directly and potently on the affected CNS tissues.

Furthermore, the ability to engineer exosomes to carry specific therapeutic cargoes opens up possibilities for highly targeted therapies. For example, exosomes could be loaded with remyelinating factors, neuroprotective agents, or immunomodulatory molecules tailored to the specific pathological features of an individual patient's MS. This level of precision in therapy delivery, combined with the direct CNS access afforded by intrathecal administration, could lead to more effective and personalised treatment approaches for MS.

The enhanced efficacy potential of intrathecal exosome administration is not limited to direct therapeutic effects. This approach could also facilitate more accurate monitoring of treatment response and disease progression. For instance, analysing the composition of CSF following intrathecal exosome therapy could provide valuable insights into the biological effects of the treatment and help guide further therapeutic decisions.

2.3. Evidence from Preclinical and Clinical Studies

2.3.1. Exosome-mediated Neuroprotection and Remyelination

Preclinical studies have demonstrated the potential of exosomes, particularly those derived from mesenchymal stem cells (MSCs), in promoting neuroprotection and remyelination in MS models. These findings provide a strong foundation for exploring intrathecal exosome administration as a therapeutic strategy in MS.

Riazifar et al. (2019) conducted a notable study examining the effects of MSC-derived exosomes in an experimental autoimmune encephalomyelitis (EAE) model of MS. They found that administration of these exosomes (given intravenously in that study) resulted in reduced neuroinflammation, decreased demyelination, and improved clinical outcomes. Specifically, the researchers observed:

A significant reduction in the number of infiltrating inflammatory cells in the CNS. Decreased levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ. Increased expression of anti-inflammatory mediators like IL-10 and TGF-β. Enhanced remyelination, as evidenced by increased expression of myelin basic protein (MBP) in treated animals.

This suggests that intrathecally administered exosomes can exert broad effects on MS pathology, addressing both the inflammatory and neurodegenerative aspects of the disease.

Building on these results, work on vesicle cargo has examined the microRNAs through which MSC-derived exosomes might promote neuroprotection and remyelination, although the specific miRNAs responsible in MS models remain incompletely defined. Two examples are often cited.

Exosomal miR-219 promotes oligodendrocyte differentiation and myelination, an effect first shown for serum exosomes from young animals rather than for MSC-derived vesicles, and exosome-associated miR-23a has been reported to act on apoptotic pathways in neurons. Neither effect has been demonstrated for MSC-derived exosomes in an MS model.

Such mechanistic work leaves exosomes as versatile candidate agents capable of addressing multiple aspects of MS pathology through their complex cargo.

In the study by Salwierak-Głośna et al. (2022), the authors investigated the effect of multiple sclerosis (MS) cerebrospinal fluid (CSF) on the secretome of human Wharton's jelly mesenchymal stem cells (WJ-MSCs). Their findings demonstrated that the CSF environment influenced the exosomal

composition, particularly enhancing its neuroprotective potential. This highlights the therapeutic promise of using MSC-derived exosomes in treating neuroinflammatory diseases like MS. Administering these exosomes intrathecally may provide targeted delivery to the central nervous system, potentially promoting remyelination and modulating immune responses within the spinal cord and brain, where demyelination occurs.

2.3.2. Immunomodulatory Effects

The immunomodulatory properties of exosomes have been a focal point of research, given the central role of immune dysregulation in MS pathogenesis. Several studies have demonstrated the potential of intrathecally administered exosomes to modulate immune responses within the CNS.

Casella et al. (2018) examined the effects of extracellular vesicles engineered to contain IL-4 in an EAE model. When administered intrathecally, these vesicles had the following effects.

Effectively suppressed ongoing EAE, resulting in significant clinical improvement. Reduced the number of inflammatory infiltrates in the CNS. Decreased production of pro-inflammatory cytokines like IFN-γ and IL-17. Promoted the expansion of regulatory T cells (Tregs), key players in maintaining immune tolerance.

Exosomes may therefore reprogramme the immune response in MS, promoting a more tolerogenic environment within the CNS.

Durable immunomodulatory effects of MSC-derived small extracellular vesicles have also been reported in models of CNS inflammation. The key findings of that work included:

MSC-sEVs **converted pathogenic Th17 cells into IL-17^low "ex-Th17" cells** by destabilizing RORγt through K63 deubiquitination and deacetylation mechanisms, mediated by Eid3 contained within the sEVs. EAE mice treated with MSC-sEVs showed **ameliorated clinical scores** and **reduced Th17 cell counts** in both peripheral lymph nodes and the central nervous system. These immunomodulatory effects correlated with **decreased neuroinflammation** and **protection against spinal cord demyelination**, with therapeutic benefits persisting beyond the treatment period.

This study highlights the **long-lasting, disease-modifying immune modulation** enabled by MSC-sEVs in chronic inflammatory CNS disease models.

2.4. Challenges and Considerations

2.4.1. Safety and Tolerability

The safety profile of intrathecal exosome administration needs to be thoroughly evaluated before widespread clinical application. While exosomes generally exhibit low immunogenicity, the potential for adverse reactions, particularly with repeated dosing, requires careful assessment.

Reported risks of intrathecal drug administration include rare but serious complications such as toxic myelitis and arachnoiditis. Proper technique and careful patient selection are important to minimise these risks, and long-term follow-up is needed to detect delayed adverse effects.

Detailed safety protocols are therefore required for intrathecal exosome administration in MS patients.

Furthermore, Boran et al. (2007) reported a case of intracerebral hematoma as a complication of intrathecal methotrexate administration, emphasizing the potential risks associated with intrathecal therapies. This case highlights:

The need for careful monitoring of patients receiving intrathecal treatments. The importance of patient education regarding potential complications and warning signs. The value of developing standardised protocols for managing potential adverse events.

To address these safety concerns, future research should focus on:

Conducting thorough preclinical toxicology studies to assess the safety profile of intrathecally administered exosomes. Developing standardised protocols for exosome preparation and characterisation to ensure consistent quality and safety. Implementing rigorous monitoring protocols in clinical trials to detect and manage potential adverse events promptly. Exploring the use of biomarkers to predict and monitor treatment response and potential toxicities.

2.4.2. Optimal Dosing and Administration Protocols

Establishing optimal dosing regimens and administration protocols for intrathecal exosome therapy in MS remains a significant challenge. Factors such as exosome concentration, frequency of administration, and duration of treatment need to be carefully evaluated to maximize therapeutic efficacy while minimising potential side effects.

Abu-Mugheisib and Zettl (2011) conducted a review of intrathecal triamcinolone acetonide administration in progressive MS, highlighting several key considerations.

The importance of individualizing treatment protocols based on patient characteristics and disease severity. The need for careful dose titration to achieve optimal therapeutic effects while minimising side effects. The potential benefits of repeated administration in maintaining therapeutic efficacy over time.

These insights can inform the development of dosing strategies for intrathecal exosome therapy in MS.

Moreover, intrathecal delivery of MSC-derived products has been examined in progressive MS. Harris et al. (2018, 2021) conducted uncontrolled phase I/IIa studies of intrathecal autologous MSC-derived neural progenitors (MSC-NPs), and reported:

No treatment-related serious adverse events in these small, uncontrolled cohorts. Exploratory changes in ambulatory, manual-dexterity and cognitive measures, which were not compared against a concurrent control group and cannot be interpreted as superiority over intravenous delivery. Exploratory cerebrospinal fluid findings, including neurofilament light chain measurements, during follow-up.

In the subsequent randomised, double-blind phase II trial, Harris et al. (2024) did not meet the primary EDSS-Plus endpoint (see Section 4.6). Data on exosome preparations administered intrathecally remain limited, and any expectation of sustained benefit from repeated intrathecal EV administration is at present a hypothesis requiring direct testing.

2.4.3. Exosome Characterization and Quality Control

Ensuring consistent production and characterisation of therapeutic exosomes is important for clinical translation. Standardised protocols for exosome isolation, purification, and quality assessment need to be developed to ensure reproducibility and safety.

Théry et al. (2018) published broad guidelines for studying extracellular vesicles, including exosomes. These guidelines provide a framework for standardising exosome research and production, which is essential for developing reliable and reproducible exosome-based therapies for MS. Key recommendations include:

Standardised methods for exosome isolation and purification to ensure consistent quality. Comprehensive characterisation of exosome size, concentration, and surface markers. Rigorous analysis of exosome cargo, including proteomic and RNA profiling. Implementation of quality control measures to ensure batch-to-batch consistency.

These technological advancements could significantly enhance our ability to produce and characterize clinical-grade exosomes for intrathecal administration in MS treatment.

Future research should focus on:

Developing and validating standardised protocols for large-scale production of clinical-grade exosomes suitable for intrathecal administration. Establishing regulatory guidelines for exosome-based therapies, including specific requirements for intrathecal administration. Investigating the stability and shelf-life of exosome preparations under various storage conditions to ensure consistent quality in clinical settings. Exploring novel engineering approaches to enhance the therapeutic properties of exosomes while maintaining consistent quality and safety profiles.

2.4.4. Long-term Efficacy and Safety

The long-term effects of intrathecal exosome administration in MS patients need to be carefully evaluated. Studies assessing the durability of therapeutic effects and potential long-term complications are essential for establishing the viability of this approach as a treatment option for MS.

While long-term data on intrathecal exosome therapy in MS is currently limited, insights can be gained from studies on other intrathecal therapies. For instance, Draulans et al. (2013) conducted a long-term follow-up study on intrathecal baclofen therapy in MS patients, revealing:

Sustained efficacy in managing spasticity over several years of treatment. The need for dose adjustments over time to maintain therapeutic effects. Potential complications associated with long-term intrathecal drug delivery systems, such as catheter-related issues.

Long-term monitoring and management strategies are therefore needed for intrathecal therapies in MS.

Furthermore, work by Cohen et al. (2023) evaluating intrathecal administration of neurotrophic factor-secreting MSCs in progressive MS provides relevant observations.

Sustained improvements in clinical measures and quality of life in some patients up to two years post-treatment.

The potential for repeated administrations to maintain therapeutic effects over time. The importance of long-term safety monitoring, including regular neuroimaging and CSF analysis.

Future research should focus on:

Conducting long-term follow-up studies to assess the durability of therapeutic effects and monitor for potential delayed adverse events. Investigating the potential for repeated administrations of exosomes to maintain therapeutic efficacy over time. Developing biomarkers to predict long-term treatment response and monitor for potential toxicities. Exploring combination therapies that could enhance the long-term efficacy of intrathecal exosome administration in MS.

2.4.5. Patient Selection and Stratification

Identifying the most suitable candidates for intrathecal exosome therapy is key. Factors such as disease subtype, stage of progression, and individual patient characteristics need to be considered to optimise treatment outcomes and minimise risks.

Harris et al. (2021) reported two-year follow-up of intrathecal MSC-derived neural progenitor treatment in progressive MS, providing observations relevant to patient selection for intrathecal therapies, including:

The importance of carefully defining inclusion criteria based on disease duration, disability level, and evidence of active inflammation. The potential benefit of using advanced imaging techniques, such as magnetization transfer ratio (MTR) mapping, to identify patients with residual repair capacity who might benefit most from regenerative therapies. The value of biomarker profiling, including CSF analysis, to predict treatment response and guide patient selection.

Patient selection therefore has to be individualized for intrathecal exosome therapy in MS.

Furthermore, Bergman et al. (2018), in their study on intrathecal rituximab treatment in progressive MS, emphasized the importance of patient stratification based on specific disease characteristics. They noted:

The potential benefit of selecting patients with evidence of leptomeningeal contrast enhancement on MRI, indicating active intrathecal inflammation. The value of CSF biomarkers, such as neurofilament light chain (NfL) levels, in identifying patients with active disease who might be more likely to respond to intrathecal therapies. The need to consider comorbidities and overall health status when selecting patients for invasive intrathecal treatments.

Building on these insights, future research on intrathecal exosome therapy for MS should focus on:

Developing full patient selection criteria that incorporate clinical, imaging, and biomarker data to identify the most suitable candidates for treatment. Investigating the potential of personalised exosome formulations tailored to individual patient characteristics and disease subtypes. Exploring the use of artificial intelligence and machine learning algorithms to analyse complex patient data and predict treatment response, facilitating more precise patient selection. Conducting studies to determine the optimal timing of intrathecal exosome therapy in the course of MS progression to maximize therapeutic benefits.

2.5. Future Directions and Emerging Approaches

2.5.1. Engineered Exosomes with Enhanced Therapeutic Properties

The development of engineered exosomes with enhanced therapeutic properties through genetic modification or cargo loading represents a further avenue for future research. This approach allows for the creation of tailored exosomes that can target specific aspects of MS pathology more effectively.

Exosomes might therefore be engineered for highly specific effects in MS.

Furthermore, Sutaria et al. (2017) explored various strategies for loading therapeutic cargo into exosomes, including:

Genetic engineering of parent cells to overexpress specific therapeutic proteins or RNAs. Post-isolation loading techniques, such as electroporation or sonication, to introduce small molecules or nucleic acids into exosomes. Surface modification of exosomes to enhance their targeting capabilities or alter their pharmacokinetic properties.

These approaches open up possibilities for creating "designer" exosomes tailored to address specific aspects of MS pathology, such as promoting remyelination, modulating neuroinflammation, or enhancing neuroprotection.

Future research in this area should focus on:

Developing exosomes engineered to express multiple therapeutic factors simultaneously, addressing the complex pathophysiology of MS more comprehensively. Exploring the use of tissue-specific promoters to achieve targeted expression of therapeutic cargo in specific CNS cell types. Investigating the potential of exosome surface modifications to enhance CNS retention following intrathecal administration. Conducting comparative studies to evaluate the efficacy and safety of engineered exosomes versus native exosomes in MS models.

2.5.2. Combination Therapies

Combining intrathecal EV administration with established MS treatment is a research hypothesis. Potential interactions, additive effects, delivery, and safety would require direct testing before any clinical benefit could be claimed.

Building on this concept, future research should explore:

The combination of intrathecal exosome therapy with other disease-modifying therapies, such as ocrelizumab or alemtuzumab, to enhance their efficacy or address aspects of MS pathology not fully targeted by these drugs. The potential of sequential treatment approaches, where intrathecal exosome administration follows or precedes other therapies to optimise therapeutic effects. The development of dual-action exosomes that can deliver both small molecule drugs and biological factors simultaneously, offering a more integrated combination therapy approach. The use of intrathecal exosome therapy in combination with emerging neuroprotective or remyelinating agents to enhance CNS repair and regeneration in MS.

2.5.3. Advanced Delivery Systems and Nanotechnology

Advancements in nanotechnology and biomaterials science are opening up new possibilities for enhancing the delivery and efficacy of intrathecal exosome therapies in MS. Several innovative approaches are being explored.

Han et al. (2022) developed a three-dimensional-cultured MSC-derived exosome-hydrogel hybrid microneedle array patch for spinal cord repair. This technology could be adapted for localised, sustained delivery of exosomes in MS treatment. Zhan et al. (2020) engineered blood exosomes for tumour-targeted combination therapy, illustrating how vesicles can be adapted as controlled delivery vehicles. Similar approaches could be developed for intrathecal exosome delivery in the CNS. Jang et al. (2013) introduced the concept of cell-derived nanovesicles that mimic the properties of natural exosomes but can be produced at a larger scale. These synthetic exosome mimetics could overcome some of the manufacturing challenges associated with natural exosomes. Busato et al. (2016) developed a method for labeling exosomes with superparamagnetic iron oxide nanoparticles, allowing for magnetic resonance imaging (MRI) tracking and potential magnetic guidance to target tissues.

These advanced delivery systems offer the potential for more precise, controlled, and sustained delivery of exosomes to affected areas in MS, potentially enhancing therapeutic efficacy while minimising off-target effects.

2.5.4. Personalized Exosome Therapies

The concept of personalised medicine is gaining traction in MS treatment, and exosome therapy could be central to this paradigm. Recent advancements in exosome engineering and characterisation techniques are supporting the development of more tailored therapeutic approaches.

Building on these insights, future research in personalized exosome therapy for MS should focus on:

Developing patient-derived exosome therapies, where exosomes are isolated from a patient's own cells and engineered to address their specific disease characteristics. Exploring the use of artificial intelligence and machine learning algorithms to analyze complex patient data and design optimised exosome formulations for individual patients. Investigating the potential of "on-demand" exosome production systems that can rapidly generate personalized treatments based on real-time patient data. Conducting large-scale, longitudinal studies to identify biomarker profiles that can predict response to different types of exosome therapies, enabling more precise treatment selection.

2.5.5. Exosome-Based Biomarkers and Liquid Biopsies

The potential of exosomes as biomarkers for MS diagnosis, prognosis, and treatment monitoring represents an active area of research that complements their therapeutic applications. Exosome-based liquid biopsies could provide valuable insights into disease activity and treatment response, potentially advancing MS management.

Manna et al. (2018) demonstrated the potential of exosome-associated miRNA profiling as a prognostic tool for monitoring therapy response in MS patients. Their study showed that:

Specific exosomal miRNA signatures correlated with disease activity and treatment response. Exosome-based biomarkers could predict treatment outcomes and guide therapy selection. Longitudinal monitoring of exosomal miRNA profiles could provide insights into disease progression and treatment efficacy.

Building on this work, future research should focus on:

Developing standardised protocols for isolating and analysing CNS-derived exosomes from easily accessible biofluids, such as blood or cerebrospinal fluid. Identifying exosomal biomarker panels that can accurately reflect different aspects of MS pathology, including inflammation, demyelination, and neurodegeneration. Integrating exosome-based biomarker analysis with other diagnostic and monitoring tools, such as MRI and clinical assessments, to provide a more detailed picture of disease status. Exploring the potential of exosome-based liquid biopsies to guide personalised treatment decisions and monitor the efficacy of intrathecal exosome therapies.

2.6. Conclusion

Intrathecal extracellular-vesicle delivery is being investigated as a means of increasing CNS exposure without relying on systemic passage across the blood-brain barrier. The rationale is largely preclinical; efficacy, dose-response relationships, product consistency, and long-term safety in people with MS remain to be established.

The evidence reviewed here suggests that intrathecal exosome administration might:

Provide targeted delivery of therapeutic agents to the CNS, overcoming the limitations of the blood-brain barrier. Modulate local immune responses and reduce neuroinflammation. Promote remyelination and neuroprotection through the delivery of specific molecular cargoes. Offer a personalised approach to MS treatment, with the potential for tailored exosome formulations based on individual patient characteristics.

Important translational problems remain before these findings can be evaluated as clinical interventions. Priority areas include:

Optimising safety profiles and developing thorough monitoring protocols for long-term intrathecal exosome therapy. Establishing standardised manufacturing processes and quality control measures for clinical-grade exosomes. Determining optimal dosing regimens and administration protocols for different MS subtypes and stages. Developing advanced delivery systems and biomarkers to enhance the precision and monitoring of intrathecal exosome therapy. Addressing regulatory considerations specific to intrathecal administration of cell-derived therapies like exosomes.

A balanced view is needed: the approach is worth investigating, and the obstacles ahead are substantial. Collaborative efforts between researchers, clinicians, and regulatory bodies will be essential in navigating these challenges and realizing the full potential of intrathecal exosome therapy for MS.

Engineered EVs, delivery systems, biomarker development, and patient-stratification methods are converging in preclinical research. Translation to MS therapy will require reproducible manufacturing, validated identity and potency assays, biodistribution and target-engagement data, and controlled evidence of safety and patient benefit.

Further evaluation of intrathecal EV administration requires well-designed clinical trials with prespecified safety and efficacy outcomes, adequate follow-up, and product characterisation sufficient to permit replication. Long-term effects, dose selection, biodistribution, and any disease-modifying signal should be tested directly rather than inferred from preclinical studies.

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3. Localized Delivery

3.1. Introduction

Localized exosome administration offers several potential advantages for MS treatment, including targeted delivery to affected CNS regions, enhanced therapeutic efficacy, and reduced systemic side effects. This approach aligns with the growing trend towards personalised medicine in MS management, offering the possibility of tailored therapies that address the specific pathophysiological mechanisms at play in individual patients.

Local delivery aims to increase exposure at a specified site. Experimental results need to be interpreted in relation to procedural risk and to the multiple, spatially distributed lesions found in MS.

3.2. Rationale for Localized Exosome Administration in MS

3.2.1. Targeted Delivery to CNS

One of the primary advantages of localised exosome administration is the ability to deliver therapeutic agents directly to the affected regions of the CNS. MS is characterised by focal areas of inflammation and demyelination throughout the brain and spinal cord, making targeted delivery important for effective treatment. Exosomes have demonstrated the ability to cross the blood-brain barrier (BBB), a significant obstacle in CNS drug delivery. Local administration is intended to raise drug levels at the affected CNS regions while limiting exposure elsewhere. That rationale comes from experimental delivery work; local and systemic dosing have not yet been compared in people with MS.

Recent studies have shown that engineered exosomes can be designed to target specific cell types within the CNS. For instance, Alvarez-Erviti et al. (2011) demonstrated that exosomes expressing a neuron-specific targeting peptide could efficiently deliver siRNA to the brain, achieving widespread gene knockdown in neurons. This level of targeting specificity could be particularly beneficial in MS, where different cell types (e.g., oligodendrocytes, neurons, and immune cells) play distinct roles in disease pathogenesis.

Furthermore, the natural homing abilities of certain types of exosomes, particularly those derived from mesenchymal stem cells (MSCs), offer an additional layer of targeting. Baharlooi et al. (2021) reported that umbilical cord MSCs and their released exosomes suppressed the proliferation of activated peripheral blood mononuclear cells from people with MS.

3.2.2. Modulation of Local Immune Responses

MS is characterised by dysregulated immune responses within the CNS, leading to inflammation, demyelination, and neurodegeneration. Localized exosome administration offers the potential to modulate these immune responses directly on the site of pathology. Exosomes, particularly those derived from immunomodulatory cells like MSCs, have demonstrated potent anti-inflammatory and immunoregulatory properties.

A notable study by Riazifar et al. (2019) showed that administration of MSC-derived exosomes, given intravenously in that study, in an experimental autoimmune encephalomyelitis (EAE) model of MS resulted in:

reduced infiltration of pro-inflammatory immune cells into the CNS; decreased production of pro-inflammatory cytokines; increased expression of anti-inflammatory mediators; and enhanced proliferation and function of regulatory T cells (Tregs).

The evidence behind this is an intravenous study in EAE, which bears on exosome-mediated immunomodulation broadly and says little about what local delivery would do in a patient. It is a reason to test localised administration, not a finding about it.

3.2.3. Promotion of Remyelination and Neuroprotection

One of the key challenges in MS treatment is promoting remyelination and neuroprotection to halt or reverse disease progression. Localized exosome administration is being explored with this in mind. Exosomes carry factors that act on oligodendrocyte differentiation, myelin synthesis, and neuronal survival in experimental systems, although no clinical study has yet shown repair of that kind in patients.

3.2.4. Enhanced Bioavailability and Reduced Systemic Effects

Localized administration of exosomes offers the advantage of enhanced bioavailability at the target site while potentially reducing systemic effects. This is particularly important in the context of MS, where long-term systemic immunomodulation can lead to various side effects and increased susceptibility to infections.

Moreover, the ability to fine-tune the release kinetics of exosomes through various delivery systems offers additional control over the therapeutic effects. Recent work by Han et al. (2022) demonstrated the development of a hydrogel-based delivery system for sustained release of MSC-derived exosomes. This approach allowed for:

controlled release of exosomes over an extended period; prolonged immunomodulatory and neuroprotective effects in the CNS; and reduced frequency of administration, potentially improving patient compliance and quality of life.

3.3. Challenges and Considerations

3.3.1. Optimization of Exosome Production and Characterization

One of the primary challenges in developing localised exosome therapies for MS is the standardisation and optimisation of exosome production and characterisation. Ensuring consistent quality and purity of therapeutic exosomes is important for their clinical application.

The reporting standards that govern this work are the MISEV recommendations, summarised in Section 2.4.3 and updated by MISEV2023; they are not repeated here.

Despite these guidelines, obstacles persist in scaling up exosome production for clinical applications. Whitford and Guterstam (2019) highlighted several key issues.

the need for large-scale, GMP-compliant production methods; difficulties in maintaining exosome stability during long-term storage; and challenges in ensuring consistent biological activity across different batches.

Addressing these challenges will be key for the successful translation of localised exosome therapies from bench to bedside in MS treatment.

3.3.2. Optimization of Delivery Methods

Developing effective methods for localised delivery of exosomes to the CNS is another significant challenge. While direct injection into the brain or spinal cord offers the most targeted approach, it is highly invasive and may not be suitable for repeated administration. Several alternative strategies are being explored.

Intrathecal administration is one route. This approach involves injecting exosomes directly into the cerebrospinal fluid (CSF), allowing for distribution throughout the CNS. Mueller et al. (2013) demonstrated the feasibility of this approach for delivering therapeutic agents into the cerebrospinal fluid in mice. Exosomes can also be administered intranasally, leveraging the olfactory and trigeminal nerve pathways to bypass the BBB. Perets et al. (2018) showed that intranasally administered MSC-derived exosomes reached the brain and ameliorated autistic-like behaviours in BTBR mice. Convection-enhanced delivery uses a pressure gradient to distribute exosomes throughout the brain parenchyma. While not yet explored specifically for exosome delivery in MS, CED has shown promise in delivering various therapeutics in brain disorders (Barua et al., 2014). Recent advances in biomaterials have led to the development of hydrogels and nanofibers that can encapsulate exosomes for sustained localised release. Han et al. (2022) reported effects of an exosome-loaded hydrogel in an experimental model of spinal cord repair; whether such a system could be adapted for MS remains untested.

Optimising these delivery methods for MS-specific applications will be essential for maximizing the therapeutic potential

of localised exosome administration while minimising invasiveness and potential side effects.

3.3.3. Targeting Specificity and Biodistribution

Ensuring precise targeting of exosomes to the desired cell types and tissues within the CNS remains a significant challenge. While exosomes have shown some natural tropism for inflammatory sites, enhancing their targeting specificity could greatly improve therapeutic efficacy.

Several strategies are being explored to enhance the targeting capabilities of exosomes.

Surface modification is one strategy. Decorating exosomes with targeting ligands or antibodies can enhance their specificity for certain cell types. For instance, Alvarez-Erviti et al. (2011) demonstrated that exosomes expressing the neuron-specific RVG peptide showed enhanced brain targeting. The selection of cell source is another. The choice of parent cells for exosome production can influence their targeting properties. MSC-derived exosomes, for example, have shown natural tropism for inflammatory sites (Baharlooi et al., 2020).

Understanding and optimising the biodistribution of exosomes following localised administration is central for maximizing therapeutic effects and minimising off-target accumulation. Advanced imaging techniques, such as those developed by Busato et al. (2016) for tracking exosome biodistribution using MRI, will be essential tools in this effort.

3.3.4. Long-term Safety and Efficacy

Assessing the long-term safety and efficacy of localised exosome administration in MS patients is a critical consideration. While preclinical studies have shown early findings, long-term data in human subjects are still lacking.

Key areas that require thorough investigation include:

Although exosomes are generally considered to have low immunogenicity, the potential for adverse immune reactions, particularly with repeated administration, needs careful evaluation. The ability of exosomes to transfer genetic material and modulate cellular signalling raises concerns about potential oncogenic effects, especially with long-term use. Understanding how localised exosome administration interacts with existing MS therapies will be important for developing optimal treatment protocols. Recent preclinical studies and reviews provide evidence that repeated dosing regimens of MSC-derived extracellular vesicles (EVs) yield durable therapeutic benefits in chronic EAE and MS models: Williams et al. (2013) demonstrated in a pregnancy-associated EAE model that serum-derived exosomes suppressed T-cell activation, enhanced oligodendrocyte precursor maturation, and accelerated disease remission (Williams et al., 2013). A narrative review by Kråkenes et al. (2024) summarized multiple rodent studies in which biweekly intravenous administration of MSC-EVs, typically at 5 × 10^10 particles twice weekly for four weeks, significantly improved clinical scores, promoted remyelination, modulated microglia from an M1 to M2 phenotype, inhibited pro-inflammatory cytokines, and supported cognitive recovery long-term (Kråkenes et al., 2024).

These findings argue for maintenance dosing schedules in designing effective exosome therapies for chronic MS, suggesting that regular, moderate-dose treatments may be key to achieving sustained immunomodulatory and neuroprotective effects.

3.3.5. Regulatory and Manufacturing Considerations

The development of localised exosome therapies for MS faces significant regulatory challenges, as this novel treatment modality does not fit neatly into existing regulatory frameworks for biologics or cell therapies.

Key regulatory considerations include:

Key regulatory questions include how exosome-based treatments should be classified, as biologics, as advanced therapy medicinal products (ATMPs) or as a new category altogether , and what Good Manufacturing Practice guidelines specific to exosome production and characterisation should require. Standardised assays are needed for purity, potency and stability, as are agreed endpoints and safety measures for clinical trials of localised exosome administration.

Lener et al. (2015) published a position paper addressing some of these regulatory challenges, emphasizing the need for a collaborative approach between researchers, industry, and regulatory bodies to establish appropriate frameworks for exosome-based therapies.

From a manufacturing perspective, scaling up exosome production while maintaining consistency and quality presents substantial difficulties. Whitford and Guterstam (2019) set out the main issues: large-scale culture systems for exosome-producing cells, isolation and purification methods suited to industrial-scale production, stable formulations for long-term storage and distribution, and quality control measures applied throughout the manufacturing process.

Addressing these regulatory and manufacturing challenges will be important for the successful translation of localised exosome therapies from bench to bedside in MS treatment.

3.4. Future Directions and Emerging Approaches

The emerging approaches relevant to localised exosome administration, engineered exosomes with enhanced targeting or cargo, combination regimens with established disease-modifying therapies, hydrogel- and nanotechnology-based delivery systems, patient-specific formulations, and exosome-based biomarkers and liquid biopsies , are the same as those discussed for intrathecal administration, and they are set out in detail in Section 2.5 rather than repeated here. Two points are specific to the localised route. First, the targeting problem is more tractable, because the vesicles are delivered close to the

intended site of action, so surface engineering serves to retain rather than to direct them. Second, sustained-release carriers are more directly applicable, since a depot placed at or near a lesion can, in principle, maintain local concentrations that systemic dosing cannot achieve. Both remain experimental propositions awaiting direct testing.

Shao et al. (2025) co-loaded exosomes with superoxide dismutase and chondroitinase ABC. Intranasal administration increased enzyme delivery to the brain and spinal cord and altered inflammatory and remyelination-related readouts in EAE mice. This tests combined cargo delivery within an experimental EV product, rather than co-administration of living MSCs with EVs. Human dose, safety, and efficacy remain undetermined.

3.5. Conclusion

Localized exosome administration is an investigational approach in the treatment of multiple sclerosis. The ability to deliver therapeutic cargo directly to affected CNS regions, modulate local immune responses, and potentially promote remyelination and neuroprotection offers a multimodal approach to addressing the complex pathology of MS. Preclinical studies and early clinical investigations have demonstrated encouraging results, suggesting that the approach might improve clinical outcomes and quality of life for MS patients.

The evidence reviewed here indicates that localised exosome administration could, in principle, deliver therapeutic cargo directly to CNS targets, modulate local immune responses and support remyelination, while limiting systemic exposure; these remain propositions derived from experimental models, as set out in Section 2.6 for the intrathecal route.

Substantial difficulties remain, however, in translating these findings into clinical applications. Production, characterisation and quality control need to be optimised; effective and minimally invasive methods for localised delivery to the CNS have yet to be established; targeting specificity and biodistribution after administration are poorly understood; long-term safety and efficacy in patients with MS have not been assessed; and regulatory and manufacturing obstacles remain to be addressed before clinical translation can proceed.

Collaborative efforts between researchers, clinicians, and regulatory bodies will be essential in navigating these challenges and realizing the full potential of localised exosome therapy for MS.

Localized EV delivery likewise requires controlled clinical evaluation with prespecified safety and efficacy outcomes, adequate follow-up, and reproducible product characterisation. Studies should define local biodistribution, retention, dose, procedure-related risks, and whether any observed biological effect translates into a clinically meaningful patient outcome.

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5.9. CHALLENGES AND CONSIDERATIONS

Extracellular-vesicle approaches are being investigated in MS for effects on immune signalling, oligodendroglial biology, and neuroprotective pathways (Baharlooi et al., 2022; Pusic et al., 2014). The evidence is predominantly mechanistic or preclinical, so these proposed effects should not be interpreted as established therapeutic outcomes. Figure 5.9.1 groups the barriers that stand between these observations and clinical use.

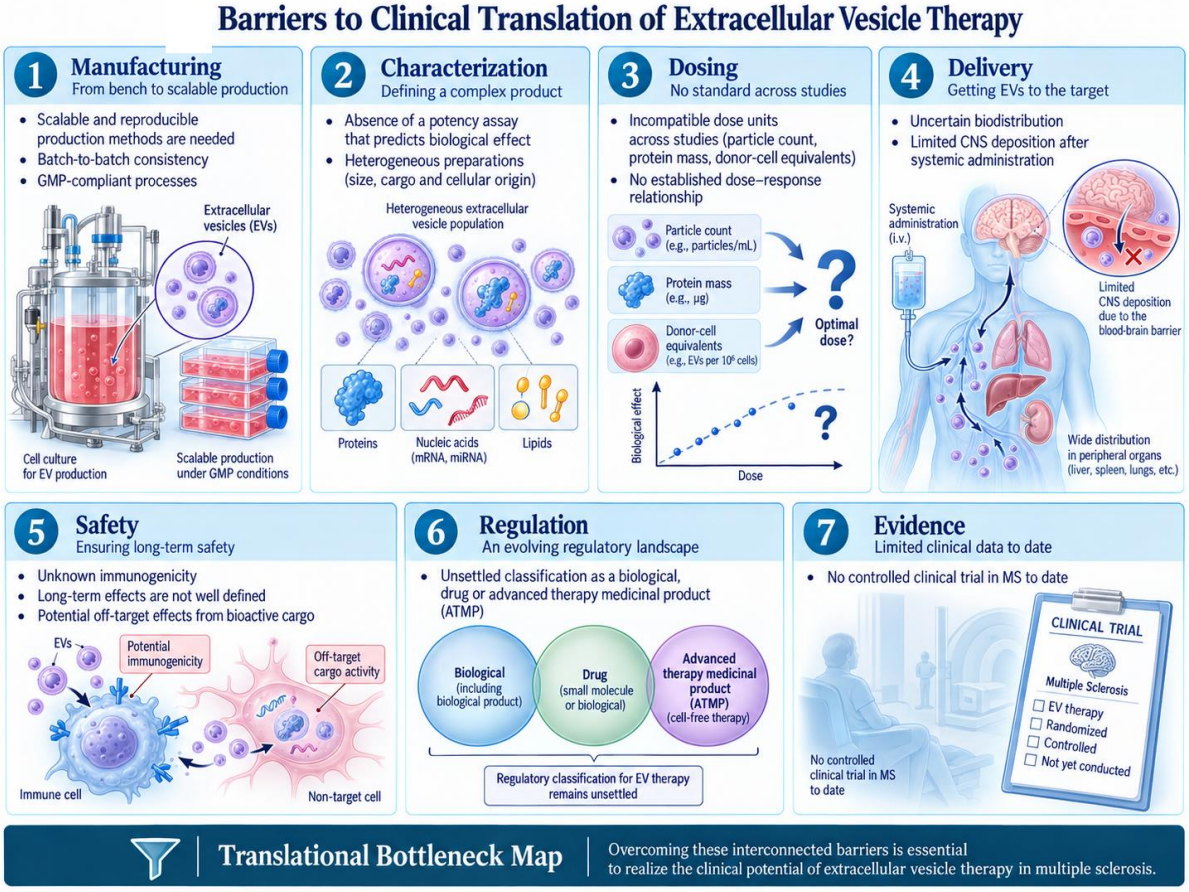


Figure 5.9.1. Barriers to clinical translation of extracellular vesicle therapy in multiple sclerosis, from manufacturing and characterisation to regulation and the current absence of controlled clinical data.

Exosomes are nano-sized extracellular vesicles (30-150 nm in diameter) that play central roles in intercellular communication by transporting proteins, lipids, and nucleic acids between cells. Their ability to cross the blood-brain barrier (BBB) and deliver therapeutic cargo to specific cell types makes them attractive candidates for CNS-targeted therapies (Frühbeis et al., 2012; Willis et al., 2017). Moreover, the immunomodulatory properties of certain exosome populations, particularly those derived from mesenchymal stem cells (MSCs), offer possible routes for managing the autoimmune component of MS (Baharlooi et al., 2022; Riazifar et al., 2019). Size alone does not establish endosomal origin; EV or small EV is the operational term when that origin has not been demonstrated.

Clinical translation requires control of dose, delivery, immune effects, and product quality. Each is considered below because variation in any one can change the interpretation of safety or activity.

The following sections examine current findings, technical strategies, and emerging technologies relevant to EV-based approaches in MS, with attention to what has been demonstrated experimentally and what remains untested in patient care.

1. DOSAGE AND DELIVERY

1.1. Introduction

Exosome and extracellular-vesicle products are being investigated in MS because they participate in intercellular communication and can transport bioactive cargo. For therapeutic use, however, dose, route, biodistribution, product identity, manufacturing consistency, and safety remain

unresolved. These variables must be defined before efficacy can be tested reliably in clinical trials.

The intricacies of exosome biology, coupled with the diverse and dynamic nature of MS, present unique challenges in establishing standardised dosing protocols and effective delivery mechanisms. Exosomes' heterogeneity, in terms of cellular origin, cargo composition, and functional properties, contributes to the complexity of dosage optimisation. At the same time, the delivery of exosomes to the central nervous system (CNS) requires overcoming physiological barriers, such as the blood-brain barrier, while ensuring targeted distribution to specific sites of inflammation or neural damage within the CNS.

A dose should describe the administered product and its measured activity, rather than volume alone. Delivery studies need to establish exposure at the intended target and the relationship between exposure and response.

Dose optimisation also requires reproducible manufacturing and a way to compare batches. Without these, apparent dose-response differences may reflect differences in the preparations themselves.

1.2. Dosage Considerations

Determining the optimal dosage for exosome-based therapies in MS is a complex endeavor that requires careful consideration of various factors. The heterogeneity of exosome populations, their diverse biological effects, and the dynamic nature of MS progression all contribute to the challenges in establishing standardised dosing protocols.

1.2.1. Dose-Response Relationship

One of the primary challenges in dosage optimisation is elucidating the dose-response relationship for exosome therapies in MS. Studies have shown that the therapeutic effects of exosomes often exhibit a non-linear relationship with dose, suggesting the existence of optimal therapeutic windows (Wiklander et al., 2019).

This non-linear relationship necessitates broad dose-escalation studies to identify the most effective dosage range for different exosome formulations. Furthermore, the optimal dose may vary depending on the specific therapeutic target (e.g., immunomodulation, remyelination, or neuroprotection) and the stage of MS progression, adding another layer of complexity to dosage determination (Pusic et al., 2014).

1.2.2. Exosome Potency and Variability

The potency of exosomes can vary significantly depending on their cellular origin, isolation method, and cargo composition. This variability poses challenges in establishing consistent dosing protocols across different exosome preparations. For example, exosomes derived from MSCs primed under hypoxic conditions have shown enhanced pro-angiogenic and tissue-regenerative properties compared to those from normoxic MSCs, potentially allowing for lower effective doses (Zhang et al., 2016).

To address this challenge, researchers are developing standardised potency assays that can assess the biological activity of exosomes in relevant in vitro or in vivo models. Lener et al. (2015) proposed a framework for developing potency assays based on the intended therapeutic application, incorporating both biochemical and functional readouts. These efforts aim to establish more reliable and comparable dosing strategies across different exosome preparations and studies.

1.2.3. Pharmacokinetics and Biodistribution

Understanding the pharmacokinetics and biodistribution of exosomes is important for optimising dosage regimens. Exosomes are rapidly cleared from circulation, primarily through the liver and spleen, which can limit their therapeutic efficacy (Lai et al., 2014). This rapid elimination necessitates strategies to enhance the circulatory half-life of exosomes or to develop sustained release formulations that can maintain therapeutic levels over extended periods.

Recent advancements in exosome engineering and formulation have shown promise in addressing these challenges. For instance, Kamerkar et al. (2017) demonstrated that displaying CD47, a "don't eat me" signal, on the surface of exosomes significantly enhanced their circulation time and reduced macrophage uptake. Similarly, the incorporation of exosomes into hydrogels or other biomaterials has been explored as a means to provide a depot for gradual release, potentially improving their therapeutic efficacy in MS treatment (Riau et al., 2019).

1.3. Delivery Strategies

The successful delivery of exosomes to target tissues and cells in MS patients presents another set of challenges that must be overcome to maximize therapeutic efficacy. The complex pathophysiology of MS, involving both CNS and peripheral components, necessitates the development of sophisticated delivery strategies that can achieve targeted distribution and optimal bioavailability.

1.3.1. Crossing the Blood-Brain Barrier

One of the primary advantages of exosomes in MS therapy is their ability to cross the blood-brain barrier (BBB), a feat

that many conventional therapeutic agents struggle to achieve. However, the efficiency of BBB crossing can vary depending on the exosome source and surface properties. Alvarez-Erviti et al. (2011) demonstrated that exosomes engineered to express the neuron-specific RVG peptide on their surface showed enhanced BBB crossing and delivery of siRNA to the brain.

Building on this concept, researchers are exploring various strategies to enhance the BBB-crossing capabilities of exosomes. For example, Jia et al. (2018) reported exosomes functionalized with a neuropilin-1-targeting peptide that improved delivery to brain tumours; equivalent formulations have yet to be tested in EAE models of MS.

1.3.2. Targeted Delivery

Achieving targeted delivery of exosomes to specific cell types or tissues affected in MS is important for maximizing therapeutic efficacy while minimising off-target effects. Various approaches have been explored to enhance the targeting capabilities of exosomes, including surface modification with targeting ligands or antibodies.

A study by Tian et al. (2018) demonstrated the potential of surface-functionalized exosomes for targeted delivery of therapeutic agents to injured brain tissue. This approach could be particularly valuable in MS therapy, where targeting exosomes to specific immune cell populations or CNS cell types (e.g., oligodendrocytes for remyelination) could enhance therapeutic outcomes.

Moreover, the concept of "exosome homing" is gaining attention in the field. This phenomenon, where exosomes preferentially accumulate in tissues of origin or sites of inflammation, could be leveraged to enhance the targeted delivery of therapeutic exosomes in MS (Wiklander et al., 2015). Further research is needed to fully elucidate the mechanisms underlying exosome homing and to develop strategies to exploit this property for improved therapeutic outcomes.

1.3.3. Route of Administration

The choice of administration route can significantly impact the biodistribution and therapeutic efficacy of exosomes in MS. While intravenous administration is the most common route, it may not be optimal for all applications due to rapid clearance and potential off-target accumulation. Alternative routes of administration are being explored to enhance the therapeutic potential of exosomes in specific contexts.

Intranasal delivery has shown promise for neurological applications, leveraging the nose-to-brain pathway to bypass the BBB. Zhuang et al. (2011) demonstrated that intranasal administration of exosomes loaded with anti-inflammatory drugs could effectively deliver the therapeutic cargo to the brain and alleviate neuroinflammation. This approach could be particularly valuable for targeting the CNS components of MS while minimising systemic exposure.

Additionally, local injection or topical application of exosomes has shown effects in experimental models of certain conditions, allowing targeted delivery with minimal systemic exposure. While these approaches may have limited applicability in systemic diseases like MS, they could be useful for addressing specific MS-related symptoms or complications.

1.3.4. Advanced Delivery Systems

The development of advanced delivery systems represents a frontier in exosome-based therapies for MS. These innovative approaches aim to overcome the limitations of traditional exosome delivery methods and enhance their therapeutic potential.

One investigational strategy involves the creation of hybrid nanoparticle-exosome systems that combine the targeting capabilities of exosomes with the drug-loading capacity and stability of synthetic nanoparticles. For example, Sato et al. (2016) developed a hybrid exosome-liposome system for targeted delivery of therapeutic agents, demonstrating enhanced drug loading capacity and improved efficacy compared to conventional liposomes.

Another emerging approach is the use of biomimetic nanoparticles that replicate key features of exosomes while offering greater control over composition and cargo loading. These synthetic exosome-mimics can be engineered with specific targeting moieties, optimised drug loading, and tailored physicochemical properties to enhance their therapeutic efficacy and overcome delivery barriers (Fang et al., 2018).

Furthermore, the integration of exosomes with stimuli-responsive materials is being explored as a means to achieve controlled release of therapeutic cargo in response to specific physiological cues. For instance, Zhao et al. (2020) showed that hypoxia alters the miRNA cargo of exosomes secreted by neural progenitor cells, illustrating how the local microenvironment can be exploited to tune vesicle content.

1.4. Future Directions and Emerging Technologies

Several emerging technologies may help to address the problems of dosage and delivery.

Genetic modification of producer cells to enhance the therapeutic cargo or targeting capabilities of exosomes offers new avenues for improving the potency and specificity of exosome-based therapies (Yim et al., 2016). Computational approaches can be used to analyse large datasets of exosome characteristics, cargo profiles, and therapeutic outcomes to identify key determinants of exosome efficacy and predict optimal dosing regimens. Advances in imaging techniques, such as intravital microscopy and advanced labeling methods, are providing marked insights into the in vivo behaviour of exosomes, informing the development of more effective delivery strategies (Lai et al., 2014). The development of microfluidic devices for exosome isolation and characterisation offers the potential for rapid, high-throughput analysis of exosome properties, facilitating the optimisation of dosage and delivery strategies (Contreras-Naranjo et al., 2017). Novel approaches to modifying the surface properties of exosomes, such as lipid engineering or the incorporation of synthetic polymers, are being explored to enhance their stability, targeting capabilities, and therapeutic efficacy (de Jong et al., 2019).

Optimising EV dosage and delivery for MS requires coordinated attention to product identity, biodistribution, route of administration, pharmacokinetics, manufacturing, and clinically relevant endpoints. These variables should be evaluated experimentally rather than inferred from vesicle biology alone.

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2. IMMUNE RESPONSE

2.1. Introduction

Exosomes, nanoscale vesicles central to intercellular communication, have become powerful modulators of the immune response, presenting both therapeutic opportunities and challenges in the treatment of autoimmune diseases like Multiple Sclerosis (MS). As mediators of immune interactions, exosomes carry a diverse array of bioactive molecules capable of influencing both innate and adaptive immune systems. In MS, an autoimmune disorder characterised by immune-mediated attacks on the central nervous system, understanding and harnessing the immunomodulatory properties of exosomes is essential for developing effective therapeutic strategies.

However, the interaction between exosomes and the immune system complicates their clinical application in MS. Exosomes interact with various immune cell types and can either enhance or suppress immune responses depending on their cellular origin, surface markers, and cargo composition. These interactions can significantly influence the therapeutic efficacy and safety profile of exosome-based treatments, posing unique challenges in achieving the desired immunomodulatory effects without triggering adverse immune reactions.

EV preparations can alter immune responses in different directions. Source-cell state, cargo, dose, and recipient characteristics need to be examined before assuming an anti-inflammatory or tolerogenic effect.

Resolving questions about EV identity, immunogenicity, and distribution would support the design of investigational products. Whether those products can be administered safely or improve MS outcomes remains to be established in prospective studies.

2.2. Exosome-Immune System Interactions

The interactions between exosomes and the immune system are complex, involving various components of both innate and adaptive immunity. These interactions can significantly influence the therapeutic efficacy and safety profile of exosome-based treatments for MS.

2.2.1. Surface Antigen Recognition

Exosomes express a diverse array of surface molecules that can be recognised by pattern recognition receptors (PRRs) on immune cells. These surface antigens include proteins, lipids, and carbohydrates that may be interpreted as foreign or danger signals by the host immune system. Théry et al. (2018), in the MISEV2018 position statement, noted phosphatidylserine exposure among the surface characteristics used to describe extracellular vesicles; this phospholipid is normally confined to the inner leaflet of the plasma membrane. This exposure can lead to recognition by macrophages through phosphatidylserine receptors, resulting in rapid clearance of exosomes from circulation.

The glycosylation patterns on exosome surfaces can also significantly influence their immunogenicity. Williams et al. (2019) reviewed metabolomic approaches to extracellular vesicles, which together with glycomic analyses are being used to define the surface features that shape vesicle interactions with immune cells. Post-translational modifications therefore matter in exosome engineering for therapeutic applications in MS.

2.2.2. Complement Activation

The complement system, a key component of innate immunity, can be activated by exosomes, leading to their opsonization and subsequent phagocytosis. Ricklefs et al. (2018) showed that glioblastoma-derived extracellular vesicles carry PD-L1 and mediate immune evasion, illustrating how vesicle surface molecules shape the host response to them. This complement activation not only marks exosomes for clearance but can also generate inflammatory mediators that further stimulate immune responses.

Interestingly, the degree of complement activation can vary depending on the cellular origin of exosomes. For example, studies have shown that exosomes derived from tumour cells tend to activate the complement system more robustly than those from normal cells, likely due to the presence of tumour-associated antigens on their surface (Théry et al., 2009). Such variation means that exosome sources must be chosen carefully when developing exosome-based therapies for conditions such as MS.

2.2.3. Antigen Presentation

Exosomes can serve as vehicles for antigen presentation, a property that has been exploited in vaccine development but poses challenges in other therapeutic contexts. Pitt et al. (2014) demonstrated that dendritic cell-derived exosomes could directly present antigens to T cells, stimulating potent immune responses. While beneficial for cancer immunotherapy, this antigen-presenting capability can be problematic when immune activation is undesirable, such as in the treatment of autoimmune disorders like MS.

Furthermore, Benites et al. (2019) showed that exosomes could transfer antigens to professional antigen-presenting cells (APCs), indirectly stimulating T cell responses. This mechanism of cross-presentation adds another layer of complexity to the immunogenicity of exosome-based therapies, as even exosomes not directly derived from immune cells can potentially initiate adaptive immune responses in MS patients.

2.2.4. Cytokine Induction

The interaction of exosomes with immune cells can trigger the production and release of various cytokines and chemokines, shaping the overall immune response. For example, studies have shown that certain exosome populations can induce the release of pro-inflammatory cytokines such as TNF-α and IL-6 from macrophages, which may exacerbate inflammatory conditions relevant to MS (Buzas et al., 2014).

Conversely, Riazifar et al. (2019) demonstrated that mesenchymal stem cell-derived exosomes could promote the production of anti-inflammatory cytokines like IL-10 and TGF-β, suggesting a potential immunomodulatory role. This dual nature of exosome-induced cytokine responses reinforces the complexity of predicting and managing immune reactions to exosome-based therapies in MS.

2.3. Factors Influencing Immune Responses to Exosomes

The nature and intensity of immune responses to administered exosomes can be influenced by a multitude of factors,

ranging from the intrinsic properties of the exosomes themselves to the physiological state of the recipient. Understanding these factors is essential for developing strategies to mitigate unwanted immune reactions and optimise the therapeutic efficacy of exosome-based interventions in MS.

2.3.1. Cellular Origin

The source of exosomes contributes substantially to determining their immunogenicity. Exosomes derived from different cell types can elicit varying degrees of immune response due to differences in their molecular composition and surface antigen profile. Robbins and Morelli (2014) reviewed how extracellular vesicles regulate immune responses, noting that vesicles from immune cells such as dendritic cells are more immunostimulatory than those from mesenchymal stromal cells or fibroblasts. This differential immunogenicity is attributed to the presence of co-stimulatory molecules and MHC complexes on immune cell-derived exosomes, which are central for T cell activation.

In the context of MS therapy, the choice of exosome source is particularly critical. For instance, exosomes derived from regulatory T cells (Tregs) have shown promise in suppressing autoimmune responses in MS models. Research has shown that Treg-derived exosomes can effectively inhibit the proliferation and activation of autoreactive T cells, as observed in experimental autoimmune encephalomyelitis (EAE) models, thereby highlighting their potential as a novel immunomodulatory therapy for MS (Okoye et al., 2014).

2.3.2. Route of Administration

The method by which exosomes are delivered can significantly impact their interaction with the immune system and, consequently, their immunogenicity. Systemic administration, such as intravenous injection, typically results in higher immune exposure compared to local or targeted delivery methods. Alptekin et al. (2021) used pulsed focused ultrasound as a non-invasive method to increase exosome delivery to the brain, one of several strategies aimed at improving CNS targeting while limiting systemic exposure in neurological applications such as MS.

Furthermore, Wiklander et al. (2015) conducted a detailed study comparing different routes of exosome administration, including intravenous, intraperitoneal, and subcutaneous injections. They found that the biodistribution and subsequent immune recognition of exosomes varied significantly depending on the administration route, with intravenous injection leading to rapid accumulation in the liver and spleen, organs rich in immune cells. The route of administration therefore has to be matched in the context of the intended therapeutic application and desired immune response profile in MS treatment.

2.3.3. Dose and Frequency

The quantity of exosomes administered, and the frequency of administration can significantly influence the likelihood and intensity of immune recognition and response. Higher doses and repeated administrations of exosomes may increase the risk of immune sensitization and the development of anti-exosome antibodies. Charoenviriyakul et al. (2018) showed that lyophilization can preserve exosomes at room temperature, a practical consideration for repeated dosing regimens.

In the context of MS therapy, where long-term treatment may be necessary, the potential for immune sensitization with repeated exosome administration poses a significant challenge. Strategies to mitigate this risk, such as alternating exosome sources or engineering "stealth" exosomes with reduced immunogenicity, are areas of active research (de Jong et al., 2019). By modifying exosomal membranes or incorporating immunomodulatory molecules, researchers are working to create exosomes that evade immune detection, thereby making them more suitable for chronic use in conditions like MS.

2.3.4. Patient-Specific Factors

Individual variations in immune status, genetic background, and pre-existing conditions can significantly influence the immune response to exosomes in MS patients. This heterogeneity poses challenges in predicting and managing immune reactions in diverse patient populations. Théry et al. (2018) emphasized rigorous characterisation and reporting of vesicle preparations, without which the influence of patient-specific factors on efficacy and immune reactions cannot be assessed.

Recent work has highlighted the role of the gut microbiome in modulating immune responses to exosomes. For example, studies have indicated that variations in the composition of the intestinal microbiota can significantly influence systemic immune responses, potentially affecting the efficacy of exosome therapies (Heintz-Buschart & Wilmes, 2018). This insight opens up new possibilities for targeting the gut microbiome to optimise exosome-based treatments for MS, as individual microbiome compositions may explain some of the observed variability in therapeutic outcomes.

2.4. Consequences of Unwanted Immune Responses

The development of unwanted immune responses to exosomes can have several detrimental effects on their therapeutic efficacy and safety, potentially limiting their clinical utility in MS treatment.

2.4.1. Reduced Efficacy

Immune recognition and clearance of exosomes can significantly diminish their therapeutic potential by reducing their bioavailability and ability to reach intended target tissues. Smyth et al. (2015) reported that unmodified tumour-derived exosomes were cleared rapidly from the circulation after

systemic administration, which limits the time available for them to reach their targets. This accelerated clearance not only reduces the overall efficacy of the treatment but may also necessitate higher or more frequent dosing, potentially exacerbating immune reactions and increasing the risk of side effects in MS patients.

2.4.2. Altered Biodistribution

Immune responses can profoundly affect the biodistribution profile of administered exosomes, potentially diverting them from their intended targets and altering their therapeutic impact. Imai et al. (2015) showed that systemically administered exosomes are cleared from the blood in a macrophage-dependent manner, accumulating in organs rich in phagocytic cells such as the liver and spleen. This altered biodistribution can result in reduced accumulation at therapeutic sites in the CNS and increased off-target effects, potentially compromising both efficacy and safety in MS treatment.

2.4.3. Inflammatory Reactions

In some cases, immune responses to exosomes can trigger inflammatory reactions, leading to adverse effects that may range from mild local inflammation to severe systemic responses. For instance, studies have shown that certain exosome formulations can induce the release of pro-inflammatory cytokines from immune cells, which may exacerbate existing inflammatory conditions or initiate new inflammatory processes. These inflammatory reactions not only pose safety concerns but can also alter the local tissue environment, potentially interfering with the intended therapeutic actions of the exosomes in MS (Gupta & Pulliam, 2014).

2.4.4. Development of Anti-Exosome Antibodies

Repeated exposure to exosomes can lead to the production of anti-exosome antibodies, which may neutralize subsequent doses and limit long-term therapeutic efficacy. This adaptive immune response poses significant challenges for chronic or repeated exosome-based therapies, which may be necessary for managing a chronic condition like MS. Studies have shown that anti-exosome antibodies can cross-react with endogenous exosomes, potentially disrupting physiological processes that rely on these vesicles (Witwer & Théry, 2019). Immune responses therefore require vigilant monitoring in exosome-based therapies to mitigate potential long-term consequences, including the disruption of normal cellular communication pathways.

2.5. Strategies to Mitigate Unwanted Immune Responses

To address the challenge of unwanted immune responses to exosomes in MS therapy, researchers are exploring various innovative approaches aimed at enhancing the immune evasion capabilities of therapeutic exosomes while preserving their functional properties.

2.5.1. Surface Modification

Modifying the surface of exosomes with immune-evading molecules has attracted attention as an investigational strategy to reduce their recognition by the immune system and prolong their circulation time. Kamerkar et al. (2017) demonstrated that displaying CD47, a "don't eat me" signal, on the surface of exosomes significantly enhanced their circulation time and reduced macrophage uptake. This approach mimics the strategy used by some cancer cells to evade immune surveillance and has shown promise in improving the pharmacokinetics of exosome-based therapies.

In the context of MS, where long-term treatment may be necessary, such surface modifications could be particularly valuable in maintaining therapeutic efficacy over extended periods. Ongoing research is exploring the potential of combining multiple immune-evading signals on exosome surfaces to further enhance their stealth properties and therapeutic potential in autoimmune conditions like MS.

2.5.2. Immunomodulatory Cargo

Loading exosomes with immunosuppressive or immunomodulatory molecules represents another approach to managing unwanted immune responses. Mokarizadeh et al. (2012) showed that exosomes carrying TGF-β could induce regulatory T cells, potentially suppressing unwanted immune reactions. This strategy leverages the natural cargo-carrying capabilities of exosomes to deliver immunomodulatory signals directly to recipient immune cells.

Other work has explored the potential of engineering exosomes to express high levels of programmed death-ligand 1 (PD-L1), a key immune checkpoint molecule. For example, Poggio et al. (2019) demonstrated that PD-L1-rich exosomes can effectively suppress T cell activation and proliferation, indicating an investigational approach for inducing localised immunosuppression, which could be beneficial in autoimmune diseases like MS.

2.5.3. Cell Source Selection

Choosing exosome sources that naturally possess immunomodulatory properties can help reduce immune recognition and response. Mesenchymal stem cells (MSCs) have been investigated as a particularly attractive source due to their inherent immunosuppressive capabilities. Bruno et al. (2015) reported that MSC-derived exosomes exhibited potent immunomodulatory effects, including the ability to suppress T cell proliferation and promote the expansion of regulatory T cells.

In the context of MS therapy, the use of MSC-derived exosomes has shown particular promise. Riazifar et al. (2019) demonstrated that MSC-derived exosomes could effectively suppress neuroinflammation and promote remyelination in

an EAE model of MS, highlighting their potential as a cell-free therapeutic approach for MS treatment.

2.5.4. Personalized Approaches

Developing patient-specific exosome formulations using autologous cells could minimise immune recognition and potentially improve therapeutic outcomes. While logistically challenging, this approach could reduce the risk of immune reactions, particularly in the context of long-term or repeated therapies for chronic conditions like MS.

A study by Besse et al. (2016) examined the use of autologous dendritic cell-derived exosomes for immunotherapy in cancer patients. By generating therapeutic exosomes from the patient's own cells, they observed a lower incidence of neutralizing antibody formation and enhanced long-term efficacy compared to exosomes derived from allogeneic sources. Although this research was focused on cancer, the principles could be applied to MS treatment, where effective long-term immune management is critical.

2.5.5. Novel Delivery Strategies

Exploring alternative delivery routes and formulations can help bypass systemic immune exposure and enhance the targeted delivery of exosomes. Gudbergsson et al. (2019) systematically reviewed targeted extracellular vesicles for drug delivery and highlighted the methodological and biological heterogeneity that complicates comparisons between delivery strategies. This approach not only minimizes exposure to circulating immune cells but also provides a controlled release mechanism, potentially prolonging the therapeutic effect of the exosomes.

In the context of MS, where targeted delivery to the CNS is important, innovative approaches such as intranasal administration or the use of focused ultrasound to temporarily open the blood-brain barrier are being explored to enhance the delivery of therapeutic exosomes while minimising systemic immune exposure (Zhuang et al., 2011; Meng et al., 2021).

2.6. Future Directions and Emerging Technologies

As research in exosome-based therapies for MS progresses, several emerging technologies and approaches may help to address the challenges related to immune responses.

Advanced genetic engineering techniques are being explored to create exosomes with improved immune-evading properties. For example, CRISPR-Cas9 technology has been employed to modify exosome-producing cells, enabling them to either knock out immunogenic epitopes or express specific immunomodulatory molecules, thereby reducing the likelihood of immune recognition and enhancing their therapeutic potential (Yim et al., 2016). These approaches could facilitate the use of exosomes in chronic conditions that require repeated administration, such as MS. Machine learning algorithms are increasingly used to predict the immunogenicity of exosomes based on their molecular composition and surface properties. This approach has the potential to enable the rational design of exosomes with optimised immune-evading characteristics, which could be highly beneficial for therapies targeting autoimmune diseases like MS. For example, Bruno et al. (2012) showed that microvesicles derived from mesenchymal stem cells enhanced survival in a lethal model of acute kidney injury, one of the observations that motivated interest in vesicle-based therapeutics. The use of exosomes alongside established immunomodulatory drugs is being investigated as a strategy to enhance therapeutic efficacy while managing immune responses. For example, research has shown that co-administration of exosomes with immunosuppressants can modulate immune activity, potentially reducing the risk of immune sensitization and optimizing therapeutic outcomes (Tian et al., 2014). Such approaches could provide a balanced way to leverage the benefits of exosome therapy while controlling immune responses. The development of synthetic nanoparticles that mimic the key immunomodulatory properties of exosomes while offering greater control over composition and cargo is an emerging area of research. These "artificial exosomes" could offer improved stability, reduced batch-to-batch variability, and enhanced immune-evading properties compared to natural exosomes (Fang et al., 2018). The use of exosomal content as biomarkers for predicting and monitoring immune responses to exosome therapy is being explored. This approach could facilitate more personalized exosome-based treatments in MS, allowing for real-time adjustments to therapy based on individual patient responses. Such advancements could improve treatment efficacy by tailoring therapy to the specific needs of each patient, thereby optimizing clinical outcomes. Advanced imaging techniques complete this group. The development of non-invasive imaging methods to track exosome biodistribution and immune interactions in real-time could provide valuable insights into the dynamics of immune responses to exosomes in MS patients. Recent advances in intravital microscopy and molecular imaging offer candidate tools for visualizing exosome-immune cell interactions in living organisms, potentially guiding the development of more effective delivery strategies with reduced immunogenicity.

2.7. Conclusion

The immune response to exosomes presents both challenges and opportunities in the development of exosome-based therapies for Multiple Sclerosis. While the immunomodulatory properties of certain exosome populations offer promising therapeutic potential, the risk of unwanted immune reactions necessitates careful consideration and innovative approaches to exosome engineering and delivery.

The integration of advanced technologies, from genetic engineering to artificial intelligence, is supporting the development of more sophisticated and targeted approaches to exosome therapy in MS.

However, several key questions remain to be addressed.

How can we reliably predict and control the immune response to exosomes in diverse MS patient populations? What are the long-term immunological consequences of repeated exosome administrations in chronic MS treatment? How can we optimise the balance between the immunomodulatory benefits and potential immunogenic risks of exosome therapy? What role can personalised medicine approaches play in tailoring exosome therapies to individual MS patients' immune profiles?

Addressing these questions will be important for realizing the full potential of exosome-based therapies in MS treatment. As research progresses, it is likely that a multimodal approach, combining various strategies to enhance immune evasion, optimise delivery, and modulate immune responses, will be necessary to overcome the immunological challenges associated with exosome therapy in MS.

EV-based therapy in MS remains preclinical. Further work should define the relevant cell of origin, cargo, dose, route, biodistribution, immune effects, and product-quality attributes, followed by controlled human studies designed to distinguish biological activity from clinically meaningful benefit.

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3. STANDARDIZATION AND QUALITY CONTROL

3.1. Introduction

Exosomes, nanoscale extracellular vesicles involved in intercellular communication, have developed into promising therapeutic agents and potential biomarkers for various diseases, including Multiple Sclerosis (MS). Their ability to cross biological barriers, including the blood-brain barrier, and deliver bioactive molecules such as proteins, lipids, and nucleic acids, has positioned them prominent in innovative treatment strategies for neurological disorders (Willis et al., 2017; Long et al., 2017). However, the translation of exosome-based therapies from bench to bedside faces significant challenges, particularly in standardisation and quality control.

Product variability affects both interpretation and safety. Isolation, characterisation, and functional validation should establish what is being administered and how consistently it can be produced.

Quality control should link product identity to purity, biological activity, and batch consistency. EV-biomaterial combinations add further variables, including retention, cargo release, and changes in distribution.

The standardisation and quality control of exosomes for MS treatments are not merely technical challenges but are fundamental to realizing the full potential of these nanoscale vesicles in advancing MS therapy. By addressing these challenges comprehensively, we support development of the development of more effective, personalised treatments that could significantly improve the lives of individuals affected by MS.

3.2. Exosome Biology and Heterogeneity

3.2.1. Exosome Biogenesis and Composition

Exosomes are formed through the inward budding of endosomal membranes, creating multivesicular bodies (MVBs) that subsequently fuse with the plasma membrane to release exosomes into the extracellular space (Théry et al., 2002). This process is intricately regulated by various molecular mechanisms, including the endosomal sorting complex required for transport (ESCRT) machinery and tetraspanin-enriched microdomains (Colombo et al., 2014).

The composition of exosomes reflects their cellular origin and the physiological state of the parent cell. They typically contain a diverse array of biomolecules, including:

The protein fraction includes membrane transport and fusion proteins (e.g., Rab GTPases, annexins), tetraspanins (CD9, CD63, CD81), heat shock proteins (HSP70, HSP90), and cell-type-specific proteins (Mathivanan et al., 2010). The lipid fraction comprises cholesterol, sphingomyelin, ceramide and phosphatidylserine, which contribute to the stability and fusogenic properties of exosomes (Skotland et al., 2017). Nucleic acids are another. Various RNA species, including mRNAs, microRNAs (miRNAs), and other non-coding RNAs, as well as DNA fragments (Valadi et al., 2007; Thakur et al., 2014).

3.2.2. Exosome Heterogeneity and Its Implications for MS Therapy

The heterogeneity of exosomes poses a significant challenge in their standardisation for therapeutic applications in MS. This heterogeneity stems from several factors.

Exosomes derived from different cell types (e.g., mesenchymal stem cells, neurons, immune cells) exhibit distinct molecular profiles that influence their biological functions (Komlakh et al., 2022; Mycko & Baranzini, 2020). The microenvironment of the parent cell, including stress conditions, inflammation, and disease states, can alter the cargo and surface characteristics of exosomes (Li et al., 2016). Different isolation techniques can yield exosome populations with varying purity and composition, affecting their downstream applications (Witwer et al., 2013).

MSC-derived EV preparations have been studied in MS-related experimental systems, but their activity varies with source, culture conditions and separation methods. Baharlooi et al. (2022) investigated human-cell immunological responses in vitro; that study did not demonstrate remyelination in patients.

Recent studies have highlighted the role of exosomal microRNAs in MS pathology and potential therapy. For example, Ebrahimkhani et al. (2017) identified specific miRNA signatures in circulating exosomes of MS patients that could serve as biomarkers for disease status and progression. Furthermore, the transfer of exosomal miRNAs has been implicated in the modulation of neuroinflammation and remyelination processes (Pusic et al., 2014).

The heterogeneity of exosomes also extends to their surface proteins, which play key roles in cellular targeting and uptake. Selmaj et al. (2017) reviewed the role of exosomes in CNS inflammation and MS, including reports that vesicles carrying CNS-derived proteins can be detected in the blood of patients, which has raised interest in their diagnostic potential.

To address the challenges posed by exosome heterogeneity, researchers are exploring various strategies.

Developing standardised protocols for exosome isolation and characterisation that account for the variability in cellular sources and physiological conditions (Théry et al., 2018). Implementing advanced analytical techniques, such as single-vesicle analysis and high-resolution proteomics, to better

characterize exosome subpopulations (Smith et al., 2015; Chiang & Chen, 2019). Exploring the use of engineered or "designer" exosomes with defined cargo and surface properties to enhance therapeutic efficacy and reduce variability (Antimisiaris et al., 2018).

Understanding and harnessing exosome heterogeneity is essential for developing effective MS therapies. By elucidating the specific exosome subpopulations that mediate beneficial effects in MS, researchers can work towards more targeted and personalised treatment approaches.

3.3. Challenges in Exosome Isolation and Characterization

3.3.1. Isolation Methods and Their Impact on Exosome Quality

The isolation of exosomes from biological fluids or cell culture supernatants is a critical step in their preparation for therapeutic applications. However, the choice of isolation method can significantly impact the yield, purity, and functionality of the obtained exosomes. Common isolation techniques include:

Ultracentrifugation, often considered the gold standard, involves sequential centrifugation steps to separate exosomes based on their density and size. While widely used, ultracentrifugation can lead to co-isolation of protein aggregates and other extracellular vesicles, potentially affecting the purity of the exosome preparation (Théry et al., 2006). Size-exclusion chromatography is one option. SEC separates particles based on their size as they pass through a porous stationary phase. This method can yield highly pure exosome preparations but may result in sample dilution (Böing et al., 2014). Immunoaffinity capture uses antibodies against specific exosome surface markers (e.g., CD63, CD9) to isolate exosomes. While highly specific, it may bias the isolation towards certain exosome subpopulations (Nakai et al., 2016). Commercial kits that use polymers to precipitate exosomes offer a quick and easy isolation method but can co-isolate contaminants and affect exosome functionality (Rider et al., 2016). Tangential flow filtration uses membrane filtration to concentrate and purify exosomes. TFF has shown promise for large-scale exosome production while maintaining their integrity (Haraszti et al., 2018).

Each isolation method has its advantages and limitations, and the choice can significantly impact downstream applications. Isolation method strongly affects yield and purity: size-exclusion chromatography and density gradient ultracentrifugation generally produce purer preparations, whereas polymer-based precipitation gives higher yields but lower purity.

3.3.2. Characterization Challenges and Emerging Technologies

Accurate characterisation of exosomes is essential for ensuring their quality and functionality in therapeutic applications. However, the nanoscale size and heterogeneity of exosomes pose significant challenges for their characterisation. Common characterisation techniques include:

Nanoparticle tracking analysis is one method. NTA provides information on particle size distribution and concentration but can be affected by sample preparation and instrument settings (Vestad et al., 2017). Dynamic light scattering (DLS) measures the size distribution of particles in suspension but may be less accurate for polydisperse samples like exosomes (Sokolova et al., 2011). Transmission electron microscopy (TEM) and cryo-electron microscopy provide high-resolution images of exosome morphology but require extensive sample preparation that can alter exosome structure (Théry et al., 2018). While traditional flow cytometry is limited by the small size of exosomes, advances in high-resolution flow cytometry have enabled the analysis of individual exosomes (Morales-Kastresana et al., 2017). Western blotting and ELISA can detect specific exosome markers but provide limited quantitative information and may not capture the heterogeneity of exosome populations (Lötvall et al., 2014).

Emerging technologies address some of these limitations.

Techniques such as nanoflow cytometry and super-resolution microscopy are enabling the characterisation of individual exosomes, providing insights into exosome heterogeneity (Tian et al., 2020; Hartjes et al., 2019). Raman spectroscopy, a label-free technique, can provide information on the biochemical composition of exosomes without extensive sample preparation (Kruglik et al., 2019). Surface plasmon resonance (SPR) allows real-time, label-free detection of exosomes and their interactions with other molecules (Rupert et al., 2014). Asymmetrical flow field-flow fractionation completes this list. This technique separates exosomes based on their size and can be coupled with various detectors for detailed characterisation (Zhang et al., 2019).

In the field of MS research, these advanced characterisation methods provide new insights into exosome biology. For example, Geraci et al. (2018) examined vesicle-mediated intercellular communication during clinical relapse and gadolinium-enhanced MRI activity in relapsing-remitting MS.

The integration of multiple characterisation techniques is central for a detailed understanding of exosome properties. A multi-parametric approach combining size, concentration, morphology, and molecular composition analysis can provide a more complete picture of exosome quality and functionality (Gandham et al., 2020).

3.4. Quality Control Measures for Exosome-Based Therapies

3.4.1. Establishing Quality Attributes for Therapeutic Exosomes

The development of robust quality control measures is essential for the successful translation of exosome-based therapies from bench to bedside. Establishing a set of critical quality attributes (CQAs) for therapeutic exosomes is essential to ensure their safety, efficacy, and consistency in clinical applications. These CQAs typically include:

These attributes cover the physical characteristics of the preparation; size distribution, concentration and morphology; its biochemical composition, meaning protein, lipid and nucleic acid content; its functional properties, including biological activity, stability and interaction with target cells; and its purity, that is, the absence of contaminants and of undesired co-isolated material.

MISEV2023 updates recommendations for EV nomenclature, separation, characterisation and functional studies. These research-reporting recommendations do not constitute regulatory authorization or certification of a therapeutic product (Welsh et al., 2024).

It should be noted that the earlier MISEV2018 recommendations have since been superseded. MISEV2023 replaces MISEV2018 as the reference framework for extracellular vesicle nomenclature, separation, characterisation and functional studies, and it is more explicit than its predecessor about the limits of inference from bulk vesicle preparations, about the need to report separation methods in terms of what they enrich rather than what they are assumed to purify, and about the distinction between research-reporting standards and evidence of therapeutic efficacy (Welsh et al., 2024). Throughout this book, where MISEV2018 is cited for a specific technical recommendation, readers preparing new work should consult the 2023 update.

Recent efforts have focused on developing more thorough quality control strategies. For instance, Rohde et al. (2019) proposed a systematic approach to defining CQAs for mesenchymal stromal cell-derived exosomes, emphasizing the importance of correlating physical and biochemical properties with functional outcomes.

In the context of MS therapy, specific quality attributes may include:

Immunomodulatory activity comes first: potency testing should measure the direction and magnitude of immune effects, since vesicle activity cannot be presumed beneficial from its source alone. Neuroprotective potential, the capacity to support neuronal survival and promote remyelination , is a second (Pusic et al., 2014), followed by efficiency in crossing the blood-brain barrier to reach CNS targets (Matsumoto et al., 2017) and the presence of MS-relevant proteins or miRNAs associated with therapeutic effects (Pusic et al., 2014).

3.4.2. Implementing Quality Control Strategies

Implementing broad quality control strategies for exosome-based therapies involves several key components.

The first is standardised production. Developing Good Manufacturing Practice (GMP) compliant protocols for exosome production, including defined culture conditions, harvest timing, and isolation methods (Witwer et al., 2019). Analytical methods must also be validated: establishing sensitive and reproducible methods for exosome characterisation, including quantitative assays for specific biomarkers (Valkonen et al., 2017). Potency assays are a third requirement, meaning functional assays that correlate with therapeutic efficacy, such as T cell suppression assays or oligodendrocyte differentiation assays for MS applications (Gimona et al., 2017). Stability testing assesses exosome preparations under various storage conditions and durations to define shelf life and optimal storage parameters (Charoenviriyakul et al., 2017). Contaminant testing detects and quantifies potential contaminants, including host cell proteins, endotoxins, and mycoplasma (Lener et al., 2015). Batch-to-batch consistency requires acceptance criteria for batch release and developing strategies to minimise variability between production runs (Lener et al., 2015).

Recent technological advancements are enhancing quality control capabilities. For example, Choi et al. (2019) used nano-flow cytometry to map subpopulations of cell-derived extracellular vesicles and particles, an approach that could support quality control during production.

In the field of MS research, novel quality control approaches are being explored. A study by Thompson et al. (2020) used cerebrospinal fluid extracellular vesicle proteomics to demonstrate altered protein homeostasis in amyotrophic lateral sclerosis, illustrating how proteomic profiling can characterize vesicle populations.

3.4.3. Challenges in Scaling Up Production

As exosome-based therapies move towards clinical applications, scaling up production while maintaining consistent quality presents significant challenges. Key considerations include:

Large-scale culture systems are needed that mimic that mimic the physiological conditions of exosome-producing cells (Watson et al., 2016). Adapting isolation methods for large-scale production while maintaining exosome quality is a significant challenge. Tangential flow filtration (TFF) has come to be seen as a promising technique for scalable exosome isolation. Haraszti et al. (2018) demonstrated that TFF can produce higher yields of exosomes from 3D-cultured mesenchymal stem cells compared to traditional ultracentrifugation methods, while also maintaining their biological activity. Ensuring batch-to-batch consistency in large-scale production is essential. Witwer et al. (2019) discussed vesicle nomenclature and the precision of terminology, which underpins consistent characterisation and reporting throughout the production process. Developing economically viable large-scale

production methods is essential for the clinical translation of exosome therapies. Colao et al. (2018) call for new approaches to reduce production costs while maintaining exosome quality. Scaling up production must also adhere to Good Manufacturing Practice (GMP) guidelines. Rohde et al. (2019) discuss the regulatory considerations for exosome manufacturing, emphasizing the need for standardised protocols that meet regulatory requirements.

3.5. Regulatory Considerations for Exosome-Based Therapies

3.5.1. Current Regulatory Landscape

The regulatory framework for exosome-based therapies is still evolving, presenting challenges for researchers and developers. Key aspects of the current regulatory landscape include:

Exosomes may be classified differently depending on their source and intended use. They could be considered biological products, cell therapy products, or even drug delivery vehicles, each with different regulatory pathways (Popowski et al., 2020). Regulatory agencies require full data on the safety and efficacy of exosome products. This includes demonstrating consistent composition, stability, and biological activity (Batrakova & Kim, 2016). Compliance with Good Manufacturing Practice (GMP) is essential for clinical-grade exosome production. This involves establishing rigorous quality control measures throughout the manufacturing process (Zhu et al., 2020). MISEV2023 supports rigorous EV research and reporting. Compliance does not itself establish product efficacy or regulatory approval (Welsh et al., 2024).

3.5.2. Challenges in Meeting Regulatory Requirements

Several challenges exist in meeting regulatory requirements for exosome-based therapies.

The lack of standardised methods for exosome isolation, characterisation, and quality control complicates regulatory compliance. Efforts are ongoing to establish consensus guidelines (Witwer et al., 2013). Product definition is another. Defining the active pharmaceutical ingredient (API) in exosome products can be challenging due to their complex and heterogeneous nature (Lener et al., 2015). Developing standardised potency assays that correlate with clinical efficacy is important but challenging due to the broad mechanisms of action of exosomes (Gimona et al., 2017). Demonstrating that quality and efficacy are maintained during scale-up from research to clinical production is a significant hurdle (Watson et al., 2016). Long-term stability is another. Establishing the shelf life and appropriate storage conditions for exosome products is essential for regulatory approval (Charoenviriyakul et al., 2018).

3.5.3. Strategies for Navigating Regulatory Pathways

To address these challenges, researchers and developers are adopting various strategies.

Proactive communication with regulatory bodies can help clarify requirements and streamline the approval process (Batrakova & Kim, 2016). Collaborative efforts between researchers, industry, and regulatory agencies are working towards establishing exosome-specific regulatory guidelines (Reiner et al., 2017). Participation in standardisation efforts, such as those led by ISEV, can help align research practices with evolving regulatory expectations (Théry et al., 2018). Multi-modal characterisation approaches can provide to provide a full understanding of exosome properties and functionality (Gandham et al., 2020). Utilizing adaptive clinical trial designs that allow for the refinement of exosome products based on early clinical data.

3.6. Innovative Approaches in Exosome Therapy for Multiple Sclerosis

3.6.1. Engineered Exosomes for Enhanced Therapeutic Efficacy

Researchers are exploring various strategies to enhance the therapeutic potential of exosomes for MS treatment.

Surface modification is one approach. Tailoring exosome surface proteins to improve targeting to specific cell types or tissues. For example, Alvarez-Erviti et al. (2011) demonstrated that exosomes engineered to express a neuron-specific peptide could efficiently deliver siRNA to the brain. Cargo loading is another. Developing methods to load exosomes with specific therapeutic molecules. Didiot et al. (2016) showed that exosomes could be loaded with hydrophobically modified siRNA silencing huntingtin mRNA, suggesting potential applications in other CNS diseases. Identifying and using cell sources that produce exosomes with enhanced therapeutic properties. Riazifar et al. (2019) found that exosomes derived from interferon-γ-primed mesenchymal stem cells exhibited superior immunomodulatory effects in an MS model.

3.6.2. Exosome-Biomaterial Combinations

Integrating exosomes with biomaterials offers new possibilities for targeted delivery and sustained release.

Vesicle activity itself depends on culture conditions: Mao et al. (2019) showed that hypoxic exosomes alter the phenotype and transcriptome of endothelial cells. Incorporating exosomes into nanofibre scaffolds can provide localised delivery and support for tissue regeneration. This approach could be adapted to promote remyelination in MS (Qian et al., 2017). Exosome-nanoparticle hybrids are another. Combining exosomes with synthetic nanoparticles can enhance their stability and targeting capabilities. Jia et al. (2018) created peptide-targeted, cargo-loaded exosomes for improved delivery to brain tumours.

3.6.3. Exosomes as Biomarkers in MS

Exosomes are being investigated as potential biomarkers for MS diagnosis, prognosis, and treatment response.

miRNA profiles: Ebrahimkhani et al. (2017) identified specific miRNA signatures in circulating exosomes of MS patients that could serve as biomarkers for disease status and progression. Galazka et al. (2018) detected myelin proteins in serum-derived exosomes from MS patients, suggesting their potential as diagnostic markers. Treatment-response monitoring is one application. Prospective longitudinal validation is required before a vesicle measurement can guide treatment. The mechanistic study by Kimura et al. (2018) does not establish this application.

3.7. Future Directions and Conclusions

3.7.1. Emerging Technologies in Exosome Research

Several advanced technologies are poised to advance exosome research and therapy development.

Techniques such as nanoflow cytometry and super-resolution microscopy are enabling the characterisation of individual exosomes, providing striking insights into exosome heterogeneity (Tian et al., 2020). Artificial intelligence and machine learning are being applied to analyse large datasets generated from exosome characterisation, potentially identifying novel biomarkers and optimising production processes. Advanced microfluidic platforms are being developed for rapid isolation and analysis of exosomes, which could streamline quality control processes (Liu et al., 2022).

3.7.2. Addressing Translational Challenges

To facilitate the clinical translation of exosome-based therapies for MS, several key areas require focused research.

Continued efforts to establish standardised protocols for exosome isolation, characterisation, and quality control are essential (Witwer et al., 2019). The development of GMP-compliant, cost-effective methods for large-scale exosome production is key for clinical applications (Watson et al., 2016). Improving methods to track exosome biodistribution and fate in vivo will enhance our understanding of their therapeutic mechanisms and safety profiles (Wiklander et al., 2015). Personalised approaches complete this list. Exploring the potential for patient-specific exosome therapies, taking into account individual disease characteristics and treatment responses (Reiner et al., 2017).

The regulatory position remains the limiting step rather than a formality. A 2025 review of vesicle-based medicinal products observed that although more than a hundred clinical studies of natural and engineered vesicle drugs have been registered worldwide, no drug regulatory authority has yet issued technical evaluation guidance specific to this class of product, which leaves sponsors working from ISEV nomenclature and the MISEV reporting framework alongside general requirements for biologics (Xu et al., 2025). For MS, where no vesicle product has yet entered a registered trial, the practical consequence is that quality specifications will be set by whoever attempts the first one.

3.7.3. Conclusions

Exosome-based therapies are being investigated for Multiple Sclerosis, offering potential advantages in targeting, biocompatibility, and multiple therapeutic effects. However, significant questions remain open in standardising their production, ensuring consistent quality, and navigating the evolving regulatory landscape.

The field of exosome research is rapidly advancing, driven by interdisciplinary collaborations and technological innovations.

Clinical development depends on reproducible products, validated potency assays, and trials capable of identifying harm as well as benefit. Engineering advances do not remove those requirements.

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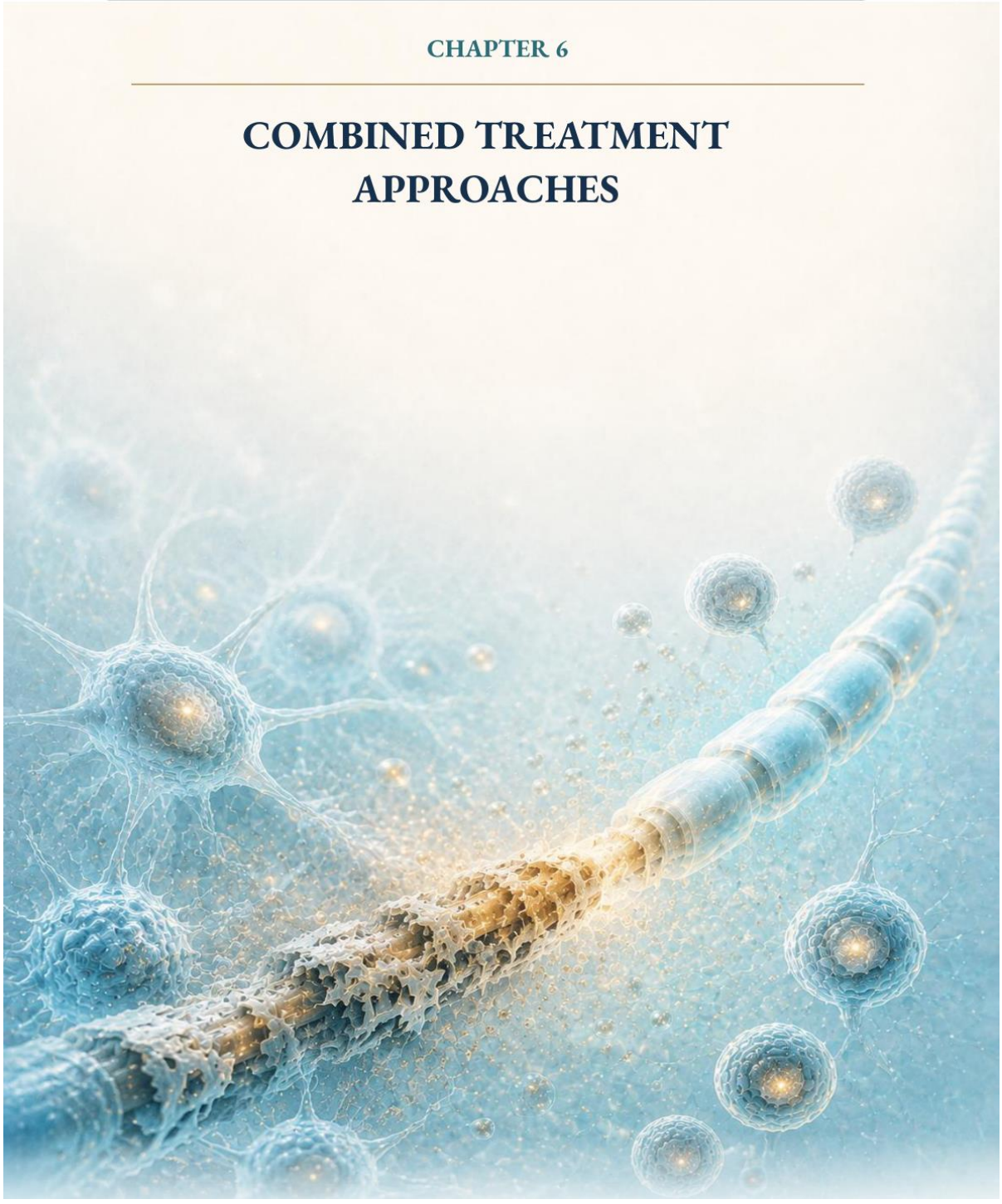
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CHAPTER 6

COMBINED TREATMENT APPROACHES



6.1. RATIONALE FOR COMBINING STEM CELL AND EXOSOME THERAPIES

Combining mesenchymal stromal cells with EV-based products is an investigational approach in MS. Proposed complementary effects on inflammatory and repair-related pathways have been explored mainly in experimental settings; clinical synergy, optimal dosing, and long-term safety have not been established. Figure 6.1.1 sets out the rationale for combination and the point at which it remains hypothesis.

Recent reviews discuss immunoregulatory cargo and cell-free repair strategies in MS (Sayed et al., 2026; Yao et al., 2026). These mechanistic syntheses help identify experimental targets, but they do not supply controlled evidence that combining MSCs with EVs improves outcomes in patients. Product identity, dosing and prospective safety assessment remain necessary.

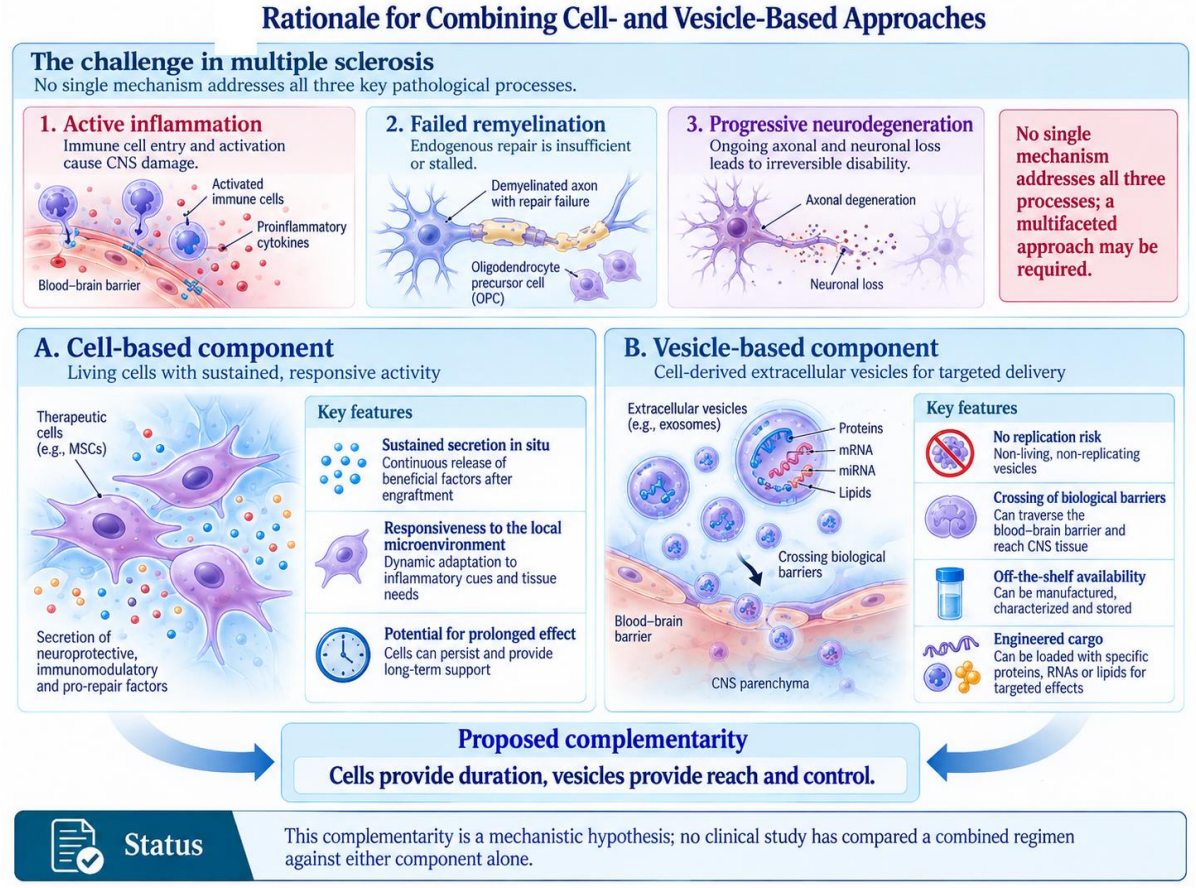


Figure 6.1.1. Rationale for combining cell- and vesicle-based approaches. The proposed complementarity is a mechanistic hypothesis; no clinical study has compared a combined regimen against either component alone.

Mesenchymal stem cells have long been recognised for their considerable plasticity and therapeutic potential in regenerative medicine. These multipotent cells, which can be isolated from various sources including bone marrow, adipose tissue, and umbilical cord, are defined by their capacity to differentiate into mesodermal lineages such as bone, cartilage and fat (Uccelli et al., 2008); reports of differentiation into neurons and oligodendrocytes rest largely on marker expression in vitro and remain contested, and the therapeutic rationale in MS is based on paracrine and immunomodulatory activity rather than on cell replacement. In experimental systems relevant to MS, MSCs have shown immunomodulatory, anti-inflammatory and trophic effects, including proposed support for neuroprotection and tissue repair (Scolding et al., 2017).

The immunomodulatory properties of MSCs are particularly relevant in the treatment of MS, an autoimmune condition characterised by aberrant immune responses against myelin and oligodendrocytes. MSCs have been shown to suppress the proliferation and activation of autoreactive T cells, induce regulatory T cells, and modulate the function of B cells and natural killer cells (Glennie et al., 2005). Furthermore, MSCs can alter the polarisation of macrophages from a pro-inflammatory M1 phenotype to an anti-

inflammatory M2 phenotype, contributing to a more favourable immunological environment in the CNS (Cho et al., 2014).

Recent research has elucidated the molecular mechanisms underlying the immunomodulatory effects of MSCs in MS. For instance, MSCs have been shown to secrete various anti-inflammatory factors, including transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), and prostaglandin E2 (PGE2), which collectively contribute to the suppression of inflammatory responses and the promotion of regulatory immune cell populations (Uccelli et al., 2011). Additionally, MSCs express indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes the degradation of tryptophan, leading to the suppression of T cell proliferation and the induction of regulatory T cells (Meisel et al., 2004).

In addition to their direct effects on the CNS, MSC-based products have been proposed to modulate the blood-brain barrier (BBB), a critical interface that is often compromised in MS. Supporting evidence is indirect: for example, conditioned medium from stem cells of human exfoliated deciduous teeth ameliorated experimental autoimmune encephalomyelitis (Shimojima et al., 2016). Whether MSCs strengthen BBB integrity in people with MS, and whether such an effect would prevent exacerbations, has not been established.

The primary endpoints in MESEMS, the Harris et al. (2024) MSC-NP trial, and SMART-MS were not met, whereas Petrou et al. (2020) reported a favourable treatment-failure result in a small randomised trial. Product and protocol differences limit comparison. MSC-derived EVs offer a way to investigate secreted biological activity, but size alone cannot identify an exosome; EV or small EV is the appropriate operational term when endosomal origin has not been established (Théry et al., 2002).

The therapeutic potential of MSC-derived exosomes in MS treatment is complex and continues to be an area of intense research. Extracellular vesicles can cross the blood-brain barrier in experimental settings, and inflammation can influence their brain uptake (Banks et al., 2020). This property could be advantageous in MS, where delivery of therapeutic agents to sites of inflammation and tissue damage is important, although direct comparisons of CNS delivery between vesicles and their parent cells are lacking.

The protein content of MSC-derived exosomes has also been studied for its therapeutic potential in MS. Proteomic analyses have revealed that these exosomes carry a diverse array of proteins involved in immune regulation, neuroprotection, and tissue repair. For instance, exosomes from human adipose MSCs modulated the response of in vitro-stimulated T cells (Blazquez et al., 2014). Galectin-1, a protein reported in MSC secretomes, can suppress T cell activation and promote the apoptosis of activated T cells, making it a candidate mediator of interest in MS (Cedeno-Laurent & Dimitroff, 2012).

In addition to their immunomodulatory effects, MSC-derived exosomes have shown neuroprotective and neuroregenerative properties in experimental models. These vesicles can carry neurotrophic factors and growth-promoting molecules that may support neuronal survival and axonal regeneration (Xin et al., 2013). Evidence for vesicle-mediated remyelination comes mainly from exosomes of interferon-γ-stimulated dendritic cells, which are enriched in miR-219 (Pusic et al., 2014); comparable data for miR-219-engineered MSC exosomes in MS models are lacking.

The neuroprotective effects of MSC-based products may extend beyond neurotrophic factors to cellular metabolism, which is often impaired in MS. Bone marrow-derived stromal cells have been shown to transfer mitochondria to pulmonary alveolar epithelium and protect against acute lung injury (Islam et al., 2012). Whether comparable mitochondrial transfer to neurons occurs, and whether it contributes to neuroprotection in MS, remains to be demonstrated.

MSC-derived EVs may offer practical advantages for storage, transport and repeated administration, but these depend on validated manufacturing and stability procedures (Phinney & Pittenger, 2017). EVs do not contain viable cells, so some cell-associated risks may be avoided; however, their immunogenicity, toxicity, cargo-related effects and long-term safety require product-specific evaluation. Neither the superiority of EVs to parent MSCs nor the added benefit of combined MSC-EV treatment has been established in people with MS.

Advances in exosome engineering have opened possibilities for enhancing the effects of MSC-derived exosomes. Strategies proposed for MS include modifying exosome content, for example by enriching vesicles with specific miRNAs or proteins that target pathways involved in disease pathogenesis; these approaches remain experimental.

The integration of biomaterials with exosome therapy represents another innovative approach in MS treatment. Encapsulating MSC-derived exosomes within biocompatible scaffolds or hydrogels can prolong their retention time in

the target tissues and provide a controlled release of therapeutic cargo. This strategy has shown promise in enhancing the regenerative effects of exosomes in various neurological conditions and may offer similar benefits in MS treatment (Mao et al., 2019). For instance, a recent study demonstrated that exosomes encapsulated in a hyaluronic acid-based hydrogel exhibited enhanced neuroprotective effects and promoted functional recovery in a mouse model of spinal cord injury, suggesting potential applications in MS treatment (Rao et al., 2019).

The use of biomaterials in exosome delivery for MS treatment extends beyond simple encapsulation. Researchers are exploring the development of "smart" biomaterials that can respond to specific environmental cues, such as changes in pH or the presence of inflammatory mediators, to trigger the release of exosomes at the optimal time and location within the CNS (Tian et al., 2018). These advanced delivery systems could significantly enhance the efficacy of exosome-based therapies by ensuring targeted and sustained release of therapeutic cargo in response to disease activity.

Another avenue in MSC-exosome research for MS is the development of "designer" exosomes engineered to target specific cell types or tissues within the CNS. By modifying the surface proteins of exosomes, researchers aim to enhance their ability to cross the blood-brain barrier and deliver therapeutic cargo to specific cell populations (Alvarez-Erviti et al., 2011). For example, exosomes targeted with a neuropilin-1-binding peptide and loaded with therapeutic cargo enabled simultaneous imaging and therapy of glioma in experimental models (Jia et al., 2018); analogous targeting strategies for MS remain to be developed.

MSC- and exosome-based approaches are also being studied in other neurodegenerative and autoimmune disorders, including Alzheimer's disease, Parkinson's disease, and arthritis (Cui et al., 2018; Reza-Zaldivar et al., 2018; Cosenza et al., 2018). For example, in an Alzheimer's disease model, exosomes from human umbilical cord MSCs alleviated neuroinflammation and reduced amyloid-β deposition by modulating microglial activation (Ding et al., 2018). In a Parkinson's disease model, designer exosomes produced by implanted engineered cells delivered therapeutic cargo into the brain (Kojima et al., 2018). These studies used single products rather than combined cell and vesicle therapy.

The application of MSC-exosome therapy in other autoimmune disorders shares similarities with its use in MS, particularly in terms of immunomodulation. In rheumatoid arthritis, for instance, MSC-derived exosomes have been shown to suppress the activation of synovial fibroblasts and reduce the production of pro-inflammatory cytokines, leading to decreased joint inflammation and cartilage degradation (Cosenza et al., 2018). What follows from this is that the immunomodulatory properties of MSC-derived exosomes could be harnessed to treat a wide range of autoimmune conditions, potentially advancing the field of immunotherapy.

While the combined use of MSCs and their derived exosomes in MS treatment warrants further investigation, several problems remain to be addressed. Standardisation of exosome isolation and characterisation methods is central for ensuring consistent therapeutic effects and facilitating clinical translation (Théry et al., 2018). The International Society for Extracellular Vesicles has published guidelines for minimal information for studies of extracellular vesicles (MISEV2018), which aim to improve the rigor and reproducibility of exosome research (Théry et al., 2018). However, the implementation of these guidelines across different research groups and the development of standardised protocols for large-scale exosome production remain ongoing challenges.

Optimising the dosing regimen and route of administration for both MSCs and exosomes in MS treatment requires further investigation. While intravenous administration is the most common route for both, intranasal delivery is being explored to improve CNS targeting. The intranasal route offers several potential advantages, including non-invasiveness, access to the CNS via the olfactory and trigeminal nerve pathways, and avoidance of first-pass metabolism (Jiang et al., 2011). Intranasal MSC-derived exosomes ameliorated autistic-like behaviours in BTBR mice (Perets et al., 2018), but whether this route is suitable, let alone preferable, for MS has not been tested clinically.

The development of biomarkers to predict and monitor treatment responses will be important for personalizing therapy and maximizing clinical outcomes. Researchers are exploring various approaches to identify reliable biomarkers for MSC-exosome therapy in MS. These include the analysis of circulating exosomes in patient blood samples, which may reflect disease activity and treatment response (Selmaj et al., 2017). Additionally, advanced imaging techniques, such as positron emission tomography (PET) with novel tracers targeting neuroinflammation or remyelination, could provide valuable insights into the therapeutic effects of MSC-exosome therapy in vivo (Bodini et al., 2016).

The long-term safety and efficacy of combined MSC and exosome therapies in MS require evaluation in well-designed clinical trials. The 2019 MESEMS publication cited in this context describes the planned trial design; it is not an efficacy or safety-results report. The long-term effects of repeated exosome administration remain unknown. Potential risks, including product-specific immunogenic, off-target, and theoretical tumour-promoting effects, require systematic monitoring (Hassanzadeh et al., 2021).

To address these safety concerns, researchers are developing strategies to enhance the control and tracking of administered exosomes. One approach involves the use of genetically engineered MSCs that produce exosomes with specific "kill switches" or degradation signals, allowing for the controlled elimination of therapeutic exosomes after they have exerted their effects. Another strategy involves the development of non-invasive imaging techniques to track the biodistribution and fate of administered exosomes in vivo, providing valuable information on their therapeutic activity and potential off-target effects (Lai et al., 2014).

Several emerging areas could strengthen the case for combined MSC and exosome therapy in MS. One such area is the exploration of exosome-mediated delivery of gene editing tools, such as CRISPR-Cas9, to correct genetic abnormalities associated with MS susceptibility or progression (Usman et al., 2018). This approach could offer a more targeted and personalised treatment strategy for MS patients with specific genetic risk factors.

The integration of artificial intelligence (AI) and machine learning algorithms in exosome research is also gaining traction. These advanced computational tools can help in predicting the most effective exosome compositions for specific MS subtypes, optimising treatment protocols, and identifying novel biomarkers for disease progression and treatment response (Zhu et al., 2017). AI-driven approaches could also facilitate the analysis of large-scale proteomics and genomics data from MSC-derived exosomes, potentially uncovering new therapeutic targets and mechanisms of action (Simeone et al., 2020).

Potential interactions between MSC-derived EVs and remyelinating or neuroprotective agents warrant preclinical investigation. Theoretical complementarity does not establish synergy, functional repair, or improved outcomes in patients. Candidate combinations such as clemastine or biotin with EV products would need direct evaluation (Plemel et al., 2017; Green et al., 2017; Tourbah et al., 2016). Clinical interpretation should also consider the later disability-worsening signal in a small advanced-progressive-MS cohort; clemastine is not an established MS repair therapy (Kocot et al., 2025).

The role of the gut microbiome in MS pathogenesis and treatment response is an emerging area of research that may have implications for MSC-exosome therapy. Recent studies have highlighted the complex interactions between the gut microbiome, the immune system, and the CNS in MS, suggesting that modulation of the gut microbiome could enhance the efficacy of MSC-exosome treatments (Corrêa-Oliveira et al., 2016). For instance, certain gut bacterial species have been shown to produce metabolites that can promote regulatory T cell differentiation and suppress inflammation, effects that could synergize with the immunomodulatory properties of MSC-derived exosomes (Furusawa et al., 2013).

Researchers are now exploring the possibility of engineering MSC-derived exosomes to target specific aspects of the gut-brain axis in MS. This could involve loading exosomes with microRNAs or proteins that modulate gut barrier function, influence microbial composition, or enhance the production of beneficial metabolites by gut bacteria (Burgelman, 2023). Such an approach could address both the CNS and peripheral aspects of MS pathology, offering a more integrated treatment strategy.

Progressive MS remains a major focus for repair-oriented research (Ontaneda et al., 2017). Product-specific preclinical evidence should be distinguished from human treatment evidence. Results from dendritic cell vesicles cannot be relabeled as neural stem cell or engineered MSC-vesicle results; the source and setting of the Pusic et al. (2014) study are described in Section 5.7.

To enhance the efficacy of MSC-exosome therapy in progressive MS, researchers are exploring various strategies to boost the neuroprotective and regenerative cargo of exosomes. One approach involves pre-conditioning MSCs under hypoxic conditions or exposing them to inflammatory stimuli before exosome isolation, which has been shown to enhance the therapeutic potential of the secreted exosomes (Xu et al., 2019). Another strategy involves genetic modification of MSCs to overexpress specific neuroprotective factors, such as brain-derived neurotrophic factor (BDNF) or insulin-like growth factor 1 (IGF-1), resulting in exosomes enriched with these beneficial proteins (Nakano et al., 2016).

Experimental studies of MSC-derived EVs have reported functional recovery and neurovascular plasticity after

stroke in rats (Xin et al., 2013). These findings provide a rationale for further research but do not establish synaptic repair, formation of functional neural circuits, or clinical recovery in MS patients. Any application to long-standing MS disability remains an investigational hypothesis.

Combining MSC-derived extracellular vesicles with physical rehabilitation or cognitive training is a future research hypothesis. It could be investigated as a way to address biological and functional aspects of recovery, but an established clinical benefit of this combination in MS has not been demonstrated.

The development of "smart" exosomes that can respond to specific environmental cues within the CNS is another active area of investigation in MSC-exosome therapy for MS. Researchers are exploring the creation of exosomes with surface proteins that can detect inflammatory mediators or oxidative stress, triggering the release of their therapeutic cargo only in areas of active MS pathology (Armstrong et al., 2017). This targeted approach could significantly enhance the efficacy of exosome therapy while minimizing off-target effects.

Another innovative approach in MSC-exosome therapy involves the use of exosomes as delivery vehicles for small molecule drugs. By loading exosomes with established MS drugs, such as fingolimod or dimethyl fumarate, researchers aim to enhance the CNS penetration and cellular uptake of these compounds, potentially improving their efficacy and reducing systemic side effects (Zhuang et al., 2011). This exosome-mediated drug delivery approach could open up new possibilities for repurposing existing MS drugs and developing more effective combination therapies.

The potential of MSC-exosome therapy in preventing or delaying the onset of MS in high-risk individuals is an area that warrants further investigation. Studies have shown that MSC-derived exosomes can modulate the immune system and promote a tolerogenic environment, effects that could prevent the development of autoimmunity in susceptible individuals (Zhang et al., 2014). Prophylactic administration of MSC-derived exosomes in individuals with a high genetic risk of MS or those with clinically isolated syndrome (CIS) could delay or prevent the onset of clinically definite MS.

As research in MSC-exosome therapy for MS continues to advance, there is growing interest in developing personalised treatment approaches. The heterogeneity of MS in terms of clinical course, pathological features, and treatment response calls for tailored therapies. Researchers are exploring the use of patient-derived induced pluripotent stem cells (iPSCs) to generate MSCs and exosomes that are immunologically matched to the patient, potentially enhancing treatment efficacy and reducing the risk of immune rejection (Zhang et al., 2011).

The scalability and manufacturing of MSC-derived exosomes for clinical use remain significant challenges that need to be addressed. Current methods for exosome isolation and purification are often labor-intensive and yield relatively small quantities of exosomes. To overcome these limitations, researchers are developing novel bioreactor systems and microfluidic devices that can produce large quantities of clinical-grade exosomes in a more efficient and standardised manner. These advancements in manufacturing technology will be important for the successful translation of MSC-exosome therapy from bench to bedside.

The regulatory environment surrounding MSC-exosome therapy is evolving rapidly as this field advances towards clinical application. Regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) are working to develop appropriate frameworks for the evaluation and approval of exosome-based therapeutics (Lener et al., 2015). Key considerations include the characterisation and quality control of exosome preparations, demonstration of their mechanism of action, and establishment of appropriate safety and efficacy endpoints in clinical trials.

International collaboration and data sharing will be key for accelerating the development and clinical translation of MSC-exosome therapy for MS. Initiatives such as the International Society for Extracellular Vesicles (ISEV) and the Extracellular RNA Communication Consortium (ERCC) are fostering collaboration and standardisation in exosome research (Witwer et al., 2013). These efforts will be essential for overcoming the challenges associated with this complex and fast-moving field.

Economic assessment of combined MSC-EV treatment would need evidence of clinical benefit, manufacturing costs, administration requirements, and subsequent care use. The financial burden of MS is substantial (Adelman et al., 2013), but cost savings from an unvalidated intervention cannot yet be assumed.

Combining MSCs with extracellular-vesicle products is a mechanistic hypothesis rather than an established MS treatment. Any claim of incremental efficacy requires studies that compare each component with the combination

and use reproducible product characterisation and prespecified clinical endpoints.

The complexity of MSC-exosome therapy, encompassing immunomodulation, neuroprotection, remyelination, and neuroplasticity promotion, aligns well with the complex and heterogeneous nature of MS. By targeting multiple disease mechanisms simultaneously, this approach offers the potential for broader and potentially sustained clinical benefits compared to traditional single-target therapies.

Future work should define vesicle identity and potency assays, optimise delivery and biodistribution, and test standardised products in appropriately controlled clinical studies. Mechanistic advances in MSC secretome biology are useful for designing such studies, but they do not by themselves predict clinical benefit in MS.

Translation of these preclinical findings will require collaboration across cell biology, neuroscience, clinical trial design, bioengineering, and manufacturing. The relevant benchmark is not biological plausibility alone, but reproducible clinical benefit with an acceptable safety profile.

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6.2. MECHANISM AND SYNERGISTIC EFFECTS OF COMBINED THERAPY

6.2.1. Enhanced immunomodulation: mechanistic rationale

This section examines mechanistic complementarity between mesenchymal stromal cells (MSCs) and extracellular vesicles (EVs). Direct clinical evidence that co-administering MSCs and EVs improves outcomes in multiple sclerosis is not established; the interactions discussed below should therefore be interpreted as hypotheses derived primarily from cell biology, in vitro studies, and preclinical models. See Figure 6.2.1.

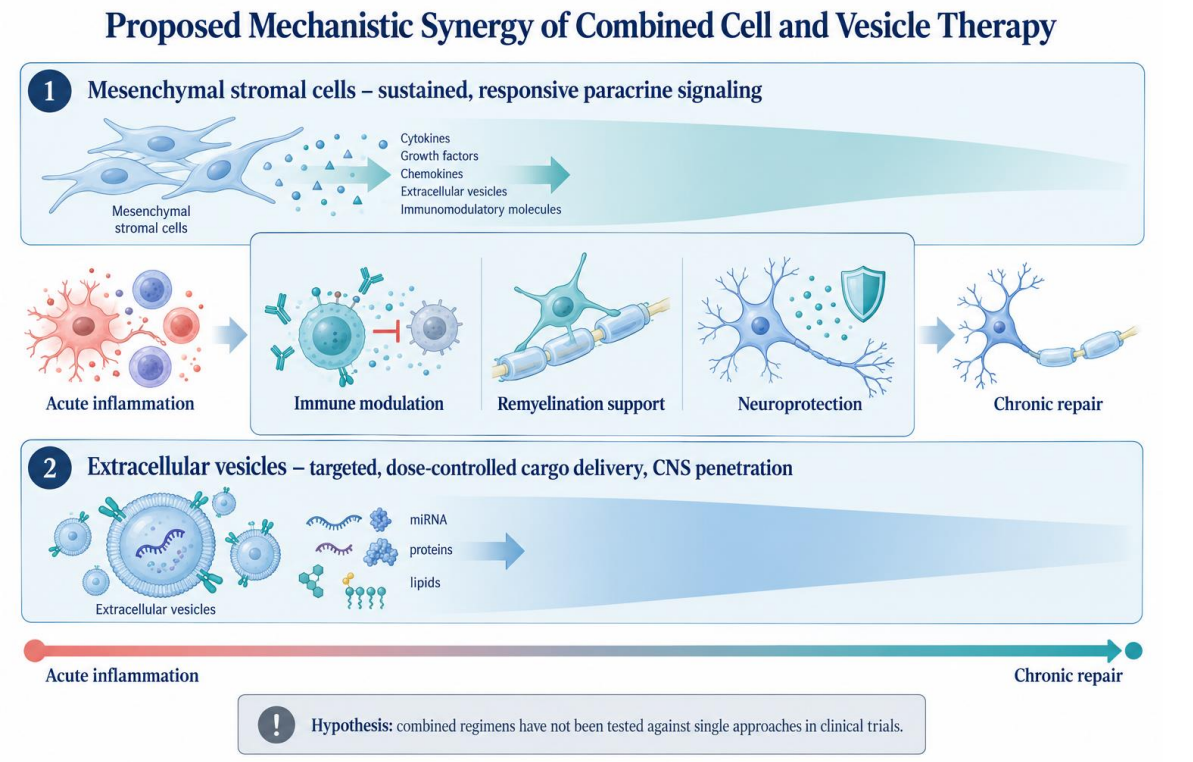


Figure 6.2.1. Proposed mechanistic synergy of combined cell and vesicle therapy, from acute inflammation to chronic repair. The combination remains a hypothesis that has not been tested clinically.

Multiple sclerosis involves inflammatory injury, demyelination, and neurodegeneration within the central nervous system (Trager et al., 2014). Because these processes are biologically interconnected, cell-based and extracellular-vesicle approaches have been investigated for effects on immune signalling and tissue repair. The rationale for combining MSCs with EVs is therefore mechanistic rather than clinical: at present, evidence does not show that co-administration improves outcomes in people with MS (Scolding et al., 2017).

Current treatment context

Established MS therapies primarily target inflammatory disease activity. Interferon-beta, glatiramer acetate, oral agents, and monoclonal antibodies reduce relapses and MRI activity to varying degrees, but effects on non-inflammatory progression are more limited (Tintore et al., 2019; Ontaneda et al., 2017). This distinction is important when considering experimental strategies intended to influence repair-related pathways.

The limitations of current therapies have motivated investigation of mesenchymal stromal cells and EV-based products (Scolding et al., 2017). Combining cells with EVs is a hypothesis-generating strategy; synergy in a clinical MS population has not been established, and the components would need to be evaluated separately and together in well-designed studies.

1. Mesenchymal stromal cells: proposed immunomodulatory mechanisms

Mesenchymal stromal cells can be isolated from bone marrow, adipose tissue, umbilical cord, and other sources. Their relevance to MS research arises mainly from paracrine and immunomodulatory effects rather than an established ability to replace damaged CNS tissue (Uccelli et al., 2011). Product source, culture conditions, potency, and

route of administration can materially influence these properties.

1.1. Immunosuppressive Capabilities

In experimental systems, MSCs can suppress or reshape T-cell, B-cell, natural-killer-cell, and antigen-presenting-cell responses (Uccelli et al., 2008). Reported mediators include prostaglandin E2, indoleamine 2,3-dioxygenase, and TGF-β, together with contact-dependent signalling (Spaggiari et al., 2009; Meisel et al., 2004; Gao et al., 2016). These mechanisms provide biological plausibility, but their relative contribution in treated patients remains uncertain.

1.2. Immunomodulatory Effects

MSCs can alter immune-cell phenotype and cytokine signalling in experimental systems. Reported effects include increased regulatory T-cell activity and changes in macrophage polarisation (Luz-Crawford et al., 2013; Cho et al., 2014). These observations support an immunomodulatory mechanism, but simplified M1/M2 models do not capture the full heterogeneity of macrophage and microglial states in vivo.

1.3. Neuroprotective and Regenerative Properties

MSCs secrete trophic factors, including BDNF, NGF, and GDNF, that can affect neuronal and glial survival in experimental systems (Liang et al., 2014). Rivera et al. (2008) reported effects on oligodendrocyte differentiation and myelin repair in an animal model. These findings support repair-related hypotheses but do not establish remyelination or neuroprotection as clinical effects of MSC treatment in MS.

1.4. Homing and Migratory Capabilities

MSC trafficking is influenced by chemokine receptors, adhesion molecules, vascular interactions, and tissue injury signals (Ullah et al., 2019). Although migration toward inflamed tissue has been demonstrated experimentally, systemic delivery does not guarantee efficient or durable localisation within MS lesions.

1.5. Paracrine Effects and Extracellular Vesicle Production

Paracrine signalling is considered an important component of MSC biology, and extracellular vesicles are one candidate mediator (Börger et al., 2017). EVs can transfer proteins, lipids, and nucleic acids between cells, but cargo detection alone does not establish a therapeutic effect or identify the relevant mechanism in vivo (Kordelas et al., 2014). These observations provide a rationale for studying MSCs and EVs within the same mechanistic framework. They do not, however, demonstrate that administering both products together is superior to either component alone.

2. Exosomes: Extracellular Vesicles as Investigational Immunomodulatory Carriers

Extracellular vesicles released by MSCs and other cell types participate in intercellular signalling and are being investigated as biomarkers and delivery vehicles (Kimura et al., 2018). Many studies use the term exosome, although endosomal origin is not always demonstrated. Claims that isolated EVs reproduce the therapeutic effects of parent cells should therefore be evaluated product by product and in the relevant experimental model (Zhang et al., 2016).

2.1. Enhanced Delivery and BBB Penetration

Experimental studies show that some engineered EV preparations can deliver cargo to the CNS, including after systemic or intranasal administration (Alvarez-Erviti et al., 2011; Zhuang et al., 2011). The extent of BBB passage depends on vesicle source, modification, route, disease state, and measurement method. These data support delivery research but do not establish efficient CNS targeting or improved efficacy in patients with MS.

2.2. Reduced Immunogenicity

Exosomes are expected to be less immunogenic than whole cells, which would matter for the repeated dosing that a chronic disease like MS demands. How far that holds depends on the particular preparation, and comparative immunogenicity has not been measured directly for the products now entering trials. The expectation of lower immunogenicity rests on their smaller size, which makes them less likely to be recognised and cleared by the immune system, and their composition, which often lacks major histocompatibility complex (MHC) molecules.

2.3. Stable Cargo Delivery

Vesicles can protect molecular cargo, but delivery, persistence, and biological effects require direct measurement (Luan et al., 2017). The presence of miRNAs does not establish sustained neuroprotection. Patient-derived vesicles can also exert potentially adverse immunological effects, as illustrated by the mechanistic study discussed in Section 5.7.

2.4. Diverse Immunomodulatory Effects

MSC-derived EVs can alter T-cell, regulatory-T-cell, antigen-presenting-cell, microglial, and macrophage responses

in experimental systems (Bruno et al., 2015). Riazifar et al. (2019) reported reduced neuroinflammation and remyelination-related effects in a mouse model using EVs from interferon-γ-stimulated MSCs. These findings are preclinical and do not establish equivalent immunomodulatory or remyelinating effects in people with MS.

2.5. Potential for Customization and Engineering

One of the most promising aspects of exosome-based therapy is the potential for customization and engineering. Researchers have explored various strategies to modify exosomes, including loading them with drugs or therapeutic molecules, altering their surface proteins to improve targeting, and generating exosomes from genetically modified cells (Luan et al., 2017). For instance, Yuan et al. (2017) used macrophage-derived exosomes as nanocarriers to deliver a therapeutic protein to the inflamed brain, illustrating an approach that could in principle be adapted to MS.

2.6. Biomarker Potential

Beyond their therapeutic applications, exosomes are also being examined as biomarkers for disease progression and treatment response in MS. The content of exosomes, particularly their miRNA profile, can reflect the physiological or pathological state of their cells of origin (Selmaj et al., 2017). This property could be leveraged to develop non-invasive diagnostic and prognostic tools for MS, potentially allowing for more personalised treatment approaches.

The unique properties of exosomes, combined with their ability to recapitulate many of the therapeutic effects of MSCs, make them an ideal complement to cell-based therapies in MS treatment. The synergistic potential of combining MSCs and exosomes represents a frontier in MS research, offering the promise of enhanced immunomodulation and neuroprotection.

3. Synergistic Effects of Combined MSC and Exosome Therapy

The combination of MSC and exosome therapies in MS treatment offers the potential for synergistic effects that could significantly enhance the overall therapeutic outcome. This synergy is based on several mechanisms and complementary properties of MSCs and exosomes.

3.1. Complementary Delivery Routes and Targeting

While MSCs have shown the ability to home to sites of inflammation in the CNS, their capacity to cross the BBB may be limited (Karussis et al., 2010). Exosomes, on the other hand, can more readily penetrate the CNS due to their small size and lipid composition (Alvarez-Erviti et al., 2011). The combination of these two modalities could enhance the overall therapeutic effect by ensuring a more detailed delivery of immunomodulatory and neuroprotective factors to the CNS. A study by Xin et al. (2013) demonstrated that exosomes derived from MSCs could enhance functional recovery in a stroke model by promoting neurite remodeling, neurogenesis, and angiogenesis, suggesting similar mechanisms could be at play in MS treatment.

3.2. Sustained and Enhanced Immunomodulation

MSCs can provide immediate and localised immunomodulatory effects through cell-to-cell contact and paracrine signalling. However, their persistence in the body may be limited due to various factors, including immune clearance (von Bahr et al., 2012). Exosomes, with their lower immunogenicity and ability to circulate systemically, could offer a more sustained and widespread immunomodulatory effect. The combination of these two approaches could provide both immediate and long-term immunomodulation, addressing different temporal aspects of MS pathology. This complementarity has not been tested directly: Laso-García et al. (2018) evaluated extracellular vesicles from human adipose-tissue MSCs given alone in a progressive MS model, Theiler's murine encephalomyelitis virus-induced demyelinating disease, and did not compare combined MSC and vesicle administration with either component.

3.3. Enhanced Neuroprotection and Regeneration

The combination of MSCs and exosomes might provide a more robust neuroprotective effect through the delivery of diverse neurotrophic factors and anti-inflammatory molecules. While MSCs can directly secrete factors such as BDNF and NGF, exosomes may deliver such factors across the BBB and to specific cellular targets (Xiong et al., 2017). Exosomes have also been shown to carry microRNAs that can promote oligodendrocyte differentiation and remyelination (Ji et al., 2022). Any synergy between MSCs and exosomes in MS lesions remains hypothetical; Reza-Zaldivar et al. (2018) reviewed the potential effects of MSC-

derived exosomes on neuroplasticity in Alzheimer's disease but did not test combined therapy.

3.4. Modulation of Multiple Immune Cell Types

While MSCs primarily interact with T cells and antigen-presenting cells, exosomes have been shown to modulate a broader range of immune cells, including B cells and natural killer cells (Zhang et al., 2017). This complementary action on different immune cell populations could offer a more thorough immunomodulatory effect, potentially addressing multiple aspects of the dysregulated immune response in MS. A study by Mokarizadeh et al. (2012) showed that exosomes derived from MSCs could induce tolerogenic dendritic cells and Tregs, suggesting a mechanism for broader immune modulation.

3.5. Priming of the Immune Microenvironment

Exosomes may help to create a more favourable immune microenvironment for the subsequent action of MSCs, potentially enhancing their therapeutic efficacy. For instance, exosomes carrying specific miRNAs or anti-inflammatory proteins could modulate the local immune environment, making it more receptive to the immunomodulatory effects of MSCs. This priming effect could enhance the overall therapeutic outcome of combined therapy.

3.6. Enhanced Tissue Repair and Remyelination

Tissue repair is the aspect of combination therapy for which the mechanistic case is clearest and the direct evidence thinnest. MSCs provide a sustained source of trophic and matrix-modifying factors at the site of injury, while isolated vesicles can be given at defined doses and carry cargo, such as pro-myelinating microRNAs, to oligodendrocyte precursors (Li et al., 2019; Pusic et al., 2014). The only experimental support for the combination itself comes from outside the central nervous system: in renal ischaemia-reperfusion injury, cells and their vesicles given together reduced tissue injury more than either component alone (Lin et al., 2016). No comparable experiment has been reported in a demyelination model, so enhanced repair after combined administration remains a hypothesis.

3.7. Potential for Personalized Therapy

The combination of MSCs and exosomes offers the potential for more personalised treatment approaches in MS. For instance, exosomes could be engineered to carry specific therapeutic molecules tailored to individual patient needs, while MSCs could be optimised for enhanced immunomodulatory or regenerative properties (Luan et al., 2017). This personalised approach could improve treatment outcomes and reduce side effects by targeting specific aspects of MS pathology in individual patients.

3.8. Mitigation of MSC-related Challenges

While MSC therapy has shown promise in MS treatment, it faces challenges, including limited cell survival and theoretical concerns about malignant transformation (von Bahr et al., 2012). Exosomes, being cell-free, do not carry a risk of malignant transformation and can be more easily stored and administered (Gimona et al., 2017). Direct evidence for combining the two comes from outside MS: in rats with acute renal ischaemia-reperfusion injury, combined ADMSC and ADMSC-derived exosome therapy reduced creatinine levels and kidney injury more than either treatment alone (Lin et al., 2016). Whether a similar effect occurs in MS is unknown.

3.9. Enhanced Anti-inflammatory Effects

The combination of MSCs and their derived exosomes has been proposed to produce a stronger anti-inflammatory effect than either therapy alone. Cosenza et al. (2018) compared MSC-derived exosomes and microparticles and found exosomes to be more immunosuppressive in a model of inflammatory arthritis; the study did not test MSCs and exosomes in combination, so a synergistic effect remains to be shown.

3.10. Improved Functional Outcomes

Improved functional outcomes with combined MSC and exosome treatment remain a hypothesis. In a rat model of stroke, Xin et al. (2013) found that systemically administered MSC-derived exosomes, compared with vehicle, improved functional recovery and were associated with increased neurite remodeling, neurogenesis, and angiogenesis. That study did not administer MSCs together with exosomes and therefore provides no evidence that combined therapy is superior to either component; whether the same repair mechanisms operate in MS also remains to be established.

3.11. Potential for Reducing Treatment Frequency

It has been proposed that combining MSCs and exosomes might allow less frequent treatment administrations, with systemically circulating exosomes sustaining the therapeutic effect between cell administrations. This proposal is

speculative: no study has compared dosing schedules of combined therapy, and any consequences for adherence or healthcare costs remain untested.

3.12. Modulation of the Gut-Brain Axis

Recent research has highlighted the importance of the gut-brain axis in MS pathogenesis (Kirby & Ochoa-Repáraz, 2018). It has been suggested that MSC-based therapies might influence this axis; for example, adipose-derived MSCs and MSC-conditioned medium showed immunomodulatory effects in chronic colitis (Heidari et al., 2018). Whether combined cell and vesicle therapy affects gut dysbiosis or neuroinflammation in MS has not been studied.

3.13. Enhanced Neuroprotection through Multiple Mechanisms

While both MSCs and exosomes have demonstrated neuroprotective properties individually, their combination might offer complementary mechanisms. MSCs can secrete neurotrophic factors and promote a regenerative microenvironment (Bai et al., 2012), while exosomes can deliver molecules such as miRNAs to target cells (Zhang et al., 2017). Sun et al. (2018) reported that exosomes from human umbilical cord MSCs promoted functional recovery in mice with spinal cord injury by attenuating inflammation; whether engineered exosomes would add to the effects of combined therapy in MS is unknown.

3.14. Potential for Addressing Progressive MS

One of the greatest challenges in MS treatment is addressing the progressive forms of the disease, which are characterised by continuous neurodegeneration with limited inflammatory activity (Ontaneda et al., 2017). Combining MSCs and exosomes has been proposed as a way to modulate residual inflammation, promote neuroprotection, and enhance remyelination simultaneously, but this rationale is derived from studies of the individual components rather than from tests of combined therapy in progressive MS models.

3.15. Synergistic Effect on Oxidative Stress Reduction

Oxidative stress contributes to both inflammation and neurodegeneration in MS (Adamczyk & Adamczyk-Sowa, 2016). The combination of MSCs and exosomes has been proposed to offer enhanced protection against oxidative stress; in renal ischaemia-reperfusion injury, combined ADMSC and exosome therapy reduced oxidative-stress markers more than either component (Lin et al., 2016). Whether this applies to oxidative damage in MS remains to be tested.

3.16. Enhanced Modulation of Microglia and Astrocytes

Microglia and astrocytes play essential roles in MS pathology, contributing to both inflammation and repair processes (Liddelow & Barres, 2017). MSC-derived exosomes have been reported to promote an anti-inflammatory microglial phenotype and reduce demyelination in EAE rats (Li et al., 2019). No study has shown that exosomes modulate glial cells more effectively than MSCs, or that combined therapy improves glial modulation in MS.

3.17. Potential for Enhancing Remyelination

While current MS therapies primarily focus on reducing inflammation and preventing relapses, promoting remyelination remains a significant challenge (Plemel et al., 2017). Exosomes from interferon-γ-stimulated dendritic cells, enriched in miR-219, increased myelination in experimental systems (Pusic et al., 2014). Engineering MSC exosomes with pro-myelinating miRNAs and combining them with MSCs is a conceivable strategy, but it has not yet been evaluated in MS models.

3.18. Addressing Multiple Aspects of MS Pathology

The complex pathology of MS involves not only immune dysregulation and demyelination but also axonal degeneration, mitochondrial dysfunction, and oxidative stress (Mahad et al., 2015). The combination of MSCs and exosomes offers the potential to address multiple aspects of this complex pathology simultaneously. While MSCs can provide broad immunomodulatory and neuroprotective effects, exosomes can deliver specific molecules targeting particular pathological processes, such as miRNAs that promote oligodendrocyte differentiation or proteins that enhance mitochondrial function.

3.19. Potential for Biomarker Discovery

The use of combined MSC and exosome therapy may also facilitate the discovery of new biomarkers for MS progression and treatment response. Exosomes contain a wealth of molecular information that reflects the state of their cells of origin (Selmaj et al., 2017). By analysing the content of circulating exosomes before, during, and after treatment, researchers may be able to identify new biomarkers that

predict treatment response or disease progression, leading to more personalised treatment approaches.

3.20. Enhanced Immunological Memory Modulation

Recent research has highlighted the role of immunological memory in MS pathogenesis, particularly the contribution of memory B cells (Li et al., 2018). MSC-derived exosomes can modulate immune responses, including through induction of regulatory T cells, as reviewed in the context of autoimmune diseases (Xu et al., 2019). Whether combined MSC and exosome therapy modifies disease-specific immune memory in MS is unknown.

Conclusion and Future Directions

The rationale for combining MSCs with MSC-derived extracellular vesicles is mechanistically plausible, but direct evidence for additive or synergistic benefit in people with MS is not available. Future studies need to define product identity, dose, route, sequencing, manufacturing controls, and long-term safety before efficacy can be tested in adequately powered comparative trials. Until such studies are available, proposed gains in immunomodulation, remyelination, or neuroprotection should be treated as hypotheses rather than expected clinical effects.

6.2.2. Remyelination and neuroprotection: evidence synthesis

The biological rationale for combining cellular and EV-based approaches is plausible but remains substantially ahead of the clinical evidence. MSCs and their secreted EVs can influence inflammatory signalling, trophic support, oligodendroglial biology, and tissue repair in experimental systems; however, these observations do not demonstrate that administering both products together produces additive or synergistic benefit in people with MS.

A clinically meaningful synergy claim would require factorial or otherwise appropriately controlled studies that compare each component alone with the combination, use prespecified patient-relevant outcomes, and characterize both products with reproducible manufacturing and potency criteria. In the absence of such evidence, improved remyelination, neuroprotection, or disability outcomes should be framed as testable hypotheses rather than expected treatment effects.

Accordingly, the discussion below focuses on mechanistic complementarity and translational requirements rather than presenting combined MSC-EV therapy as an established therapeutic strategy.

1. Mechanistic complementarity

Combining stem-cell and EV-based products remains an investigational concept. Proposed complementary effects on inflammatory and repair-related pathways have been explored mainly in experimental systems; clinical synergy, optimal sequencing, and long-term safety have not been established.

1.1. Enhanced Immunomodulation

Both MSCs and MSC-derived EVs can influence immune signalling in experimental systems, but whether co-administration produces greater or more durable immunomodulation than either component alone has not been established.

Potential complementarity could arise from cellular contact-dependent and paracrine effects together with vesicle-mediated transfer of regulatory cargo. Experimental studies support these mechanisms individually, but they do not establish that administering cells and isolated EVs together produces broader immune control in MS (Bruno et al., 2015; Riazifar et al., 2017; Kordelas et al., 2014).

1.2. Improved Remyelination

Remyelination-related effects provide a further mechanistic hypothesis, although synergy has not been demonstrated clinically.

MSCs may influence oligodendroglial survival and repair through trophic and immunomodulatory signalling, while EV cargo can affect OPC differentiation in experimental systems. These observations support mechanistic complementarity but do not establish direct cell replacement, enhanced remyelination, or superior repair after combined administration in people with MS (Xin et al., 2013; Laso-García et al., 2018).

1.3. Enhanced Neuroprotection

A combined cell-and-EV strategy could, in principle, influence several neuroprotective pathways, but comparative evidence showing benefit over either component alone is lacking.

Cellular and EV products can each carry trophic or regulatory signals that affect neuronal stress responses in experimental models. Whether combining the two produces greater neuroprotection, sustained axonal benefit, or clinically meaningful effects in progressive MS remains

unknown (Bonafede et al., 2019; Xin et al., 2012; Phinney et al., 2015).

1.4. Modulation of the Microenvironment

Stem cells and exosomes might act in complementary ways on the local microenvironment in MS lesions, although this has not been demonstrated directly.

Stem cells and exosomes can deliver a range of matrix-modifying enzymes and factors that collectively create a more permissive environment for repair and regeneration (Shiue et al., 2019). The combination of pro-angiogenic factors secreted by stem cells and those delivered by exosomes may lead to more robust vascular repair and stabilization of the BBB (Zacharek et al., 2007). Together, stem cells and exosomes may more effectively modulate the phenotype and function of microglia and astrocytes, promoting a more regenerative and less inflammatory environment (Jafarinia et al., 2020).

1.5. Potential for Personalized Therapy

The combination of stem cells and exosomes offers the potential for more personalized therapeutic approaches.

Stem cells can be engineered to produce exosomes with specific cargo tailored to individual patient needs. When combined with patient-derived stem cells, this approach could offer a highly personalised treatment strategy for MS. The ability to modify exosome content based on disease progression or treatment response could allow for more adaptive and responsive therapy over time (Luan et al., 2017).

Combination with biomarkers: Integrating exosome-based liquid biopsy techniques with stem cell therapy could provide real-time monitoring of treatment efficacy and disease progression, enabling more personalised treatment decisions (Selmaj et al., 2017). For instance, exosomes isolated from cerebrospinal fluid or blood could be analysed for specific miRNA or protein signatures indicative of disease activity or treatment response.

Patient-specific cell sources: The use of autologous induced pluripotent stem cells (iPSCs) as a source of both cells and exosomes could further personalize treatment, potentially reducing immune rejection and allowing for genetic correction of MS-associated risk variants (Thiruvalluvan et al., 2016).

2. Integration with established therapies as a research question

The integration of stem cell and exosome therapies with current MS treatments presents an opportunity to leverage the strengths of each approach and potentially achieve superior therapeutic outcomes.

2.1. Complementary Mechanisms of Action

Current disease-modifying therapies (DMTs) for MS primarily target the inflammatory component of the disease. The addition of stem cell and exosome therapies could complement these treatments by addressing the neurodegenerative aspects of MS and promoting remyelination.

Enhanced immunomodulation: Combining stem cell/exosome therapies with traditional immunomodulatory drugs may lead to more thorough control of the autoimmune response. For example, the immunomodulatory effects of interferon-β could be augmented by the diverse immunoregulatory factors provided by stem cells and their exosomes (Kieseier, 2011; Riazifar et al., 2017).

Neuroprotection and DMTs: While some DMTs, like dimethyl fumarate, have shown neuroprotective properties, the addition of stem cell/exosome therapies could provide broader and more robust neuroprotection (Linker et al., 2011; Bonafede et al., 2019).

Remyelination and immunomodulation: Combining remyelination-promoting effects of stem cells and exosomes with the inflammation-reducing effects of DMTs could create a more favourable environment for sustained myelin repair (Lublin et al., 2014; Li et al., 2019).

2.2. Potential Synergies with Specific MS Therapies

2.2.1. Natalizumab and Stem Cell/Exosome Therapy

Natalizumab, a monoclonal antibody that blocks lymphocyte migration into the CNS, could be combined with stem cell/exosome therapy to enhance its efficacy.

While natalizumab reduces inflammation by preventing lymphocyte entry, stem cells and exosomes could promote repair and remyelination within the CNS (Polman et al., 2006; Rivera et al., 2008).

This combination could address both the inflammatory and neurodegenerative aspects of MS simultaneously.

2.2.2. Sphingosine-1-phosphate receptor modulators and stem cell/exosome therapy

Drugs like fingolimod, which sequester lymphocytes in lymph nodes, could be complemented by stem cell/exosome therapies.

The reduction in circulating lymphocytes provided by fingolimod could create a window of opportunity for stem cells and exosomes to exert their regenerative effects with less interference from the peripheral immune system (Chun & Hartung, 2010; Laso-García et al., 2018).

2.2.3. B cell-depleting therapies and stem cell/exosome therapy

Combining B cell-depleting therapies like ocrelizumab with stem cell/exosome approaches could offer synergistic benefits.

While ocrelizumab targets B cells, stem cells and exosomes could modulate other aspects of the immune response and promote repair, potentially leading to broader disease control (Hauser et al., 2017; Li et al., 2019).

2.3. Timing and Sequencing of Therapies

The optimal timing and sequencing of combined therapies will be important for maximizing their synergistic effects.

Induction and maintenance strategies: Whether an MSC or EV product adds benefit to a DMT regimen requires a prospective controlled trial. AHSCT evidence concerns a distinct intervention and cannot establish the efficacy of an MSC/EV sequence.

Cyclical treatment: Alternating periods of stem cell/exosome therapy with traditional DMTs might balance the need for ongoing immunomodulation with promoting repair and regeneration (Muraro et al., 2017).

Adaptive treatment protocols remain a research objective for investigational cell and EV products. Studies should prespecify adjustment rules, safety stopping criteria, and outcomes. Evidence of individualized benefit requires prospective comparison; cell-culture remyelination experiments cannot validate a patient-specific dosing strategy.

Combination based on disease stage: The specific combination of therapies might be tailored to the stage of disease, with more emphasis on immunomodulation in early RRMS and greater focus on neuroprotection and remyelination in progressive forms of MS (Cree et al., 2022).

2.4. Potential for Reducing Side Effects

Combining stem cell/exosome therapies with current treatments might allow for dose reductions of traditional DMTs, potentially minimising side effects.

The immunomodulatory effects of stem cells and exosomes might allow for lower doses of immunosuppressive drugs, reducing the risk of infections and other complications (Riazifar et al., 2017).

The neuroprotective effects of stem cell/exosome therapies might mitigate some of the CNS-related side effects of certain MS drugs (Ontaneda et al., 2017).

3. Translational limitations and research priorities

While the potential of combined stem cell and exosome therapy for MS is promising, several challenges need to be addressed to fully realize its therapeutic potential.

3.1. Standardization and Quality Control

One of the primary challenges in the development of stem cell and exosome therapies is the need for standardisation and quality control.

Heterogeneity of stem cell populations: Developing standardised protocols for isolating, expanding, and characterising stem cells is important for ensuring consistent therapeutic effects (Galipeau & Sensébé, 2018).

Exosome isolation and characterisation: Standardizing methods for exosome isolation, quantification, and characterisation is essential for developing reliable exosome-based therapies (Théry et al., 2018).

Potency assays: Developing robust potency assays that can predict the therapeutic efficacy of stem cells and exosomes in vivo is a key challenge (Galipeau et al., 2016).

3.2. Optimizing Delivery Methods

Determining the most effective methods for delivering stem cells and exosomes to the CNS remains a significant challenge.

Systemic vs. local administration: While systemic administration offers simplicity, it may result in limited CNS penetration. Direct CNS administration, while potentially more effective, is more invasive and carries additional risks (Scolding et al., 2017).

Enhancing BBB penetration: Developing strategies to enhance the ability of stem cells and exosomes to cross the BBB, such as cell surface modification or exosome engineering, could improve their therapeutic efficacy (Matsumoto et al., 2017).

Targeted delivery: Creating methods for targeted delivery of stem cells and exosomes to specific regions of the CNS affected by MS could enhance their therapeutic effects while minimising off-target effects (Alvarez-Erviti et al., 2011).

3.3. Long-Term Safety and Efficacy

Establishing the long-term safety and efficacy of combined stem cell and exosome therapy is key.

Long-term follow-up studies: Conducting extended follow-up studies to assess the durability of therapeutic effects and monitor for potential late-onset adverse events is essential (Muraro et al., 2017).

Immunogenicity concerns: While stem cells and exosomes are generally considered to have low immunogenicity, the potential for immune reactions, especially with repeated administrations, needs to be carefully evaluated.

Tumourigenicity risk: Although the risk of tumour formation is considered low, especially with exosomes, long-term studies are needed to definitively establish the safety profile of these therapies (Barkholt et al., 2013).

3.4. Personalized Treatment Approaches

Developing personalised treatment approaches that take into account individual patient characteristics represents both a challenge and an opportunity.

Biomarker development: Identifying predictive biomarkers of treatment response could help in patient selection and treatment optimisation (Housley et al., 2015).

Patient stratification: Developing tools for patient stratification based on disease subtype, genetic factors, and biomarker profiles will be essential for optimising the use of combined stem cell and exosome therapy in MS (Bielekova & Pranzatelli, 2017).

Adaptive treatment protocols: Creating flexible treatment protocols that can be adjusted based on individual patient responses and disease progression will be important for maximizing therapeutic benefits (Negoescu et al., 2018). Establishing flexible treatment strategies that adjust based on individual patient responses and disease progression is important for optimising multiple sclerosis (MS) therapy. This approach can help clinicians adapt to the varied and often unpredictable nature of immune responses seen in MS, enabling timely adjustments to maximize therapeutic effects. Tailoring protocols, whether by increasing treatment intensity or modifying therapy altogether, may improve symptom control and potentially altering disease courses more effectively.

3.5. Regulatory Considerations

The regulatory environment for stem cell and exosome therapies is complex and evolving.

Standardised regulatory frameworks: Developing clear and standardised regulatory frameworks that ensure safety and efficacy while facilitating innovation will be central for advancing these therapies from the laboratory to the clinic (Dominici et al., 2015).

Manufacturing and quality control guidelines: Establishing full guidelines for the manufacturing and quality control of stem cell and exosome products will be essential for their clinical translation (Gimona et al., 2017).

Harmonization of international regulations: Given the global nature of MS research and treatment, harmonizing regulatory approaches across different countries and regions will be important for facilitating multicentre trials and global access to these therapies (Dominici et al., 2015).

3.6. Future Research Directions

Several key areas of research will be critical for advancing the field of combined stem cell and exosome therapy for MS.

Elucidating the precise mechanisms by which stem cells and exosomes promote remyelination and neuroprotection in MS, including the identification of key molecules and pathways involved.

Developing engineered exosomes with enhanced therapeutic properties, such as improved targeting to specific cell types or enhanced cargo delivery.

Investigating the potential of induced pluripotent stem cells (iPSCs) as a source of patient-specific stem cells and exosomes for MS treatment.

Exploring the use of artificial intelligence and machine learning algorithms to optimise combination therapies and predict treatment outcomes.

Conducting large-scale, well-designed clinical trials to establish the safety and efficacy of combined stem cell and exosome therapy in different MS subtypes and stages of disease progression.

Investigating the potential of exosome-based biomarkers for monitoring disease activity and treatment response in MS.

Exploring novel bio-manufacturing approaches, such as 3D bioprinting and microfluidic technologies, to enhance the production and quality control of stem cells and exosomes for therapeutic use.

Studying the long-term effects of combined stem cell and exosome therapy on neuronal plasticity and CNS repair in MS.

Investigating the potential of combining stem cell and exosome therapies with emerging treatments, such as remyelination-promoting drugs or neuroprotective agents.

Developing novel imaging techniques to track the distribution and effects of transplanted stem cells and administered exosomes in vivo.

4. Section synthesis

The combination of stem cell and exosome therapies is a candidate approach for enhancing remyelination and neuroprotection in MS. By leveraging the unique properties of both stem cells and exosomes, this combined therapy offers the potential to address multiple aspects of MS pathology, including inflammation, demyelination, and neurodegeneration. The integration of these innovative therapies with current MS treatments presents an opportunity to develop more complete and effective treatment strategies.

While significant obstacles persist in translating these therapies from the laboratory to the clinic, ongoing research and technological advancements are supporting the development of new therapeutic possibilities.

The future of MS therapy lies in personalised, multi-modal approaches that combine the best of current treatments with innovative cellular and molecular therapies. By continuing to explore the synergistic effects of combined therapies and addressing the challenges that lie ahead, we can hope to significantly improve outcomes for individuals living with MS, potentially altering the course of this challenging disease.

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6.3. ADVANTAGES AND DISADVANTAGES OF COMBINED THERAPY

Combining cellular or EV products with DMTs is intended to address complementary mechanisms (Baharlooi et al., 2020). The relevant questions are whether the combination adds benefit, changes toxicity, or complicates attribution of an effect. Figure 6.3.1 sets out these proposed advantages and limitations.

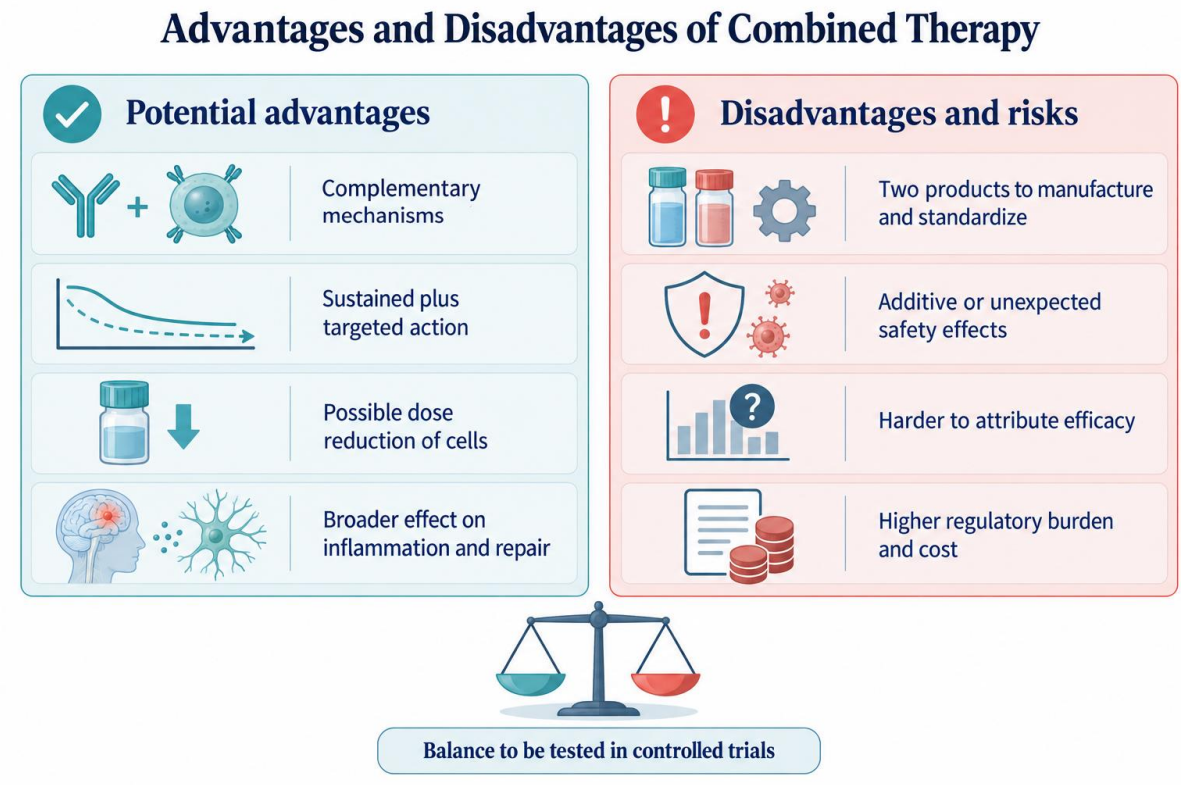


Figure 6.3.1. Potential advantages and disadvantages of combined cell- and vesicle-based therapy.

1. Advantages of Combined Therapy

1.1. Enhanced Therapeutic Efficacy

Potential complementarity is a research rationale, not demonstrated clinical synergy. Experimental MSC immunomodulation does not establish prevention of axonal degeneration, oligodendrocyte replacement or remyelination in patients. MESEMS did not meet its primary MRI efficacy endpoint (*Uccelli et al.*, 2021).

Recent studies have shown that MSCs can modulate the function of various immune cells, including T cells, B cells, and natural killer cells, through both cell-cell contact and the secretion of soluble factors (Uccelli et al., 2019). This broad immunomodulatory effect could address multiple aspects of MS pathogenesis, including both the adaptive and innate immune responses involved in the disease.

Exosomes, small extracellular vesicles secreted by various cell types including MSCs, have gained attention for their role in intercellular communication and potential therapeutic applications in MS (Ojeda-Hernández et al., 2022). These nanovesicles carry bioactive molecules, including microRNAs, proteins, and lipids, which can influence cellular behaviour and tissue repair processes (Pusic et al., 2014). The integration of exosomes with biomaterials presents a novel therapeutic strategy for MS, offering the possibility of targeted delivery and enhanced efficacy in promoting remyelination and neuroprotection (Ojeda-Hernández et al., 2022).

Research has shown that exosomes derived from MSCs can modulate the immune response in MS by promoting the expansion of regulatory T cells and suppressing the activation of pro-inflammatory T cells (Li et al., 2019). This immunomodulatory effect, combined with the potential of exosomes to cross the blood-brain barrier, makes them an attractive therapeutic option for CNS disorders like MS.

The combination of stem cell-derived exosomes with other therapies has shown promise in ameliorating neurodegenerative changes, as evidenced in studies focusing on Parkinson's disease (Samir et al., 2022). This synergistic

approach could be applied to MS treatment, leveraging the neuroprotective and regenerative properties of both stem cells and exosomes. Furthermore, exosomes have been implicated in enhancing myelination and remyelination processes by promoting the differentiation of oligodendrocyte precursor cells into mature myelinating oligodendrocytes, addressing one of the key pathological features of MS (Pusic & Kraig, 2014).

Current disease-modifying therapies for MS primarily target the inflammatory response to prevent relapses and disease progression (Cross & Riley, 2022). By combining these established treatments with stem cell and exosome therapies, a broader approach to MS management can be achieved. This multi-modal strategy has the potential to address not only the inflammatory component of MS but also promote tissue repair and neuroprotection, potentially leading to improved patient outcomes (Kråkenes et al., 2024).

1.2. Personalized Treatment Approaches

Another significant advantage of combined therapy is the possibility of personalised treatment approaches. MS is a heterogeneous disease with varying clinical presentations and responses to treatment (Ciccarelli, 2019). By integrating multiple therapeutic modalities, healthcare providers can tailor interventions to individual patients' needs, potentially leading to better symptom management, disease control, and overall quality of life for individuals living with MS.

The concept of precision medicine in MS has gained traction in recent years, with researchers exploring various biomarkers and imaging techniques to predict treatment response and disease progression (Comabella & Montalban, 2014). The combination of stem cell therapy, exosome therapy, and conventional DMTs offers an opportunity to further refine this approach, allowing for more nuanced and individualized treatment strategies.

Stratification by inflammatory activity and disease phenotype may be relevant to future combination trials. MSC/EV combinations with DMTs should be regarded as research hypotheses, with incremental benefit and additional toxicity evaluated against an appropriate control.

1.3. Reduced Side Effects and Improved Safety Profile

EV-only administration is cell-free; co-administration of EVs and viable MSCs is not. Avoiding viable-cell transplantation may change the risk profile, but the comparative safety of an EV product requires direct evaluation and cannot be assumed from its cell-free composition.

A compassionate-use EV case in graft-versus-host disease does not establish comparative safety in MS (Kordelas et al., 2014). Absence of viable cells may change risks, but immune effects, contamination, thrombogenicity and unintended cargo activity remain product-specific questions.

1.4. Addressing Treatment Gaps

Moreover, the combination of these therapies may address some of the limitations of individual treatments. For instance, while current DMTs are effective in managing inflammation and reducing relapse rates, they have limited efficacy in promoting tissue repair and remyelination (Duignan et al., 2019). The addition of stem cell and exosome therapies could fill this gap, offering a more holistic approach to MS treatment that addresses both the inflammatory and neurodegenerative aspects of the disease.

Recent research has highlighted the potential of exosomes in promoting neuroregeneration and remyelination. Xin et al. (2012) showed that exosome-mediated transfer of miR-133b from MSCs to neural cells contributes to neurite outgrowth, and in rats after stroke, miR-133b-overexpressing MSCs increased neural plasticity and functional recovery, partly through exosome-enriched transfer of miR-133b (Xin et al., 2013). Whether similar approaches could benefit people with MS remains to be investigated.

1.5. Targeting Multiple Pathways

The combined therapy approach allows for the targeting of multiple pathways involved in MS pathogenesis simultaneously. For example, while conventional DMTs primarily target the adaptive immune response, stem cells and their derived exosomes can modulate both adaptive and innate immune responses (Uccelli et al., 2019). Additionally, these therapies can potentially promote neuroprotection and remyelination through various mechanisms, including the secretion of neurotrophic factors and the modulation of local microenvironments in the CNS (Scolding et al., 2017).

This multi-targeted approach may be particularly beneficial in addressing the multifactorial nature of MS pathology. A combination acting at once on inflammation, tissue repair, and neuroprotection is attractive for that reason, but whether it controls disease better than either component alone is an open question that only a comparative trial can settle.

1.6. Potential for Long-term Disease Modification

Established DMTs can reduce relapses and disability accumulation in appropriate populations. Persistent progression motivates investigation of additional approaches, but longer-lasting benefit from adding MSCs or EVs has not been established.

Stem cell therapies, particularly haematopoietic stem cell transplantation (HSCT), have shown promise in inducing long-term remission in some MS patients (Burt et al., 2019). Whether adding exosome therapy on top of these would improve on them, or make the benefit last longer, has never been tried in a controlled setting and cannot be assumed. Repair of damage already present is the more ambitious aim, and the work behind it is largely experimental (Scolding et al., 2017); recovery of lost neurological function has not been shown in MS trials of either cells or exosomes.

1.7. Enhanced Drug Delivery and Blood-Brain Barrier Penetration

One of the challenges in treating MS is the delivery of therapeutic agents across the blood-brain barrier (BBB). Exosomes have shown the ability to cross the BBB and deliver their cargo to the CNS, making them promising vehicles for drug delivery in MS (Alvarez-Erviti et al., 2011). By combining exosome-mediated drug delivery with conventional therapies, it may be possible to enhance the CNS penetration of therapeutic agents, potentially improving their efficacy and reducing systemic side effects.

Furthermore, the natural targeting properties of exosomes can be exploited to deliver therapeutic molecules to specific cell types within the CNS. For example, exosomes can be engineered to express surface proteins that target them to oligodendrocytes or neurons, allowing for more precise delivery of remyelinating or neuroprotective agents (Osorio-Querejeta et al., 2018).

2. Disadvantages and Challenges of Combined Therapy

Despite the potential advantages, there are significant challenges and limitations associated with combining stem cell, exosome, and current therapies in MS treatment.

2.1. Limited Long-term Safety and Efficacy Data

One of the primary concerns is the lack of long-term safety and efficacy data for stem cell and exosome therapies in MS. While these novel approaches warrant further investigation, their long-term effects on disease progression, immune function, and overall patient outcomes remain to be fully elucidated. The potential for unexpected long-term complications, such as malignant transformation or autoimmune phenomena, cannot be ruled out without extensive longitudinal studies (Burt et al., 2015). Furthermore, the effects of exosome therapy on the immune system in combination with traditional therapies add complexity, as exosomes derived from mesenchymal stem cells (MSCs) have shown immunomodulatory effects, potentially altering the therapeutic landscape (Baharlooi et al., 2020).

Moreover, the optimal timing, dosing, and administration routes for combined therapies in MS remain unclear. Given the heterogeneous nature of MS, determining the most effective combination and sequence of treatments for individual patients presents a significant challenge. Long-term follow-up studies and large-scale clinical trials will be necessary to establish the safety and efficacy of these combined approaches over extended periods.

2.2. Treatment Complexity and Patient Burden

The complexity of combined treatment regimens poses another challenge in MS management. MS is already a complex condition to treat, with multiple disease-modifying therapies available, each with its own set of considerations and side effects (Duignan et al., 2019). Integrating novel therapies like stem cell and exosome treatments into this landscape may introduce challenges related to treatment monitoring, drug interactions, and patient compliance. Exosomes, for instance, are implicated in neuroinflammation regulation, which plays a critical role in MS pathology; this interaction could yield unpredictable inflammatory outcomes when combined with standard therapies (Guo et al., 2023). The potential for increased treatment burden on patients, both in terms of financial cost and time commitment, must also be carefully considered.

Patients may need to undergo multiple procedures, adhere to complex treatment schedules, and manage potential side effects from various therapies simultaneously. This increased complexity could lead to reduced adherence and quality of life for some patients.

2.3. Risk of Adverse Events and Interactions

Combination strategies require direct safety evaluation. Cell and EV products may interact with concomitant therapies, but the clinical direction and magnitude of these interactions cannot be inferred from isolated PBMC assays.

Potential infection, immune, or other risks should be described as hypotheses until supported by combination-specific data (Műzes & Sipos, 2019; Baharlooi et al., 2020).

2.4. Scalability and Standardization Challenges

The scalability and standardisation of stem cell and exosome therapies present additional challenges. Issues such as standardisation of isolation techniques, scalability of production, and targeted delivery to the central nervous system need to be addressed to optimise the therapeutic potential of these approaches in MS treatment (Rahimi Darehbagh et al., 2024). Additionally, ensuring consistent quality and potency of stem cell and exosome products across different manufacturing batches and facilities remains a significant challenge. The biological variability inherent in these therapies can make it difficult to achieve the level of standardisation required for widespread clinical use (Reiner et al., 2017). Exosomes containing bioactive molecules like microRNAs, which could theoretically influence MS pathology, further complicate this standardisation challenge (Mycko & Baranzini, 2020).

2.5. Cost and Accessibility Issues

Another potential drawback is the high cost associated with stem cell and exosome therapies. These advanced treatments often require specialized facilities and expertise, which can make them prohibitively expensive for many patients (Burt et al., 2003). The issue of accessibility and equity in healthcare becomes particularly pertinent when considering the integration of these novel therapies into standard MS care. The economic burden of combined therapies could exacerbate existing disparities in MS care, with advanced treatments becoming available only to a select group of patients who can afford them. This raises important ethical considerations regarding equitable access to potentially life-changing therapies.

2.6. Regulatory and Ethical Considerations

The regulatory environment for combined therapies in MS treatment is also complex and evolving. The approval process for novel therapies, particularly those involving stem cell and exosomes, can be lengthy and challenging. This may delay the availability of these combined treatment approaches to patients who could benefit from them (Rahimi Darehbagh et al., 2024). Moreover, the use of stem cells and their derivatives raises ethical questions, particularly when considering the source of these materials. While many current approaches use autologous or adult stem cells, the potential future use of embryonic or fetal stem cells in MS treatment could raise ethical concerns for some patients and healthcare providers (Hyun, 2010).

2.7. Potential for Exosome-Mediated Transfer of Pathogenic Factors

The same properties that make vesicles attractive as carriers also make them a potential route for transferring harmful material. Preparations from patients or from cells under inflammatory or stressed conditions can carry inflammatory mediators, myelin antigens or microRNAs that are themselves associated with disease activity, so the cargo of a therapeutic preparation cannot be assumed to be benign on the basis of its cell source alone (Mycko & Baranzini, 2020). This is primarily a product-characterisation problem: cargo profiling, defined culture conditions and release criteria are needed for each batch, and the absence of documented harm in small experimental studies is not evidence that such transfer does not occur (Reiner et al., 2017).

2.8. Challenges in Predicting Treatment Response

Lastly, the complexity of MS pathophysiology and the heterogeneity of patient responses to treatment make it challenging to predict the efficacy of combined therapies on an individual basis. The intricate interactions between different treatment modalities and their effects on various aspects of MS pathology require extensive research to develop reliable predictive models for treatment outcomes (Ciccarelli, 2019). Developing biomarkers and imaging techniques to predict and monitor response to combined therapies will be important for optimising treatment strategies for individual patients. However, the complexity of MS pathology and the varied nature of combined therapies make this a particularly challenging endeavor (Comabella & Montalban, 2014).

3. Future Directions and Research Priorities

To fully realize the potential of combined therapies in MS treatment while mitigating their risks, several key areas of research and development should be prioritized:

3.1. Long-term Safety and Efficacy Studies

Comprehensive, long-term follow-up studies are essential to establish the safety profile and sustained efficacy of combined therapies. These studies should include diverse patient populations and assess a wide range of outcomes, including disease progression, quality of life, and potential long-term complications (Sormani et al., 2017).

3.2. Biomarker Development

The identification and validation of biomarkers that can predict treatment response and monitor disease activity in the context of combined therapies is important. This may include molecular biomarkers, imaging techniques, and functional assessments that can guide treatment decisions and optimise patient outcomes (Comabella & Montalban, 2014).

3.3. Optimization of Treatment Protocols

Research is needed to determine the optimal timing, dosing, and sequencing of combined therapies. This includes investigating potential synergies and antagonisms between different treatment modalities and developing strategies to maximize therapeutic benefits while minimising risks (Lubetzki et al., 2020).

3.4. Targeted Delivery Systems

Advancing technologies for targeted delivery of stem cells, exosomes, and therapeutic agents to the CNS is essential. This may involve the development of novel biomaterials, engineered exosomes, or other innovative delivery systems that can enhance the efficacy and specificity of treatment (Osorio-Querejeta et al., 2018).

3.5. Standardization and Quality Control

Establishing robust protocols for the production, characterisation, and quality control of stem cell and exosome therapies is critical for their successful translation to clinical practice. This includes developing standardised methods for isolation, expansion, and modification of these biological products (Reiner et al., 2017).

3.6. Health Economic Analyses

Comprehensive cost-effectiveness studies are needed to assess the economic impact of combined therapies and inform policy decisions regarding their implementation in healthcare systems. These analyses should consider both short-term costs and potential long-term savings from improved disease management.

3.7. Ethical and Regulatory Frameworks

Developing clear ethical guidelines and regulatory frameworks for the development and use of advanced therapies in MS is essential. This includes addressing issues of equitable access, informed consent, and the responsible use of biological materials (Hyun, 2010).

3.8. Mechanisms of Action Studies

Further research into the precise mechanisms by which combined therapies exert their effects in MS is key. This includes investigating the complex interactions between stem cells, exosomes and the host immune system, as well as their effects on CNS repair and remyelination (Uccelli et al., 2019).

3.9. Patient-Centered Outcomes Research

Incorporating patient perspectives and priorities into the development and evaluation of combined therapies is essential. This includes assessing patient-reported outcomes, preferences, and experiences with these novel treatment approaches.

3.10. Comparative Effectiveness Research

Large-scale studies comparing the effectiveness of combined therapies with current standard-of-care treatments are needed to guide clinical decision-making and policy development (Kalincik et al., 2017).

4. Conclusion

Integrating investigational cell- or EV-based interventions with established DMTs is a research question rather than a clinical strategy. Combination studies would need to show that adding the investigational component improves patient-relevant outcomes without unacceptable toxicity and to distinguish additive benefit from the effect of the background DMT.

The benefits of this integrated therapeutic strategy are numerous and potentially important. Cells are being investigated for their capacity to rebuild damaged tissue and exosomes for their ability to carry agents across the blood-brain barrier, though neither function has yet translated into measured clinical gain in MS. Taking viable cells out of the product also takes away some of their risks, which is part of the appeal, but no comparative study in MS has shown exosomes to be the safer option overall. Moreover, the prospect of personalised treatment based on patient-specific responses points towards more tailored and effective care, addressing individual variations in disease progression and treatment outcomes.

However, the challenges posed by these advanced therapies are considerable. MS is a complex and heterogeneous disease, and the variability in patient responses to treatment requires careful consideration. Long-term safety remains a critical concern, particularly in the context of the immune system's delicate balance. Potential risks include

exacerbating autoimmune reactions or triggering unforeseen adverse events. Furthermore, issues related to scalability, cost, regulatory approval, and the logistical complexities of combining multiple therapies must be thoroughly addressed before these treatments can become widely available.

Ethical concerns also drive the development of these therapies. Ensuring equitable access to emerging treatments, managing the ethical sourcing of biological materials, and addressing concerns over the exosome-mediated transfer of pathogenic factors are all pressing issues. The standardisation of production and quality control for these biological therapies adds another layer of complexity, as variability in the manufacturing process could impact their efficacy and safety.

Even so, combined cell- and EV-based strategies remain a research hypothesis rather than an established component of MS management. Their ability to improve remyelination, neuroprotection, long-term disability, or durable disease control has not been demonstrated in controlled clinical studies. These aims should therefore be framed as targets for future investigation, not as evidence for remission or cure.

Future MS care will continue to integrate advances in immunotherapy, repair biology, imaging, biomarkers, and digital methods. The contribution of cell- or EV-based approaches will depend on comparative clinical evidence, reproducible manufacturing, and long-term safety data rather than mechanistic plausibility alone.

Overall, integrating cell- or EV-based interventions with established MS therapies is an investigational concept that requires careful clinical evaluation. Progress will depend on controlled comparisons, reproducible product characterisation, prespecified patient-relevant outcomes, and long-term assessment of safety, scalability, and access.

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CHAPTER 7

CLINICAL STUDIES AND TRANSLATIONAL EXPERIENCE



7.1. PUBLISHED CLINICAL STUDIES OF CELL-BASED THERAPIES AND LONG-TERM OUTCOMES

Multiple sclerosis is an immune-mediated disorder in which inflammatory activity and neurodegeneration contribute differently across disease stages. Cell-based interventions have therefore been investigated for immune reconstitution, immunomodulation, and repair-related mechanisms, but the evidence base varies substantially by product and indication (Reich et al., 2018).

AHSCT, MSC products, and neural progenitor approaches differ in mechanism and evidence maturity. Their published outcomes are assessed separately below; uncontrolled observations and EV laboratory findings are given weight appropriate to their design.

1. Historical Context and Early Studies

The exploration of stem cell therapies for MS began in the late 1990s and early 2000s, with initial studies focusing on haematopoietic stem cell transplantation (HSCT) as a means of "resetting" the immune system. These early studies were primarily conducted in patients with aggressive forms of MS who had failed to respond to conventional treatments (Fassas et al., 1997; Burt et al., 2009).

Subsequent work examined mesenchymal stromal cells because of their immunomodulatory and trophic properties. The 2007 report by Mohyeddin Bonab et al. was among the early clinical studies to evaluate this approach in people with MS.

2. Mesenchymal stromal cell evidence: critical synthesis

Section 4.6 provides the study-level descriptions of MSC and MSC-NP trials. The discussion here focuses on what those findings imply for clinical use and for the design of subsequent studies.

2.1. Safety and feasibility

Early uncontrolled and phase I studies support procedural feasibility and provide short-term tolerability observations. Their small sample sizes, heterogeneous products, and limited follow-up do not establish a general safety profile and cannot reliably quantify uncommon or delayed adverse events.

2.2. Efficacy

Efficacy signals from uncontrolled series should be weighted below randomised evidence because spontaneous disease fluctuation, regression to the mean, concomitant treatment, and selective follow-up can influence apparent improvement. The multicentre MESEMS trial did not meet its prespecified MRI primary endpoint, and the more recent randomised MSC-NP trial likewise did not meet its primary EDSS-Plus comparison. These negative primary outcomes do not exclude biological activity, but they prevent claims of established clinical efficacy.

Two pooled analyses published since reach the same place by different routes. One, restricted to randomised trials of mesenchymal stem cell transplantation in progressive MS, combined three trials and 143 participants; it found no significant effect on EDSS and no excess of adverse events, and its authors concluded that the available evidence does not support benefit in unselected patients (Guo et al., 2026b). The other took in eleven randomised trials and 691 participants across both haematopoietic and mesenchymal products, again finding no significant difference in EDSS (mean difference −0.35, 95% CI −0.79 to 0.08) or in T2 lesion change, with comparable rates of treatment-emergent adverse events (Maji et al., 2025). In the same interval the recent randomised, placebo-controlled SMART-MS trial of intrathecally delivered mesenchymal stem cells in progressive MS failed to separate from placebo, and reported spinal imaging abnormalities that had not been anticipated (Kvistad et al., 2026). The correction to the Guo et al. (2026b) article concerned author order (Guo et al., 2026a).

2.3. Durability and long-term evidence

Longer follow-up cohorts, including MSC-NP and repeated-MSC series, are useful for surveillance and hypothesis generation. In the absence of concurrent randomised controls, however, stability or improvement cannot be attributed confidently to treatment, and durability of benefit remains unproven.

Biomarker sampling has begun to add a different kind of follow-up. Across a phase 2 trial and an expanded-access cohort, four cerebrospinal fluid markers, CCL2, MMP9, SCF and CHIT1, changed significantly after intrathecal treatment with mesenchymal stem cell-derived neural progenitors, while the corresponding serum levels did not and other established MS markers were unchanged (Harris et al., 2026). A measurable biological response in the compartment being treated is a precondition for benefit rather

than evidence of it, and these observations carry no concurrent control.

2.4. Translational interpretation

The central limitation of the MSC literature is heterogeneity: cell source, culture and release criteria, dose, route, treatment frequency, disease phenotype, concomitant therapy, and outcome selection differ substantially across studies. A clinically useful conclusion therefore requires more than pooling nominally similar interventions. Product identity and potency must be linked to prespecified endpoints in reproducible, adequately powered comparative trials.

3. Evidence hierarchy and clinical positioning

The cell-based approaches reviewed in this book should not be treated as a single evidence category. Autologous haematopoietic stem cell transplantation (AHSCT) has a distinct mechanism and a more mature clinical evidence base in carefully selected patients with highly active inflammatory relapsing MS, whereas MSC and MSC-NP products remain investigational and therapeutic EV administration in MS remains predominantly preclinical. This hierarchy is an interpretation of evidence maturity, not a recommendation to substitute one intervention for another.

4. Hematopoietic Stem Cell Transplantation (HSCT) in Multiple Sclerosis

Hematopoietic stem cell transplantation (HSCT) has been explored as a treatment for MS since the mid-1990s. The rationale behind this approach is to "reset" the immune system by eliminating autoreactive immune cells and reconstructing a new, tolerant immune system. Figure 7.1.1 shows the treatment pathway from patient selection to long-term follow-up.

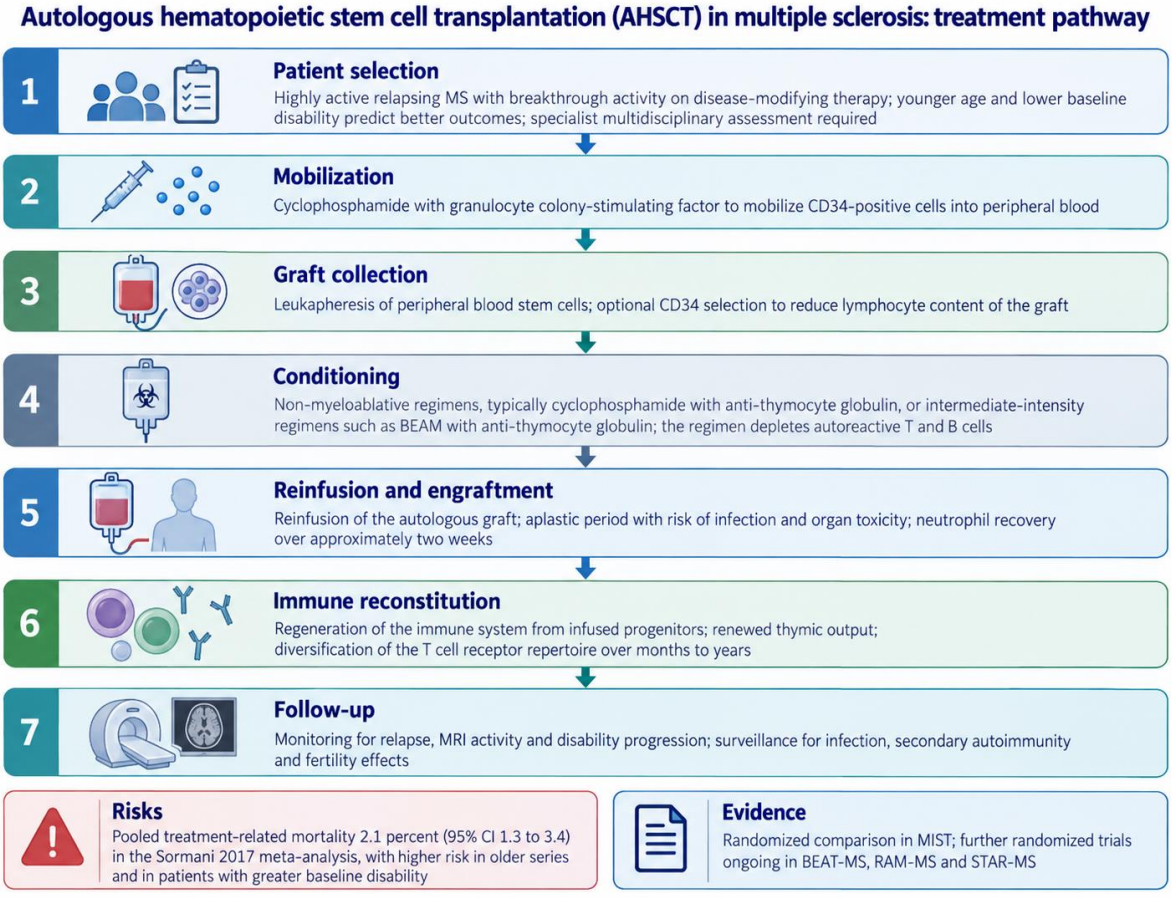


Figure 7.1.1. Treatment pathway for autologous haematopoietic stem cell transplantation in multiple sclerosis, from patient selection to long-term follow-up. Conditioning regimens and mortality estimates vary by centre, cohort and era.

4.1. Early Studies and Proof of Concept

An early clinical report by Fassas et al. (1997) described autologous HSCT in 15 patients with progressive MS. See Figure 7.1.2.

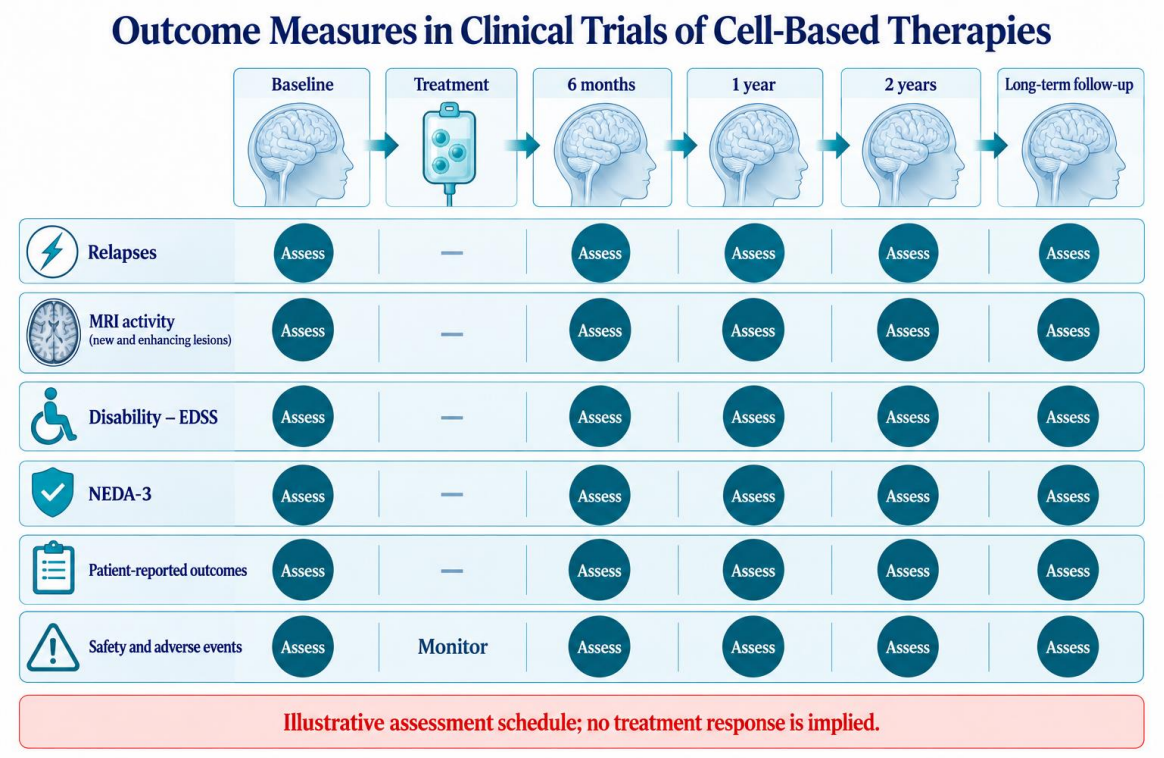


Figure 7.1.2. Outcome measures in clinical trials of cell-based therapies, shown along a schematic follow-up timeline. The timeline is a schematic assessment framework and should not be interpreted as an observed or predicted clinical course.

The procedure was feasible in that cohort, although clinically important toxicities occurred. Neurological outcomes varied, with stabilization or improvement reported in some participants; the uncontrolled design prevents causal interpretation.

The report helped establish the feasibility questions addressed in later HSCT studies.

4.2. Subsequent Studies and Refinement of Techniques

Over the next decade, several studies refined the HSCT approach for MS.

Burt et al. (2009) conducted a study on 21 patients with relapsing-remitting MS (RRMS). They reported: 81% of patients remained relapse-free at 3 years post-transplant. Significant improvement in disability scores in 62% of patients. Mancardi et al. (2015) conducted the ASTIMS trial, a randomised study comparing HSCT to mitoxantrone in secondary progressive MS and relapsing-remitting MS. Key findings included: HSCT was superior to mitoxantrone in reducing MRI activity. No difference in clinical progression was observed, possibly due to the advanced disease stage of enrolled patients. Burt et al. (2019) reported a randomised comparison of nonmyeloablative autologous HSCT with continued DMT in 110 patients with highly active RRMS. The primary report found: Disease progression occurred in 3 patients in the HSCT group and 34 in the DMT group during the trial follow-up. Time to progression was significantly longer with HSCT (hazard ratio 0.07, 95% CI 0.02–0.24; $p<0.001$). Quality-of-life outcomes were secondary and should not be generalized beyond the selected trial population.

4.3. Mechanism of Action

HSCT is believed to work through several mechanisms.

The conditioning regimen eliminates existing immune cells, including those that are autoreactive. The newly reconstituted immune system may develop tolerance to myelin antigens. HSCT may also reactivate the thymus, potentially improving central tolerance mechanisms.

4.4. Challenges and Future Directions

While HSCT has shown promise, several difficulties remain.

Identifying the most appropriate candidates for HSCT is essential. Current evidence suggests that patients with highly active RRMS and younger patients may benefit most. HSCT carries significant risks, including infections and treatment-related mortality. Ongoing research aims to develop less toxic conditioning regimens. More long-term follow-up studies are needed to establish the durability of treatment effects.

Registry and real-world series reported since have sharpened these figures. A twenty-year national analysis from the United Kingdom covering 364 transplants gave progression-free survival of 83.5% at two years and 62.4% at five, with transplant-related mortality of 1.4% occurring only in patients who already had advanced disability (Kazmi et al., 2025). A two-centre German cohort of 109 patients reported NEDA-3 in 82.8% and transplant-related mortality of 0.9%, with better results in relapsing than in progressive disease and in those who met consensus eligibility criteria (Fischbach et al., 2026). Comparative work has also begun to appear, including a Turkish cohort that examined five-year outcomes after transplantation against those after alemtuzumab (Manazoğlu et al., 2025). Whether the procedure should on this basis move to first line in highly active disease is now argued openly on both sides, and the disagreement rests less on the size of the effect than on how much early risk is acceptable and which comparator the effect is measured against (Mariottini & Muraro, 2026; Toljan & Jacob, 2026).

A 2026 individual-patient-data meta-analysis compared AHSCT with alemtuzumab using observational comparative studies. AHSCT was associated with greater EDSS improvement, relapse-free survival, and NEDA, but not with a statistically significant difference in disability progression or MRI activity. Because the analysis relied on non-randomised evidence and the certainty was rated very low, the authors treated the findings as hypothesis-generating rather than proof of superiority (Kebir et al., 2026).

The clinical position of AHSCT has also been formalized more clearly. Joint ECTRIMS-EBMT recommendations published in 2025 support AHSCT as a treatment option for relapsing MS that remains highly active despite high-efficacy disease-modifying therapy, while emphasizing specialist patient selection, transplant protocols, rehabilitation, and long-term monitoring (Muraro et al., 2025). A 2025 EBMT review focused on aggressive MS similarly argued for earlier consideration in patients with poor prognostic features when disease remains insufficiently controlled (Boffa et al., 2025). Subsequent 2026 real-world cohorts from the United Kingdom and Italy reported durable suppression of inflammatory activity in selected patients, with outcomes generally better in relapsing than progressive disease; these observational data strengthen effectiveness estimates but do not remove the need for randomised comparisons with contemporary high-efficacy DMTs (Muraro et al., 2026; Malucchi et al., 2026).

Evidence in progressive MS remains less certain. A 2024 systematic review assembled individual data from 665 patients with progressive MS across 15 studies and found wide variation in five-year progression-free and disease-activity outcomes, with an average transplant-related mortality of 1.9% in the studies reporting that outcome. The authors concluded that AHSCT does not reliably halt progression in unselected progressive MS and that any benefit is likely to depend on careful patient selection (Braun et al., 2024).

5. Neural stem/progenitor cell approaches in multiple sclerosis

Neural stem cells have been explored as a potential treatment for MS due to their ability to differentiate into various neural cell types and their potential to promote repair and remyelination.

5.1. Preclinical Studies

Numerous preclinical studies have demonstrated the potential of NSCs in MS models.

Pluchino et al. (2003) showed that NSC transplantation in an experimental autoimmune encephalomyelitis (EAE) model reduced inflammation and promoted remyelination. Einstein et al. (2007) reported that neural precursors attenuated EAE predominantly through peripheral immunosuppression rather than cell replacement.

5.2. Clinical Studies

Clinical evidence for neural stem/progenitor approaches in MS remains limited, and the available early-phase studies are best interpreted as feasibility and safety investigations.

Harris and colleagues (2016) reported a small uncontrolled pilot series using intrathecal injection of autologous mesenchymal stem cell-derived neural progenitors in progressive MS patients. They reported that the procedure was tolerated in this small uncontrolled cohort, and that changes

in walking speed and hand function in some participants were exploratory observations. In their review and consensus recommendations on cell-based strategies, Scolding et al. (2017) summarized early-phase studies of intravenous autologous bone marrow-derived MSCs, in which treatment was feasible and some structural, functional, and physiological measures changed; these uncontrolled findings likewise require confirmation in randomised comparisons.

The intrathecal fetal neural precursor-cell cohort reported by Genchi et al. (2023), which is distinct from MSC-derived neural progenitor cohorts, has now been followed much further. In the extension of the STEMS study, participants given intrathecal fetal neural precursor cells were followed for a median of 5.7 years with no serious transplantation-related adverse events; cognitive measures stayed stable and the rate of grey-matter atrophy was lower than in the period immediately after transplantation (Rocca et al., 2026). This speaks to the durability of safety, which was the main question left open by the phase 1 report. It leaves efficacy where it was, since there was no randomised comparator.

A 2026 review of neural stem-cell approaches reached a similarly cautious translational assessment: preclinical data support neuroprotective, immunomodulatory and remyelination-related mechanisms, but clinical development remains early and unresolved questions include cell source, delivery, survival, integration, immune response and long-term safety (Razzaghi et al., 2026).

5.3. Challenges and Future Directions

Several unresolved issues limit the clinical development of neural stem/progenitor approaches in MS.

Key uncertainties include the choice of cell source, route and timing of administration, survival and integration within host tissue, and long-term risks such as ectopic tissue formation or tumourigenicity. These questions require standardised products and prospective follow-up before efficacy can be assessed reliably.

Future research directions include:

Optimising cell preparation and delivery protocols. Combining NSC therapy with other treatments to enhance efficacy. Exploring the use of induced pluripotent stem cell (iPSC)-derived neural stem cells.

6. Synthesis

Among the cellular approaches reviewed here, AHSCT has the most mature clinical evidence for selected patients with highly active inflammatory relapsing MS, but it remains a complex intervention with material acute and long-term risks. MSC and neural progenitor approaches remain investigational, and their feasibility or exploratory signals should not be interpreted as established efficacy.

For extracellular vesicles, the evidence gap is wider: biomarker studies, human-sample experiments, and animal models support biological relevance but do not establish therapeutic benefit from EV administration in people with MS. Future trials should prioritise well-defined products, prespecified clinical endpoints, appropriate comparators, and long-term safety surveillance.

The translational question is therefore not whether cell and EV biology is interesting, but which precisely characterised intervention can produce a reproducible patient-relevant benefit that exceeds its risks and the performance of contemporary standard care.

7. Exosome Therapies in Multiple Sclerosis: Emerging Frontiers in Treatment

7.1. Introduction

Exosomes, small extracellular vesicles ranging from 30 to 150 nm in diameter, have been investigated as a significant area of interest in multiple sclerosis (MS) research over the past decade. These nanovesicles, secreted by various cell types, play central roles in intercellular communication by transferring proteins, lipids, and nucleic acids between cells (Théry et al., 2002). Their potential as diagnostic biomarkers and therapeutic agents has been explored in numerous diseases, including MS, a chronic autoimmune disease affecting the central nervous system (CNS). Size alone does not establish endosomal origin; EV or small EV is the operational term when that origin has not been demonstrated.

The exploration of exosomes in MS began to gain traction in the early 2010s, with pioneering studies investigating their role in disease pathogenesis and potential therapeutic applications. Exosomes have since shown promise both as biomarkers of disease activity and as candidate agents for modulating immunity and supporting neural repair in MS.

7.2. Exosomes as Biomarkers in Multiple Sclerosis

One of the most promising applications of exosome research in MS is their potential use as biomarkers for disease activity, progression, and treatment response. Several studies have investigated the utility of exosomes and other extracellular vesicles in this context.

7.2.1. Sáenz-Cuesta et al. (2014) Study

Sáenz-Cuesta et al. (2014) examined whether circulating microparticles, a class of larger extracellular vesicles distinct from exosomes, reflect treatment effects and clinical status in MS. The researchers collected blood samples from MS patients with different disease subtypes (relapsing-remitting, secondary progressive, and primary progressive) and from healthy controls.

Key findings were as follows. In untreated MS, platelet-derived microparticle levels exceeded those in controls. Across clinical subtypes, relapsing-remitting MS showed the highest levels, whereas levels in secondary progressive MS were similar to those in controls. Treated patient groups showed increased, rather than decreased, microparticle counts.

These observations may inform biomarker research in MS. However, microparticles cannot be equated with exosomes, and the reported associations do not establish a validated measure of disease progression, subtype, or treatment response; prospective analytical and clinical validation is required.

7.2.2. Geraci et al. (2018) Study

Building on this work, Geraci et al. (2018) investigated the potential of plasma-derived extracellular vesicles as biomarkers in MS. They found that:

The concentration of extracellular vesicles was significantly higher in MS patients compared to healthy controls. Specific miRNA profiles within these vesicles correlated with disease activity. Certain exosomal miRNAs showed potential as biomarkers for distinguishing between relapsing-remitting and progressive forms of MS.

This study highlighted the potential of exosomal miRNAs as biomarkers for MS diagnosis and prognosis, opening up new possibilities for non-invasive monitoring of disease activity.

7.2.3. Galazka et al. (2018) Study

In a notable study, Galazka et al. (2018) examined the presence of myelin proteins in serum-derived exosomes from MS patients. Their key findings included:

Exosomes isolated from serum, cerebrospinal fluid (CSF), and peripheral blood mononuclear cells (PBMCs) of MS patients contained central nervous system (CNS)-derived myelin proteins, including myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). Exosomal MOG content strongly correlated with disease activity in MS patients, with the highest levels detected in relapsing-remitting MS (RRMS) patients during relapse and in secondary progressive MS (SPMS) patients. Serum-derived exosomes retained the immunogenicity of MOG, as they induced the proliferation of MOG-specific T cells in vitro.

This study was particularly significant as it demonstrated that exosomes could carry CNS-specific proteins into peripheral circulation, potentially providing a "liquid biopsy" of CNS pathology in MS. The strong correlation between exosomal MOG levels and disease activity suggested that these exosomes could serve as valuable biomarkers for monitoring disease progression and treatment response.

7.2.4. Pieragostino et al. (2019) Study

Pieragostino et al. (2019) approached the same question by a different route. Rather than analysing bulk vesicle preparations, they sorted extracellular vesicles by flow cytometry and then characterised the proteome of the sorted populations, working with two fluids drawn from the same patients: cerebrospinal fluid and tears.

The pairing of those two fluids is the point of the design. Tears can be collected without a lumbar puncture, so the question being asked is whether the vesicle proteome of an accessible peripheral fluid carries any of the molecular information present in the compartment adjacent to the lesions. The authors report a disease-specific molecular cross-talk between the two fluids, which is a statement about shared proteomic features across compartments rather than about diagnostic accuracy in an individual patient.

What this establishes is narrower than a biomarker. Cohorts in extracellular-vesicle proteomics of this kind are small, vesicle sorting and proteomic workflows are not yet standardised between laboratories, and no threshold has been validated prospectively against a clinical endpoint. The contribution is methodological: it shows that a peripheral fluid can be interrogated for CNS-associated vesicle content, and it makes the case for testing that idea in larger, prospectively designed cohorts.

7.3. Exosomes in MS Pathogenesis

Understanding the role of exosomes in MS pathogenesis is important for developing targeted therapies and identifying new biomarkers. Several studies indicate how exosomes may contribute to the autoimmune processes driving MS.

7.3.1. Kimura et al. (2018) Study

Kimura et al. (2018) conducted an important study examining the effect of circulating exosomes from MS patients on regulatory T cells (Tregs). Their key findings included:

Exosomes from MS patients suppressed the induction of Treg cells, an effect mediated by let-7i microRNA present in the exosomes. Let-7i was found to target IGF1R and TGFBR1 genes, which are important in Treg cell development. Inhibition of let-7i counteracted the effect in T-cell culture; this result should not be described as EAE treatment evidence.

This study provided insights into how circulating exosomes might contribute to MS pathogenesis by suppressing regulatory T cell function, which leaves exosomes as candidate therapeutic targets and biomarkers in MS.

7.3.2. Azimi et al. (2018) Study

Expanding on this work, Azimi et al. (2018) investigated the immunomodulatory function of Treg-derived exosomes in RRMS patients. They found that:

Exosomes derived from Tregs of RRMS patients were significantly less effective in suppressing conventional T cell proliferation and inducing apoptosis compared to those from healthy controls. The mean percentage of proliferation suppression by exosomes was 8.04% in RRMS patients versus 12.5% in healthy controls. The apoptosis rate of conventional T cells induced by exosomes was lower in the RRMS group (0.68%) compared to healthy individuals (1.29%).

This impaired function of Treg-derived exosomes could contribute to the insufficient control of autoreactive T cells in MS, providing another potential mechanism by which exosomes influence disease progression.

7.3.3. Marcos-Ramiro et al. (2014) Study

In a study focusing on the blood-brain barrier (BBB), Marcos-Ramiro et al. (2014) examined circulating plasma microparticles from patients with MS and clinically isolated syndrome and their effect on the barrier function of cultured human brain endothelial cells.

The vesicles studied were circulating microparticles rather than endothelial exosomes, and the experiments were performed in vitro.

The findings suggest that circulating vesicles could influence endothelial barrier function, but they do not establish a causal role in BBB breakdown in people with MS.

7.4. Therapeutic Potential of Exosomes in MS

MSC-derived EVs have altered immune and repair-related readouts in preclinical studies. Human-cell experiments and isolated treatment reports provide different levels of evidence and should not be grouped as if they demonstrate clinical efficacy.

7.4.1. Riazifar et al. (2019) Study

Riazifar et al. (2019) conducted a wide-ranging study on the therapeutic effects of MSC-derived exosomes in an EAE model of MS. Their findings included:

Intravenous administration of MSC-derived exosomes significantly ameliorated EAE symptoms. Exosome treatment reduced demyelination and promoted remyelination in the CNS. The therapeutic effects were associated with a reduction in neuroinflammation and an increase in regulatory T cells. Exosomes were found to carry anti-inflammatory proteins and miRNAs that could contribute to their therapeutic effects.

This study provided strong preclinical evidence for the potential of MSC-derived exosomes as a cell-free therapy for MS.

7.4.2. Gurunathan et al. (2019) Study

Gurunathan et al. (2019) reviewed exosome isolation, characterisation, biological functions, and possible applications. The review supplies background for EV research; it does not demonstrate that MSC-derived EV administration restores BBB integrity or improves neurological outcomes in people with MS.

7.5. Challenges and Future Directions

Despite the early findings in exosome research for MS, several questions remain open.

7.5.1. Standardization of Isolation and Characterization Methods

The lack of standardised protocols for exosome isolation and characterisation makes it difficult to compare results across studies. Efforts are underway to develop consensus guidelines for exosome research (Théry et al., 2018).

7.5.2. Scalability and Manufacturing

Producing clinical-grade exosomes in sufficient quantities for therapeutic use remains a challenge. Research is ongoing to develop more efficient and scalable production methods (Whitford & Guterstam, 2019).

7.5.3. Targeting and Delivery

Ensuring that therapeutic exosomes reach their intended targets in the CNS is key. Strategies being explored include engineering exosomes with specific surface proteins to enhance BBB crossing and target cell uptake (Alvarez-Erviti et al., 2011).

7.5.4. Understanding Mechanism of Action

While exosomes have shown therapeutic potential, the precise mechanisms by which they exert their effects in MS are not fully understood. Further research is needed to elucidate these mechanisms and optimise therapeutic strategies.

7.5.5. Long-term Safety

As with any new therapeutic approach, the long-term safety of exosome therapies needs to be carefully evaluated in clinical trials.

7.6. Future Research Directions

Future research directions in exosome therapies for MS include:

Several directions stand out. Larger, well-designed clinical trials are needed to establish the efficacy and safety of exosome therapies in people with MS. Beyond that, exosome therapies might be tailored to individual patient characteristics and disease subtypes; combined with existing MS treatments or other emerging therapies to test for synergy; engineered for improved targeting, greater potency or a longer half-life; and used as delivery vehicles for small-molecule drugs or RNA therapeutics. Exosome-based biomarkers for diagnosis, prognosis and treatment monitoring also warrant further refinement.

7.7. Conclusion

Exosome research in MS has expanded understanding of disease biology, candidate biomarkers, and experimental delivery systems. As biomarkers, extracellular vesicles remain candidate tools requiring analytical and prospective validation. Therapeutically, MSC-derived exosomes have shown immunomodulatory and repair-related effects in preclinical models and human-cell laboratory systems; no controlled clinical trial has established efficacy of exosome administration in MS.

The main translational gap is prospective evidence linking a defined EV product to acceptable safety and clinically meaningful benefit.

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7.2. COMPARISON WITH TRADITIONAL TREATMENTS OF MS

1. Introduction

Multiple Sclerosis (MS) is a complex autoimmune disorder characterised by demyelination and neurodegeneration within the central nervous system (CNS). Affecting approximately 3.1 million people worldwide, MS is influenced by a combination of genetic susceptibility and environmental factors, which trigger an autoimmune response against myelin, leading to progressive neurodegeneration (King et al., 2026; Thompson et al., 2018). Clinically, MS presents in various forms, including relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS), each requiring distinct management strategies (Lublin et al., 2014).

Traditionally, MS treatments have centered around immunomodulatory therapies designed to reduce inflammation, manage relapses, and delay disease progression. These treatments, including interferons, glatiramer acetate, and more recently, monoclonal antibodies like natalizumab and ocrelizumab, focus primarily on modulating the immune system to limit the autoimmune attack on the CNS. While these therapies have demonstrated efficacy in controlling inflammation and reducing relapse rates, they fall short in addressing the underlying neurodegeneration and promoting neural repair, leaving an unmet need in MS treatment.

MSC and EV-based strategies are being studied for immunomodulation, delivery, and possible repair-related effects. Results in experimental systems have not established that EV products regenerate tissue or provide safer or more effective treatment than whole-cell approaches in MS. Product-specific characterisation and clinical comparison would be required to assess those possibilities.

Comparison with established treatment requires attention to study design, patient selection, endpoints, and follow-up. Mechanistic plausibility alone cannot establish the relative efficacy or safety of a cellular or EV product.

These approaches warrant further research on long-term safety, efficacy, scalability, and patient selection. Any comparison with established MS therapies must account for differences in study design, endpoints, disease stage, and follow-up. Figure 7.2.1 compares these approaches by strength of evidence.

2. Traditional Treatments for Multiple Sclerosis

2.1. Immunomodulatory Therapies

The cornerstone of established MS treatment is a set of immunomodulatory and immunosuppressive agents whose mechanisms, central trials, adverse effects and practical limitations are set out in detail in Section 3.1 and are not repeated here. For the purposes of this comparison, three features of that evidence base matter. First, these agents have been evaluated in large randomised controlled trials with radiological and disability endpoints, so their efficacy estimates rest on a level of evidence that cell- and vesicle-based approaches have not yet reached. Second, their benefit is concentrated in the inflammatory, relapsing phase of the disease, and effects on progression independent of relapse activity are modest at best. Third, their risks are characterised and manageable but real, ranging from injection-related reactions and hepatotoxicity to progressive multifocal leukoencephalopathy with cell-trafficking inhibitors. Any comparison with investigational cellular therapies must therefore weigh established, quantified benefit against potential that has not yet been quantified.

While these traditional treatments have shown efficacy in reducing relapse rates and slowing disability progression, they primarily target the inflammatory component of MS and have limited effects on neurodegeneration and repair. This limitation has driven the search for novel therapies that can address both aspects of the disease.

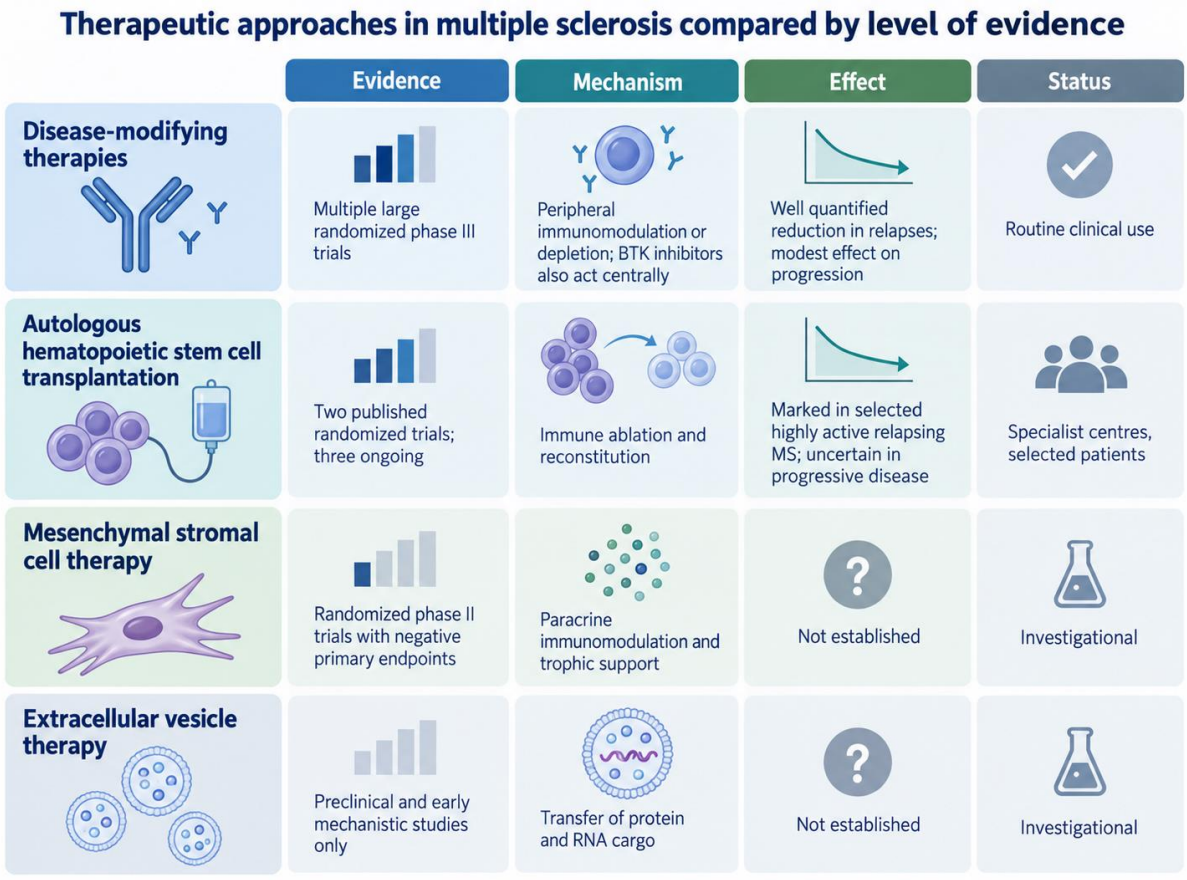


Figure 7.2.1. Established and investigational approaches in multiple sclerosis, compared by strength of evidence rather than by presumed potential. Detailed entries are given in Table 7.2.1. Evidence symbols are qualitative categories and do not represent numerical effect estimates.

3. Stem Cell Therapies in Multiple Sclerosis

AHSCT and MSC therapy should be assessed separately. AHSCT has a defined role in selected patients with highly active relapsing MS under specialist guidance; MSC therapy remains investigational. The approaches differ in mechanism, risks, and strength of evidence (Muraro et al., 2025).

3.1. Hematopoietic Stem Cell Transplantation (HSCT)

HSCT involves the ablation of the autoreactive immune system followed by reconstitution with autologous haematopoietic stem cells. This approach aims to "reset" the immune system, potentially eliminating autoreactive cells and inducing long-term remission.

A randomised clinical trial by Burt et al. (2019) compared nonmyeloablative autologous HSCT with continued disease-modifying therapy in a selected population with highly active relapsing-remitting MS.

Among 110 randomised patients, disease progression occurred in 3 patients in the HSCT group and 34 in the DMT group. Time to progression was significantly longer with HSCT (hazard ratio 0.07, 95% CI 0.02–0.24; $p<0.001$). This result applies to the selected trial population and is not a universal estimate for all MS patients.

The Sormani et al. (2017) meta-analysis pooled 15 studies involving 764 transplanted patients. It estimated progression at 17.1% at 2 years and 23.3% at 5 years; NEDA was 83% at 2 years and 67% at 5 years. These largely observational pooled estimates are not directly comparable with the randomised MIST trial because populations, designs, and outcome definitions differ.

HSCT remains an intensive treatment with potentially serious complications. In the Sormani et al. (2017) meta-analysis, pooled treatment-related mortality was 2.1% (95% CI 1.3%–3.4%), with higher risk in older studies and in patients with higher baseline disability. Contemporary estimates may be lower, but mortality varies with patient selection, conditioning regimen, centre experience, and

follow-up and should not be reduced to a single universal figure.

The long-term outcomes of HSCT in MS were reported in a recent study by Kvistad et al. (2024), which followed Norwegian patients with aggressive relapsing-remitting multiple sclerosis (RRMS). In this retrospective study, 69% of patients achieved no evidence of disease activity (NEDA-3), while 89.7% remained free from disease progression. Furthermore, there was a notable increase in work participation, with 52% of patients returning to full-time employment within five years post-HSCT. These findings suggest that HSCT can produce lasting remission and improve quality of life in patients with aggressive MS.

The randomised evidence base for AHSCT is, however, still thinner than its observational literature suggests, and this distinction matters for any comparison with disease-modifying therapy. Only two randomised trials have been completed and published to date: ASTIMS, which compared AHSCT with mitoxantrone in a small cohort, and MIST, which reported markedly less progression with AHSCT than with continued disease-modifying therapy but was conducted largely at a single centre in a selected relapsing population. The uncontrolled HALT-MS phase II study contributed important long-term event-free-survival data in highly active relapsing MS but, being single-arm, cannot establish comparative efficacy. Three further randomised trials are designed to address exactly this gap and will determine how AHSCT should be positioned relative to high-efficacy drugs: BEAT-MS (NCT04047628), a multicentre rater-blinded trial of 156 participants comparing AHSCT with best available therapy in treatment-resistant relapsing MS over six years of follow-up; RAM-MS (NCT03477500), a Nordic and Dutch open-label trial of approximately 100 participants comparing AHSCT with alemtuzumab, cladribine or ocrelizumab, with NEDA at two years as the primary endpoint and a five-year extension; and STAR-MS in the United Kingdom, which completed recruitment in 2024 and compares AHSCT with alemtuzumab, ocrelizumab, ofatumumab and cladribine. Until these trials report, statements that AHSCT is superior to high-efficacy disease-modifying therapy rest on retrospective and registry comparisons rather than on randomised evidence.

3.2. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells have shown promise due to their immunomodulatory and potentially neuroprotective properties. Unlike HSCT, MSC therapy does not require ablation of the immune system, which removes the risks that conditioning carries. No study has put the two side by side for safety, so a general claim of advantage would be premature.

The completed phase II MESEMS trial by Uccelli et al. (2021) evaluated a single intravenous dose of autologous bone-marrow-derived MSCs in adults with active MS using a randomised, double-blind, placebo-controlled crossover design. The prespecified primary efficacy outcome was cumulative gadolinium-enhancing lesion count over 24 weeks.

The primary efficacy comparison was negative: rate ratio 0.94 (95% CI 0.58–1.50; p=0.78). No reliable clinical disability benefit was established; adverse events were broadly similar between groups, and no treatment-related serious adverse events were reported.

These findings support feasibility and short-term tolerability but do not establish efficacy, durable neural repair, or superiority to HSCT. Mechanistic and preclinical findings provide a rationale for further study, not proof of clinical benefit. The mechanisms proposed for MSCs include:

MSCs have been shown to suppress T cell proliferation, induce regulatory T cells, and modulate B cell and natural killer cell function (Genc et al., 2019). Neuroprotection is another. MSCs secrete neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which may promote neuronal survival and axonal growth (Volkman & Offen, 2017). Recent evidence suggests that MSCs can enhance remyelination by promoting oligodendrocyte differentiation and maturation (Rivera et al., 2019).

The potential of MSCs to address both the inflammatory and neurodegenerative aspects of MS has led to increased interest in optimising MSC therapy. A recent study by Lin et al. (2023) demonstrated that upregulating CXCL12 in the spinal cord significantly alleviated symptoms of experimental autoimmune encephalomyelitis (EAE) in rats. This upregulation reduced leukocyte infiltration and promoted remyelination, suggesting that enhancing CXCL12 expression in MSCs may improve their therapeutic efficacy in MS by targeting neuroinflammation and promoting CNS repair.

Another approach is the use of MSC-derived neural progenitors (MSC-NPs), which are intended to combine the immunomodulatory properties of MSCs with trophic support for repair (Xiao et al., 2015). Clinically, however, repeated intrathecal MSC-NP treatment did not improve

the primary EDSS-Plus outcome compared with placebo in a randomised phase II trial (Harris et al., 2024).

4. Exosome Therapies in Multiple Sclerosis

Exosome therapy is an investigational area of MS research. Exosomes are extracellular vesicles that may cross the blood-brain barrier and carry therapeutic molecules. Their use as cell-free products may offer manufacturing and storage advantages over live-cell approaches, but their safety, pharmacology, and clinical efficacy remain unestablished.

4.1. MSC-derived Exosomes

A notable study by Li et al. (2019) demonstrated that exosomes derived from MSCs could attenuate inflammation and demyelination in an experimental autoimmune encephalomyelitis (EAE) rat model of MS. The study showed:

MSC-derived exosomes modulate microglial polarisation, shifting the balance towards an anti-inflammatory phenotype. Reduced demyelination and improved functional outcomes in treated animals.

In preclinical work, vesicles enriched in microRNA-219 promoted oligodendrocyte differentiation and myelination (Pusic et al., 2014), and Riazifar et al. (2019) reported that MSC-derived exosomes reduced neuroinflammation and demyelination in EAE, with an increase in regulatory T cells in the CNS. Effects comparable to those of whole MSCs were observed in those experimental models; they do not establish equivalence or clinical efficacy in people with MS.

4.2. Engineered Exosomes

Engineered exosomes are also being evaluated in experimental models. Studies have reported that vesicles carrying therapeutic molecules can improve CNS delivery and reduce neuroinflammation or promote remyelination in animal models. These findings support further preclinical development but do not establish clinical efficacy in MS (Riazifar et al., 2019; Li et al., 2019).

4.3. Clinical Translation of Exosome Therapy

Exosomes are extracellular vesicles that have gained attention in MS research because of their roles in cell-to-cell communication and immune modulation. Studies have examined extracellular-vesicle cargo in remyelination-related biology, immune regulation, and biomarker development. Pusic et al. (2014) studied IFN-γ-stimulated dendritic-cell exosomes in a preclinical remyelination model, while Kimura et al. (2018) examined circulating exosome effects on regulatory T-cell induction. These studies provide biological and biomarker context, but they do not demonstrate therapeutic efficacy in people with MS.

Baharlooi and colleagues studied MSCs and MSC-derived EVs in cultures of cells obtained from people with MS and controls. These are laboratory investigations, not trials of therapeutic EV administration. The 2022 report found cytokine effects that differed between patient and control cells, so findings should not be generalized uniformly across both groups (Baharlooi et al., 2021, 2022).

Exosomes have also been studied as candidate biomarkers for disease activity in MS. Sáenz-Cuesta et al. (2014) reported differences in circulating microparticles, and Galazka et al. (2018) reported myelin proteins in serum-derived exosomes. These observations are exploratory and require analytical and prospective clinical validation before they can guide treatment monitoring.

Current research explores engineering extracellular vesicles through modification of their parent MSCs or by loading specific therapeutic molecules, such as anti-inflammatory cytokines or microRNAs. Pistono et al. (2023) discussed the potential of EVs in immune regulation and CNS homeostasis. These approaches aim to improve targeting and potency, but most evidence remains preclinical; standardised isolation and characterisation, dose selection, and controlled human studies are still needed.

Exosome therapy offers several potential advantages over cellular therapy. It carries a potentially lower risk of replication-associated tumour formation than live-cell products, subject to product-specific evaluation; manufacturing and quality control are simpler; off-the-shelf availability becomes feasible; and bioactive cargo can be distributed across biological barriers, with efficiency depending on the product and the route of administration.

However, obstacles persist in the standardisation of exosome isolation and characterisation, as well as in optimising dosing and administration protocols. Ongoing research is focused on addressing these challenges to facilitate the clinical translation of exosome therapy for MS.

5. Comparative Analysis of MS Therapies

Table 7.2.1 gives the detailed entries behind that comparison.

Table 7.2.1. Comparative summary of established and investigational approaches in MS. Rows reflect the strength of available evidence rather than presumed potential.

	Established DMTs	AHSCT	MSC therapy	EV / exosome therapy
Evidence level	Multiple large randomised phase III trials	Two published randomised trials (ASTIMS, MIST); three ongoing (BEAT-MS, RAM-MS, STAR-MS)	Randomized phase II evidence available; MESEMS and the MSC-NP phase II trial negative on primary endpoints	Preclinical and early human mechanistic studies only; no controlled clinical trial
Principal mechanism	Peripheral immunomodulation or depletion; BTK inhibitors also act centrally	Immune ablation and reconstitution	Paracrine immunomodulation and trophic support	Transfer of proteins and RNA cargo; immunomodulation
Effect on relapses	Well quantified, substantial for high-efficacy agents	Marked in selected highly active relapsing MS	Not established	Not established
Effect on progression	Modest; tolebrutinib positive in non-relapsing SPMS	Uncertain in progressive disease	Not established	Not established
Principal risks	Agent-specific: PML, hepatotoxicity, infection, infusion reactions	Treatment-related mortality (2.1% in pooled older series), infection, secondary autoimmunity	Procedure-related risks; long-term safety data limited	Unknown; immunogenicity and manufacturing consistency unresolved
Practical status	Routine clinical use	Specialist centres, selected patients	Investigational	Investigational

5.1. Efficacy

Relapse effects require trial-specific comparison because populations, drugs, comparators, and follow-up differ. AHSCT relapse-free estimates cannot be compared directly with DMT annualised relapse-rate reductions. MSC trials have not established a consistent relapse benefit. For EV therapy, an isolated treatment report provides no controlled estimate of relapse prevention (Mohammad Jafari et al., 2025).

Disability progression is subject to the same caution. For disease-modifying therapies, confirmed-progression effects depend on the endpoint definition, the confirmation interval and the length of follow-up. In the MIST trial the hazard ratio for time to progression was 0.07 in a selected population (Burt et al., 2019), an estimate that cannot be read alongside percentage reductions reported in DMT trials. For mesenchymal stem cell therapy, durable stabilisation or improvement has not been established as a routine clinical benefit; heterogeneous results from small randomised trials require confirmation, and for extracellular vesicle therapy the evidence is preclinical.

5.2. Mechanism of Action

The mechanisms differ substantially across these treatment categories. Established DMTs primarily suppress or modulate immune activity. AHSCT uses conditioning followed by immune reconstitution. MSC-based interventions are being studied mainly for immunomodulatory and trophic effects rather than direct tissue replacement. Extracellular-vesicle approaches remain preclinical in MS and are investigated as carriers of biologically active cargo and modulators of intercellular signalling.

5.3. Safety Profile

The safety profile of established DMTs is agent-specific. Risks range from common laboratory or tolerability effects to uncommon but serious complications, such as progressive multifocal leukoencephalopathy with natalizumab in susceptible patients.

Autologous haematopoietic stem cell transplantation (AHSCT) is an intensive treatment with potentially

serious complications. In the Sormani et al. (2017) meta-analysis, pooled treatment-related mortality was 2.1% (95% CI 1.3%–3.4%), with higher risk in older studies and in patients with higher baseline disability. Contemporary estimates may be lower, but mortality varies with patient selection, conditioning regimen, centre experience, and follow-up and should not be reduced to a single universal figure. For mesenchymal stem cell therapy, early clinical studies have generally reported acceptable short-term tolerability, but samples are small, products and routes differ, and long-term safety is not established. Human laboratory studies should not be counted as clinical safety studies. Exosome therapy remains investigational. Preclinical studies may show a favourable short-term safety profile, but human safety data in MS are sparse and product-specific. Exosome preparations do not replicate as cells do, yet they can still have biological, immunological, manufacturing, and off-target risks. Registry studies in other diseases cannot establish long-term safety in MS.

5.4. Potential for Neural Repair

1. Traditional treatments: Limited to no direct effect on neural repair. However, some newer drugs may have indirect effects on repair processes. For instance, dimethyl fumarate has been shown to activate the Nrf2 pathway, which may promote neuroprotection and potentially enhance endogenous repair mechanisms (Linker & Gold, 2013).

2. Haematopoietic stem cell transplantation (HSCT): Although primarily aimed at resetting the immune system, HSCT has been proposed to create a favourable environment for natural repair processes. Long-term registry data show sustained freedom from progression in a proportion of patients after autologous HSCT, particularly those with relapsing disease (Muraro et al., 2017); these outcomes reflect suppression of inflammation and do not by themselves demonstrate neural repair or regeneration.

3. Mesenchymal stem cell therapy remains investigational. Preclinical studies suggest possible immunomodulatory and neuroprotective mechanisms, including trophic support and effects on oligodendrocyte-lineage cells, but clinical evidence has not established remyelination or disability improvement. 4. For exosome therapy, preclinical studies have reported that MSC-derived EVs carrying microRNAs can support oligodendrocyte precursor-cell differentiation in experimental models. These results are hypothesis-generating and do not establish remyelination or neural repair in people with MS (Riazifar et al., 2019).

6. Emerging Combination Therapies

As the understanding of MS pathophysiology advances, there is a growing interest in combination therapies targeting multiple disease aspects. Various innovative strategies are under exploration.

6.1. Sequential HSCT and MSC Therapy

Sequential or combined HSCT and MSC strategies remain hypothetical in MS. Boffa et al. (2023) studied autologous HSCT in active secondary progressive MS, not a combined HSCT–MSC intervention; their observed time to confirmed disability progression and quality-of-life findings therefore cannot be attributed to MSCs or to combination therapy. A controlled study would be needed to test whether adding MSCs provides incremental neuroprotection or functional benefit.

6.2. Exosome-Enhanced Pharmacotherapy

Exosomes, small extracellular vesicles, are being investigated as drug-delivery vehicles because experimental studies suggest they can cross the blood-brain barrier (BBB). Their potential for delivering therapeutic agents in MS remains unproven.

A study by Chen et al. (2016) investigated the migration of exosomes across an in vitro BBB model. The researchers found that exosomes could cross the BBB under inflammatory conditions, primarily via transcellular routes involving endocytosis. This finding suggests that exosomes can serve as carriers for therapeutic agents targeting the central nervous system (CNS).

While specific studies on exosomes loaded with fingolimod are limited, the general capability of exosomes to enhance drug delivery across the BBB has been demonstrated. For instance, Alvarez-Erviti et al. (2011) showed that exosomes could deliver siRNA to the mouse brain after systemic injection, indicating their potential as delivery vehicles for various therapeutic agents.

Together, these findings provide a preclinical rationale for studying exosome-based delivery of MS therapies; they do not establish improved efficacy or reduced systemic side effects in patients.

6.3. Nanoparticle-Mediated Remyelination Therapy

Remyelination is emerging as an investigational therapeutic strategy in managing multiple sclerosis (MS), a chronic autoimmune disease that deteriorates myelin sheaths in the central nervous system (CNS). This process of remyelination, involving critical cells such as oligodendrocytes, oligodendrocyte precursor cells (OPCs), and microglia, holds potential for restoring damaged myelin and slowing disease progression (Maciak et al., 2023). Stimulating remyelination could reduce further axonal injury and long-term disability in MS patients. Various microRNAs (miRNAs), including miR-223 and miR-124, are key regulatory molecules in remyelination, controlling gene expression related to myelin repair and immune modulation. For example, miR-223 facilitates myelin debris clearance by microglia, while miR-124 assists in restoring activated microglia to a resting state, both actions that support remyelination (Maciak et al., 2023).

Remyelination involves temporally regulated signalling pathways, including Wnt/β-catenin and PI3K/AKT/mTOR. Their effects depend on cell state and timing; pathway activation should not be assumed uniformly to promote OPC differentiation. Myelin-protein expression is one component of repair and does not alone establish functional restoration (Maciak et al., 2023).

Additionally, miRNAs warrant further investigation as biomarkers for monitoring disease progression in MS. For example, miR-21 and miR-146a/b have been associated with active MS lesions in cerebrospinal-fluid studies, making them potential indicators for treatment response (Muñoz-San Martín et al., 2019). Although miRNAs offer a possible route for diagnostics and therapeutics in MS, practical challenges, including standardised detection methods and reproducibility across patient samples, remain significant barriers to clinical application.

The article further discusses innovative experimental therapies utilising delivery systems such as EVs and poly(lactic-co-glycolic acid) (PLGA) nanoparticles to target remyelination. For example, recent studies have shown success in using EVs to deliver miR-219a-5p, stimulating OPC differentiation and myelination in animal models of MS. These therapeutic approaches represent significant progress in enabling effective miRNA delivery and traversing the blood-brain barrier (Osorio-Querejeta et al., 2020). Despite promising outcomes in preclinical studies, miRNA-based therapies for MS are still largely experimental, with more research needed to establish their viability for standard clinical practice (Maciak et al., 2023).

7. Future Directions and Challenges

Several areas warrant further investigation.

7.1. Personalized Treatment Approaches

The heterogeneity of MS necessitates more personalised treatment strategies. Emerging biomarker research, such as the work on serum neurofilament light chain levels, suggests that sNfL may help guide treatment decisions and predict response to specific therapies in MS. Integration of genetic, immunological, and imaging biomarkers could lead to more tailored treatment regimens (Jakimovski et al., 2020).

7.2. Targeting Progressive MS

Harris et al. (2024) did not meet the primary EDSS-Plus comparison in their randomised MSC-NP trial. Exploratory ambulatory and bladder findings, summarized in Section 4.6, justify further study but do not establish overall efficacy in progressive MS.

7.3. Long-term Safety and Efficacy

As novel therapies enter clinical practice, long-term follow-up studies are essential to establish their safety and durability of effect. Pivotal randomised trials, such as the ORATORIO trial of ocrelizumab in primary progressive MS (Montalban et al., 2017), provide controlled evidence over a limited period. Registries and long-term extension studies are therefore needed to assess real-world effectiveness and the sustainability of therapeutic effects across diverse patient populations, including for cellular and molecular therapies.

7.4. Regulatory and Manufacturing Challenges

Cellular and EV-based products face substantial manufacturing and regulatory challenges before any wider clinical use in MS. Cell source, preparation, preservation, and administration can alter product characteristics. Validated quality-control and potency assays are needed to assess consistency and safety; therapeutic efficacy must be established separately in appropriately designed clinical studies.

The International Society for Cell and Gene Therapy (ISCT) has responded to these challenges by forming specialized committees focused on establishing guidelines and standards specifically for mesenchymal stromal cell (MSC)-based treatments. These ISCT recommendations are aimed at facilitating regulatory approval and clinical

application by providing a framework that outlines best practices in MSC production, quality control, and clinical translation. The ISCT's guidance emphasizes collaboration among scientists, clinicians, and regulatory agencies to address the complexities of manufacturing cellular therapies on a large scale and under diverse clinical conditions. This collaborative approach is essential for overcoming the regulatory and manufacturing hurdles that have traditionally hindered the transition of these innovative treatments from research to routine clinical use.

As such, the establishment of these consensus-based guidelines not only helps streamline regulatory processes but also supports the consistent clinical application of MSC-based therapies. Through such efforts, the ISCT aims to enhance the reliability, safety, and accessibility of cellular therapies, supporting the development of their more extensive use in treating conditions such as graft-versus-host disease and autoimmune disorders. Addressing these regulatory and manufacturing hurdles is necessary to ensure that cellular therapies are produced, tested, and delivered with a degree of consistency and rigor comparable to that of traditional pharmaceuticals, ultimately contributing to more predictable patient outcomes and advancing the field of regenerative medicine (Alsultan et al., 2024).

8. Conclusion

The range of MS treatments is undergoing a profound transformation, driven by advances in our understanding of disease pathophysiology and the emergence of novel therapeutic modalities. While traditional immunomodulatory treatments remain essential to managing MS, stem cell and exosome therapies are being investigated for additional roles for broader disease modification, including the potential for neural repair.

AHSCT has controlled clinical evidence in selected patients with highly active relapsing MS and requires specialist assessment of substantial risks. MSC and neural cell approaches remain investigational, with mixed efficacy findings and limited safety follow-up. Therapeutic EV use in MS lacks established patient benefit. These approaches should therefore be compared according to their distinct mechanisms, study designs and evidence maturity.

Integration of investigational regenerative approaches with established MS therapy is a research question rather than a demonstrated clinical strategy. Combination approaches, including sequential HSCT and MSC therapy or EV-enhanced pharmacotherapy, should therefore be tested prospectively, with biomarkers and advanced imaging used to support, rather than replace, clinically meaningful outcomes.

Important gaps remain, particularly in progressive MS and in the long-term assessment of safety and disability outcomes. Progress will depend on adequately powered trials, clinically meaningful endpoints, independent replication, and transparent reporting rather than on the number of experimental approaches entering development.

Established disease-modifying therapies and regenerative strategies should not be presented as if they were competing versions of the same treatment. They address different problems and are supported by different levels of proof. DMTs have a defined role in controlling inflammatory disease activity; AHSCT has controlled evidence in selected patients but carries substantial treatment burden and risk; MSC, neural-cell and EV approaches remain investigational. A repair-oriented treatment would justify a place alongside established therapy only if it adds a reproducible patient-level benefit beyond inflammatory control, without an unacceptable safety or manufacturing penalty. Until that point, biological breadth is a reason to study an intervention, not a reason to assume that it works.

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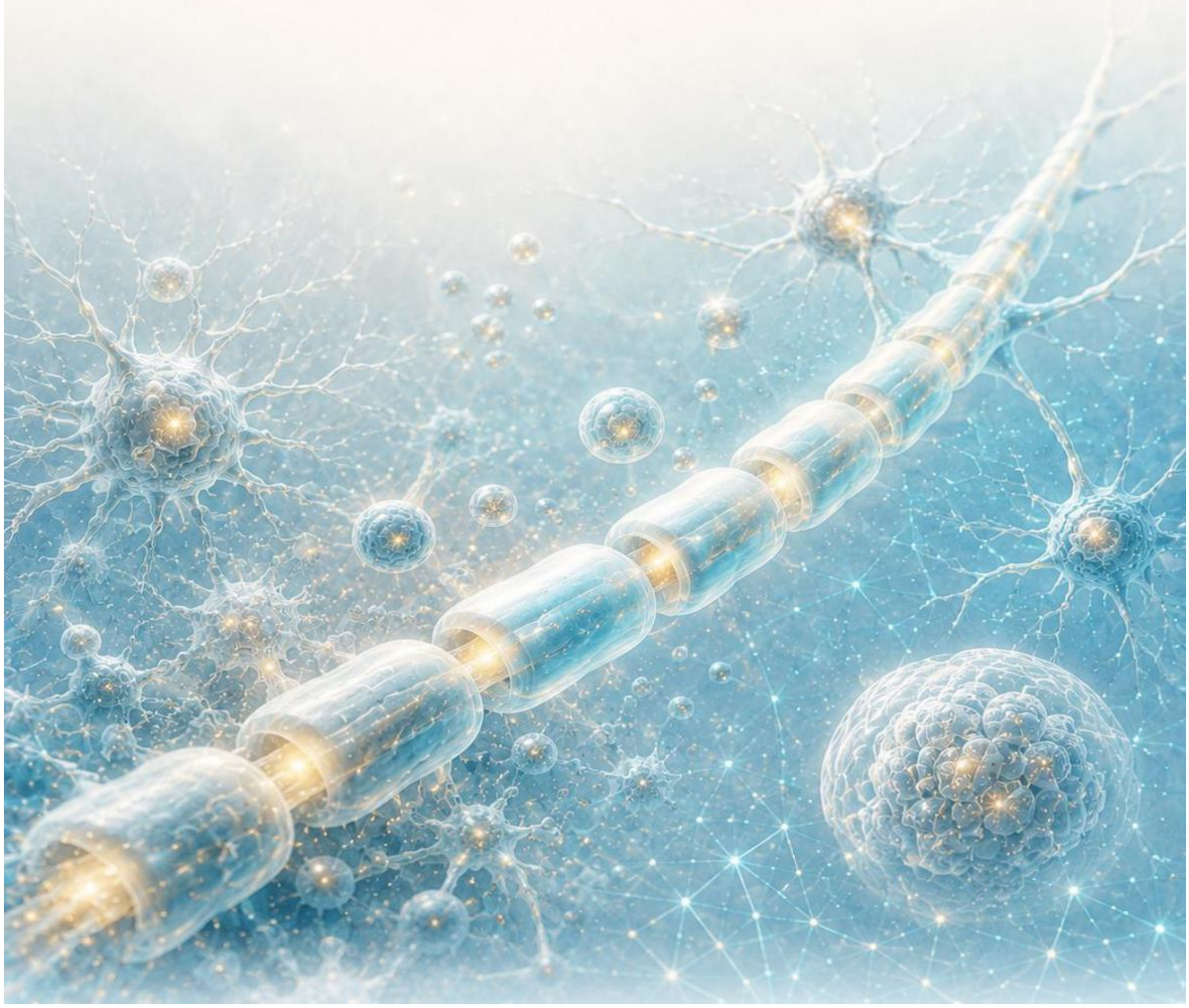
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CHAPTER 8

CONCLUSION AND FUTURE PERSPECTIVES



8.1. Introduction

Multiple sclerosis research is evolving across immunology, neurobiology, repair, biomarkers, and technology. The sections that follow distinguish established clinical advances from experimental strategies and identify the evidence needed before emerging approaches can be integrated into routine care. Figure 8.1.1 groups these priorities by time horizon.

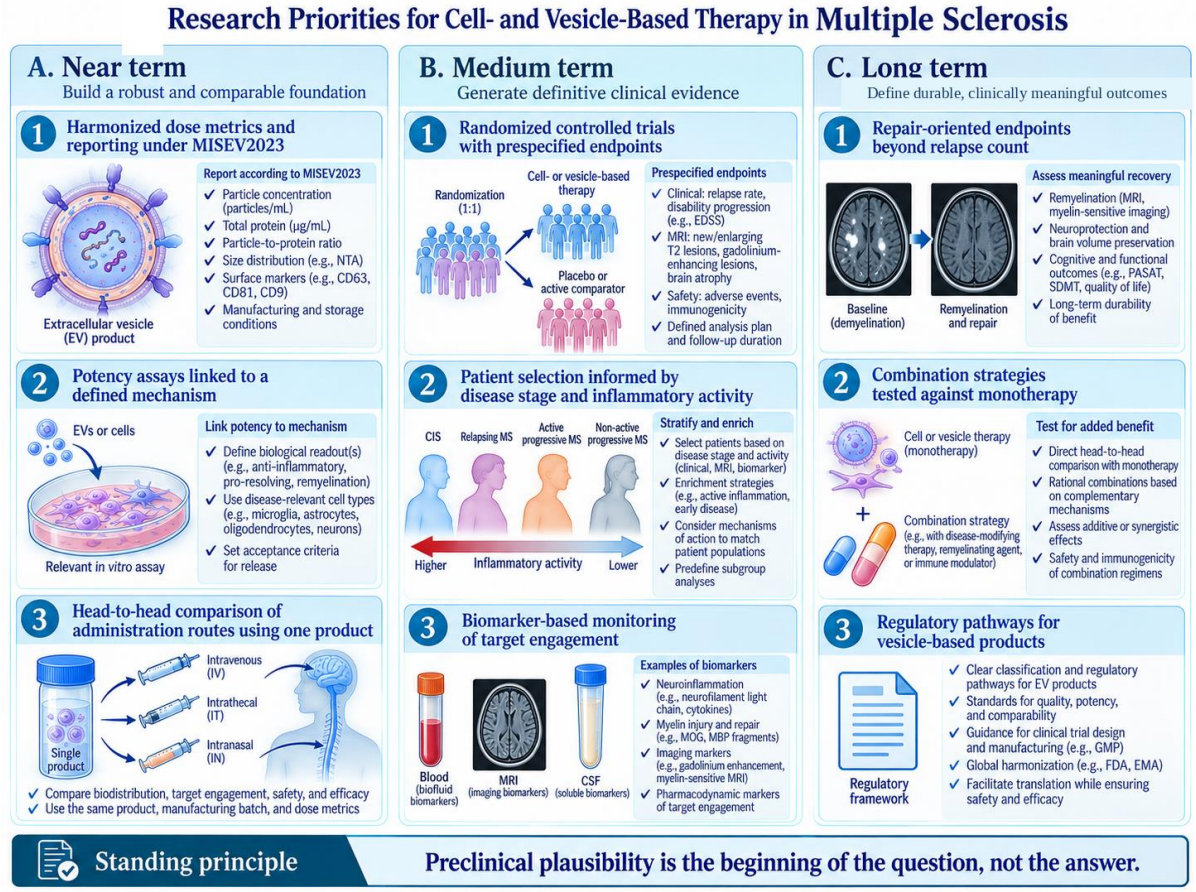


Figure 8.1.1. Research priorities for cell- and vesicle-based therapy in multiple sclerosis, grouped by time horizon. The near-term items are prerequisites for interpreting the clinical trials listed under the medium term. MRI-like panels and pathway elements are illustrative and do not represent individual patient data.

8.1.1. Neuroprotection and Remyelination Strategies

Multiple sclerosis involves both immune-mediated injury and incomplete tissue repair within the central nervous system (Höftberger & Lassmann, 2018; Zveik et al., 2024). Immunomodulatory and repair-oriented strategies are often discussed separately, but the underlying biology overlaps: inflammation influences oligodendroglial function, while repair processes occur within an immune-active microenvironment.

The Dual Role of Oligodendrocyte Progenitor Cells

Oligodendrocyte progenitor cells (OPCs) have long been recognised primarily for their ability to generate mature myelinating oligodendrocytes in the CNS (Chang et al., 2000; Hughes et al., 2013). These cells, which constitute approximately 6% of the total adult brain cell population, are especially abundant in areas such as the subventricular zone and corpus callosum (Dawson et al., 2003).

Beyond generating oligodendrocytes, OPCs can interact with immune pathways, including antigen presentation and cytokine signalling (Akay et al., 2021). Experimental work in the cuprizone model also links OPC biology to remyelination and endogenous repair (Leo & Kipp, 2022).

Reported OPC functions include expression of MHC-I and MHC-II, modulation of T-cell responses, and release of cytokines and chemokines in response to tissue injury. These observations support an immunological role for OPCs in addition to their contribution to remyelination.

In line with these findings, a recent study by Zveik et al. (2022) demonstrated that cerebrospinal fluid (CSF)

from progressive multiple sclerosis (pMS) patients impaired OPC differentiation into mature oligodendrocytes. Furthermore, pMS-derived CSF reduced immune-related gene expression in OPCs, including MHC-II expression, TNF-α secretion, and NFκB pathway activation. The implication is that the microenvironment in pMS may hinder both remyelination and immune functions of OPCs, pointing to potential therapeutic targets for progressive forms of MS.

Additionally emphasizing the therapeutic potential of OPCs, research by Mei et al. (2016) demonstrated that accelerated remyelination can prevent axonal loss and promote functional recovery in a murine model of inflammatory demyelination. By genetically targeting the M1 muscarinic receptor, the study highlighted the ability of OPCs to enhance remyelination, reduce neurodegeneration, and improve outcomes in experimental autoimmune encephalomyelitis (EAE). These findings argue for remyelination therapies that can offer neuroprotection in multiple sclerosis.

Emerging findings highlight the critical role of neuronal cholesterol synthesis in facilitating remyelination during chronic demyelination. A study by Berghoff et al. (2021) demonstrated that neurons increase cholesterol synthesis in response to chronic myelin injury, which promotes oligodendrocyte precursor cell (OPC) proliferation and remyelination. In experimental models, the absence of neuronal cholesterol synthesis significantly impaired remyelination and reduced OPC density, pointing to the necessity of local cholesterol production for effective repair mechanisms in MS.

ReBUILD reported a small improvement in visual-evoked potential latency with clemastine, a physiological signal compatible with remyelination; it did not establish broad disability recovery (Green et al., 2017). A later small TRAP-MS cohort with advanced progressive MS raised a disability-worsening safety signal during clemastine treatment (Kocot et al., 2025). The different populations and designs require careful interpretation and do not support routine clemastine use as an established MS repair therapy.

Although OPCs can differentiate within active demyelinated lesions, endogenous repair is often incomplete in MS (Heß et al., 2020). Several mechanisms may contribute to this limitation.

These include inhibitory lesion microenvironments, reduced or functionally impaired OPC pools, inefficient recruitment, and failure of differentiation despite successful migration into lesions.

Attempting to address these challenges, particularly the failed differentiation of OPCs, Cadavid et al. (2019) conducted a randomised, placebo-controlled phase 2 trial investigating opicinumab, a human monoclonal antibody against LINGO-1, which is an inhibitor of oligodendrocyte differentiation and axonal regeneration (Mi et al., 2005**)**. The study, involving 419 patients with relapsing multiple sclerosis, tested different doses of opicinumab (3, 10, 30, and 100 mg/kg) against placebo. While confirmed disability improvement was observed in different treatment groups (47% in 3 mg/kg, 63% in 10 mg/kg, 65% in 30 mg/kg, and 40% in 100 mg/kg) compared to placebo (49%), the study did not demonstrate a significant dose-linear improvement in disability. However, the identification of potentially responsive subpopulations suggested the need for further investigation to optimise opicinumab treatment in specific patient groups.

Contrary to traditional views, recent evidence suggests that controlled inflammation may be necessary for effective remyelination. Studies have shown the following.

Pro-inflammatory environments can enhance OPC differentiation through microglial crosstalk; acute inflammation may be required for effective remyelination in chronic demyelination models; and the timing and intensity of inflammatory responses are important factors.

More than 16 disease-modifying therapies (DMTs) have been approved by the FDA, all targeting systemic dysregulated immune responses. However, these treatments have shown limited efficacy in chronic progressive MS (CPMS), demonstrating the need for alternative approaches (Zveik et al., 2024).

A new therapeutic paradigm is emerging that recognizes the need to balance several elements:

pro-myelinating activity; immune modulation; OPC recruitment and differentiation; and optimisation of the lesion environment.

This approach suggests that successful treatment strategies must consider both the regenerative and immunological functions of OPCs, rather than targeting them independently.

The field is moving toward more integrated treatment approaches that recognize the interaction between inflammation and regeneration. Key areas for future research include:

development of targeted therapies that optimise both the immune and the regenerative functions of OPCs; better understanding of the temporal and spatial requirements for controlled inflammation; and investigation of combination therapies that can address multiple aspects of disease progression.

8.1.2. Targeted Immunotherapies

The development of more specific immunomodulatory drugs that can selectively target pathogenic immune cells while preserving protective immunity is a growing area of research. This approach aims to improve upon current broad-spectrum immunosuppressive therapies by offering more targeted intervention with potentially fewer side effects.

The phase 3 OPERA trials evaluated ocrelizumab against interferon beta-1a in relapsing MS (Hauser et al., 2017). Evidence in PPMS came from the separate placebo-controlled ORATORIO trial (Montalban et al., 2017). Efficacy in one disease phenotype should not be used as proof of efficacy in the other.

ULTIMATE I and II were two phase 3 trials involving 1,094 participants with relapsing MS, comparing ublituximab with teriflunomide over 96 weeks. Ublituximab reduced annualised relapse rates and MRI lesion activity, but the pooled confirmed disability-progression comparison was not statistically significant (Steinman et al., 2022).

Targeting immune pathways linked to endogenous retroviral elements has also been studied. GNbAC1, a humanized monoclonal antibody directed against the multiple sclerosis-associated retrovirus envelope protein, was evaluated in early-phase studies. Tolerability and pharmacokinetic findings supported further investigation, but early MRI and molecular observations were not sufficient to establish clinical efficacy (Derfuss et al., 2015).

Multiple sclerosis (MS) treatment has seen significant advancements with the development of targeted immunotherapies that modulate pathogenic immune responses while preserving protective immunity. A notable innovation in this area is the engineering of chimeric antigen receptor (CAR) regulatory T cells (Tregs) to target specific autoantigens involved in MS pathology. A recent preclinical study by Frikeche et al. (2024) developed a myelin oligodendrocyte glycoprotein (MOG)-specific CAR Treg cell therapy intended for MS. MOG is expressed on the outer membrane of the myelin sheath, making it an ideal target for CAR Treg therapy. The lead candidate is a second-generation CAR, composed of an anti-MOG single-chain variable fragment (scFv) screened from a large human library. In vitro, CAR-dependent functionality was demonstrated, and efficacy was shown in vivo using a passive experimental autoimmune encephalomyelitis (EAE) mouse model. Additionally, the MOG-CAR Tregs exhibited very low tonic signalling with a desirable signal-to-noise ratio, resulting in a highly potent CAR. These findings support further preclinical development of MOG-CAR Tregs. They do not establish a treatment option, durable immune tolerance, safety, or efficacy in patients with MS.

8.1.3. Gene Therapy Approaches

Understanding the genetic foundations of Multiple Sclerosis (MS) is important for gene therapy approaches. A thorough study by the International Multiple Sclerosis Genetics Consortium, analysing genetic data from 47,429 MS cases and 68,374 control subjects, has revealed the complex genetic architecture of MS. The study identified 200 autosomal susceptibility variants outside the major histocompatibility complex (MHC), one chromosome X variant, and 32 variants within the extended MHC. These findings explain approximately 48% of MS's genetic heritability. Notably, enrichment for MS susceptibility genes was observed in microglia and various immune cell types, highlighting their significant role in the autoimmune process targeting the central nervous system (International Multiple Sclerosis Genetics Consortium, 2019).

CRISPR-Cas9 approaches provide experimental tools for studying neurological disease mechanisms (Guan et al., 2022). MS susceptibility involves many loci and environmental influences. Target validity and the consequences of editing require direct investigation before a therapeutic strategy can be supported.

Lee et al. (2019) studied CRISPR-edited, soluble RAGE-secreting MSCs in a Parkinson disease animal model. Its possible relevance to MS remains a hypothesis requiring direct investigation in appropriate models.

Villiger et al. (2024) reviewed technologies for genome, epigenome and transcriptome editing. These methods offer research opportunities, but their capacity to modify MS progression remains to be established.

These technologies support mechanism research and the development of testable therapeutic hypotheses. MS-specific target validity, delivery, off-target effects and durable benefit require direct evidence before clinical translation can be justified.

8.1.4. Nanoparticle-based Therapies

Nanoparticle-based delivery systems are being investigated in MS as experimental methods for improving tissue targeting and drug distribution. Their possible relevance spans inflammatory and neurodegenerative mechanisms, but clinical utility remains to be established.

Fundamentals and Therapeutic Mechanisms

Nanoparticle delivery systems can be engineered to modify cargo distribution and release. CNS entry depends on composition, size, surface properties, route and barrier state; nanoscale dimensions do not ensure blood-brain barrier passage. Reduced systemic toxicity or improved clinical efficacy must be demonstrated for each product.

Liposomal nanoparticles, among the most widely studied delivery systems, demonstrate considerable versatility in MS treatment. Their phospholipid bilayer structure closely mimics cellular membranes, facilitating improved drug delivery across biological barriers. These carriers can be modified to encapsulate both hydrophilic and hydrophobic therapeutic agents, offering considerable flexibility in drug delivery design.

Polymeric nanoparticles represent another promising category, characterised by their biodegradability and biocompatibility. These systems provide sustained drug release profiles and can be engineered to respond to specific physiological triggers, enabling precise temporal and spatial control over drug delivery. The ability to modify their surface properties allows for enhanced targeting capabilities and improved therapeutic outcomes.

Clinical Applications and Treatment Strategies

In MS research, nanoparticle systems are being investigated for delivery of immunomodulatory agents to relevant cells or tissues. Selective targeting, preservation of healthy tissue and effects on relapse or progression remain product-specific questions requiring experimental and clinical validation.

The application of nanoparticle technology in delivering neuroprotective agents presents an active area of investigation in MS treatment. These systems can be designed to cross the blood-brain barrier efficiently, delivering therapeutic agents directly to damaged neurons and supporting cells. This targeted approach holds particular promise for addressing the neurodegenerative aspects of MS that have traditionally been difficult to treat with conventional therapies.

Current Research and Future Directions

Stimulus-responsive nanoparticle delivery systems can be designed to release cargo under specified laboratory conditions. Their targeting accuracy, safety, and clinically meaningful effects in MS require direct experimental and clinical validation.

The potential for nanoparticle-based systems to facilitate combination therapy is particularly noteworthy. These platforms can be designed to carry multiple therapeutic agents simultaneously, potentially addressing different aspects of MS pathology in a coordinated manner. This multi-modal approach may prove especially valuable in treating progressive forms of MS, where both inflammatory and neurodegenerative processes need to be addressed concurrently.

Technical Considerations and Clinical Implementation

The successful implementation of nanoparticle-based therapies in clinical practice requires careful consideration of various technical aspects. Manufacturing processes must be standardised to ensure consistent particle size distribution and drug loading efficiency. Additionally, the stability of these systems under physiological conditions and their long-term safety profiles require thorough evaluation.

While the potential benefits of nanoparticle-based therapies are substantial, several challenges need to be addressed before widespread clinical adoption. These include optimising production methods for large-scale manufacturing, establishing broad safety profiles, and developing cost-effective formulation strategies. Regulatory considerations also contribute substantially to the development pathway, requiring careful attention to safety documentation and approval processes.

Nanoparticle-based systems are an active area of MS research. Their potential value depends on product-specific evidence for biodistribution, target engagement, safety, manufacturing consistency, and clinically meaningful benefit; current preclinical or platform-level advantages should not be assumed to translate into superior patient outcomes.

The continued development of nanoparticle-based therapies, coupled with advances in our understanding of MS pathophysiology, suggests a promising future for this therapeutic approach. While significant work remains to be done, the potential benefits of these systems warrant continued investigation and development.

8.1.5. Stem cell-based Approaches

Over the past decades, stem cell-based therapies have developed into an investigational treatment strategy for multiple sclerosis (MS), offering potential for both neuroprotection and regeneration (Pluchino et al., 2020). Neural stem cells (NSCs) have shown particular potential due to their ability to differentiate into oligodendrocytes and promote remyelination of damaged axons. Multiple studies have demonstrated that transplanted NSCs can survive, integrate into host tissue, and improve functional outcomes in animal models. Human experience includes at least two distinct open-label phase 1 studies: intrathecal transplantation in 12 participants (Genchi et al., 2023) and intracerebroventricular transplantation in 15 participants (Leone et al., 2023). These studies assessed feasibility and safety and do not establish efficacy.

Several complementary mechanisms have been proposed for how NSCs might act in MS. Research has shown that NSCs can directly differentiate into oligodendrocytes to replace lost myelin-producing cells and restore saltatory conduction (Li et al., 2024). Additionally, transplanted NSCs secrete a wide array of neurotrophic factors that support survival of existing neural cells and promote endogenous repair processes. These factors include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and various cytokines that create a supportive microenvironment for repair (Zveik et al., 2024).

A key advantage of NSC-based approaches is their immunomodulatory properties. Studies have demonstrated that NSCs can regulate T-cell responses, modulate microglial activation, and reduce inflammatory mediators in the CNS (Pluchino et al., 2020). This dual action of promoting repair while dampening inflammation makes them particularly attractive for MS treatment.

Both endogenous and transplanted NSC populations have been extensively investigated. While strategies to activate and enhance endogenous NSC populations warrant further investigation, they face challenges with limited regenerative capacity in chronic MS (Li et al., 2024). Transplantation approaches using various NSC sources have progressed further, including adult NSCs, embryonic stem cell-derived NSCs, and induced pluripotent stem cell-derived NSCs. Each source has distinct advantages and considerations regarding accessibility, scalability, and ethical implications (Genchi et al., 2023).

Genchi et al. (2023) reported an open-label phase 1 study of intrathecal human fetal neural precursor cells in twelve patients with progressive MS. The study assessed feasibility, safety and tolerability over two years. Exploratory imaging and CSF findings did not establish clinical efficacy, neurological restoration or slowed progression in the absence of a randomised comparator.

Looking ahead, combination approaches that integrate NSC therapy with other therapeutic strategies may prove most effective. For instance, combining NSC transplantation with targeted immunomodulation or factors that enhance cell survival and integration could maximize therapeutic benefit (Zveik et al., 2024). Additionally, advances in cell engineering and delivery methods continue to improve the potential of NSC-based approaches.

While significant questions remain open in translating NSC therapies to widespread clinical use, this approach represents an important frontier in MS therapeutics. Continued research to better understand NSC biology, optimise their application, and identify ideal therapeutic windows will be critical for realizing their full potential in treating MS (Pluchino et al., 2020).

8.1.6. Microbiome-based Interventions

Recent research has established a significant role for the gut-brain axis in multiple sclerosis (MS), leading to increased interest in microbiome-based therapies. For instance, a meta-analysis identified common gut microbiota associated with MS across diverse studies, revealing a lower relative abundance of Prevotella and a higher abundance of Actinomyces in MS patients compared to controls (Jin et al., 2024).

Building on these findings, clinical trials have explored the therapeutic potential of probiotics in MS management. A systematic review and meta-analysis of randomised controlled trials demonstrated that probiotic supplementation significantly improved mental health outcomes in MS patients, including reductions in Expanded Disability Status Scale (EDSS) and depression scores (Rahimlou et al., 2022).

Additionally, a 12-week double-blind clinical trial investigated the effect of probiotic supplementation on systemic inflammation in MS patients. The study found that probiotic intake markedly improved insulin resistance and reduced inflammatory and oxidative stress markers, suggesting a beneficial impact on systemic inflammation in MS (Rahimlou et al., 2022).

These studies leave microbiome-based interventions worth pursuing, such as probiotics, in modulating disease activity and improving the quality of life for individuals with MS.

These emerging research areas and potential developments represent opportunities for advancing MS treatment. As these novel approaches move from preclinical studies to clinical trials, they hold the promise of more effective, targeted, and personalised therapies for individuals living with MS. The coming years are likely to see significant progress in translating these innovative concepts into practical treatments that can improve the lives of MS patients.

8.2. Integration of Treatment Strategies into Clinical Practice

Translating research findings into clinical practice remains a significant challenge in MS management. The implementation of new treatment strategies requires careful consideration of efficacy, safety, and cost-effectiveness, as well as the development of infrastructure and protocols to support their use.

8.2.1. Multi-disciplinary Approaches

Multidisciplinary care addresses neurological symptoms, rehabilitation needs and psychosocial consequences. The broad review by Thompson et al. (2018) provides clinical context. Specific care models require evaluation using comparative designs and patient-relevant outcomes.

The ECTRIMS/EAN guideline addresses pharmacological treatment decisions in MS (Montalban et al., 2018). Care coordination and individualized treatment are related components of management, with their effectiveness assessed using outcomes appropriate to each intervention.

8.2.2. Clinical Decision Support Tools

The integration of artificial intelligence (AI) into clinical decision support (CDS) tools is transforming the management of multiple sclerosis (MS). These tools enable healthcare providers to make more informed treatment decisions by analysing individual patient characteristics and disease patterns.

A notable example is the MS BioScreen, developed by the University of California, San Francisco (UCSF). This platform consolidates clinical, imaging, and biomarker data to offer individualized risk assessments and treatment guidance. By visualizing a patient's disease trajectory alongside data from a large cohort, MS BioScreen facilitates more informed decision-making for both clinicians and patients (Gourraud et al., 2014).

Further advancements include AI-driven models that predict patient-specific responses to various MS treatments. For instance, a study by Naji et al. (2023) reviewed the application of AI in MS, highlighting its potential to enhance diagnostic accuracy, monitor disease progression, and optimise treatment strategies (Naji et al., 2023).

These AI-powered systems synthesize complex datasets to deliver tailored treatment recommendations, considering genetic markers, MRI findings, and clinical histories. By identifying patterns indicative of treatment efficacy or potential side effects, these models assist clinicians in selecting optimal therapies for individual patients. Moreover, the continuous learning capabilities of deep learning approaches enable these systems to refine their predictive accuracy over time, incorporating new patient data to enhance the relevance of their recommendations.

AI-based clinical decision-support tools are being evaluated for patient stratification, monitoring, and treatment selection in MS. Their clinical value will depend on external validation, calibration, transparency, data quality, and demonstration that model-assisted decisions improve outcomes or safety.

8.2.3. Telemedicine and Remote Monitoring

The COVID-19 pandemic has accelerated the adoption of telemedicine in MS care, and emerging evidence suggests that this approach can be effective for many aspects of MS management.

Bove et al. (2019) evaluated a telemedicine-enabled disability examination in 41 adults with MS. This proof-of-principle study supported the feasibility of remote assessment in selected patients. Generalizability, implementation costs and effects on care access require additional evaluation.

Midaglia et al. (2019) assessed smartphone- and smartwatch-based monitoring over 24 weeks in 76 people with MS and 25 controls. The study focused on adherence and satisfaction. Demonstrating feasibility does not establish reliable prediction of future clinical worsening.

Digital monitoring and retinal imaging are distinct assessment approaches. Zimmermann et al. (2018) studied retinal ganglion cell layer thickness and subsequent disease activity in clinically isolated syndrome using optical coherence tomography.

Retinal imaging provides candidate markers of tissue injury. Associations with subsequent disease activity require interpretation within the studied population and independent validation before use in individualized treatment decisions (Zimmermann et al., 2018).

The integration of advanced monitoring technologies with telemedicine platforms has led to a new paradigm in

multiple sclerosis (MS) care. A study by Rodríguez-Fuentes et al. (2024) evaluated the feasibility of an immersive virtual reality exercise program for MS patients. This program combined telemedicine consultations, remote monitoring, and AI-driven decision support tools into a seamless digital ecosystem. The study, which followed 18 MS patients over a period, found that this integrated virtual care approach was feasible and safe, with participants completing the program without adverse effects. Notably, patients in the virtual care group reported significant improvements in functional capacities, including increased lower limb strength and improved functional mobility, compared to those receiving traditional care.

This study indicates the potential of virtual care models to enhance MS management by leveraging technology to provide more complete, patient-centered care.

8.2.4. Patient Education and Self-Management

Patient education should explain treatment goals, uncertainties, adverse effects and monitoring in language suited to the individual. Digital educational tools require direct assessment of comprehension, accessibility and patient-relevant outcomes; their effectiveness should not be inferred from technical capability alone.

Building on this concept, recent efforts have focused on the integration of patient involvement and digital tools in MS care. For example, Gray et al. (2023) highlighted the importance of inclusive and participatory approaches, including digital strategies, in enhancing patient education and trial engagement in multiple sclerosis research.

8.2.5. Integration of Complementary Therapies

Multiple sclerosis (MS) is a chronic, autoimmune, and neurodegenerative disease of the central nervous system that can lead to significant disability (Podlecka-Piętowska et al., 2022). While disease-modifying therapies (DMTs) have been developed to target the inflammatory and neurodegenerative aspects of MS, many patients continue to experience debilitating symptoms (Thompson et al., 2018). As a result, a significant proportion of individuals with MS have turned to complementary and alternative medicine (CAM) therapies to manage their condition (Weld-Blundell et al., 2022; Namjooyan et al., 2014).

The use of CAM therapies among individuals with MS is well-documented, with studies reporting that 31-82% of patients utilize these approaches (Weld-Blundell et al., 2022; Skovgaard et al., 2012). The majority of CAM use has been observed in patients with relapsing-remitting MS (RRMS) who are mildly or moderately affected (Campbell et al., 2017; Podlecka-Piętowska et al., 2022). This suggests that CAM therapies may be particularly appealing to those in the earlier stages of the disease.

Some of the most promising CAM therapies that have been investigated for MS include vitamin D supplementation, omega-3 fatty acids, lipoic acid, and low-fat diets (Yadav et al., 2010; Namjooyan et al., 2014). Vitamin D, in particular, has shown potential benefits in reducing disease activity and disability progression (Yadav et al., 2010). However, the evidence for the efficacy of these CAM approaches remains limited, and more high-quality research is needed to establish their safety and effectiveness (Podlecka-Piętowska et al., 2022).

Despite the widespread use of CAM therapies, current clinical practice guidelines do not recommend their regular use due to the lack of robust evidence (Podlecka-Piętowska et al., 2022). The American Academy of Neurology guidelines, for example, state that there is insufficient evidence to support the routine use of CAM therapies in MS (Podlecka-Piętowska et al., 2022). Further research is therefore needed to better understand the role of these therapies in the management of MS.

Importantly, the integration of CAM therapies into the overall treatment plan for individuals with MS should be done in close collaboration with their healthcare providers (Skovgaard et al., 2012). This is key to ensure that the use of CAM does not interfere with the effectiveness of conventional DMTs or pose any safety risks (Frohman et al., 2011). Healthcare providers should also be aware of the potential benefits and limitations of CAM therapies and be prepared to discuss these with their patients (Yadav et al., 2014).

The use of CAM therapies is common among individuals with MS, particularly in the earlier stages of the disease. While some CAM approaches, such as vitamin D supplementation, warrant further investigation, the evidence for their efficacy and safety remains limited. Healthcare providers should work closely with their patients to carefully evaluate the potential role of CAM therapies within the overall treatment plan while also advocating for more high-quality research in this area.

Simpson, Mair and Mercer (2017) conducted a feasibility study on Mindfulness-Based Stress Reduction (MBSR) for people with multiple sclerosis (MS). Fifty participants were randomised into MBSR or a wait-list control group. The study found significant improvements in stress,

depression, and anxiety immediately post-intervention, with diminished effects at three months, but sustained gains in mindfulness and self-compassion. Retention was high (90%), though only 60% completed the program. The study supports MBSR as a feasible intervention for MS, recommending further trials for optimisation and efficacy.

8.2.6. Pharmacoeconomic Considerations

As new and often costly treatments enter the market, there is an increasing focus on pharmacoeconomic analyses to guide treatment decisions and resource allocation. A detailed study by Martins et al. (2023) conducted a cost-effectiveness analysis of ocrelizumab for the treatment of multiple sclerosis (MS) in the Portuguese healthcare system. The study found that while ocrelizumab demonstrated significant clinical benefits, its cost-effectiveness varied depending on factors such as disease severity and patient age (Martins et al., 2023).

8.2.7. Implementation Science in MS Care

As multiple sclerosis (MS) treatments rapidly advance, effectively integrating personalised medicine into clinical practice has become increasingly important. Implementation science offers valuable frameworks to facilitate this translation. For instance, the MultiSCRIPT project at the University of Basel has established a trial platform that continuously evaluates novel personalised treatments for MS against standard care; commentary on personalised MS management describes the AI-assisted monitoring tools on which such platforms depend (Health Europa, 2021).

This initiative identifies key barriers and facilitators to implementation, including healthcare provider training needs, health system infrastructure requirements, and patient acceptance factors. Based on these findings, researchers have developed thorough toolkits for implementing personalised MS care, which are currently being evaluated in large-scale, multi-centre trials.

Introducing a treatment into practice requires evidence of benefit, a reproducible product, a feasible care pathway, and appropriate monitoring. Costs, access, and the patient's priorities also influence whether an intervention improves care.

8.3. Regulatory and Ethical Considerations

As novel therapies and technologies emerge in MS care, regulatory bodies must adapt to ensure patient safety while facilitating innovation. This evolving landscape presents a range of ethical challenges that require careful consideration and ongoing dialogue among stakeholders.

8.3.1. Regulatory Challenges in Stem Cell Therapies

The development of clear guidelines for stem cell therapies in MS is central. The International Society for Stem Cell Research (ISSCR) has published updated guidelines for stem cell research and clinical translation (Lovell-Badge et al., 2021), which provide a framework for responsible development of these therapies. These guidelines emphasize the importance of rigorous preclinical studies, careful patient selection, and long-term follow-up in clinical trials of stem cell therapies.

In their review of cell-based therapeutic strategies for MS, Scolding et al. (2017) set out the evidence requirements and oversight needed before such treatments enter practice. Regulatory approaches still vary between jurisdictions, and harmonized international standards would help to protect patients while allowing research to advance.

8.3.2. Ethical Implications of Gene Editing

The rapid advancement of gene editing technologies, particularly CRISPR-Cas9, has ignited extensive ethical debates, especially concerning germline modifications that can be inherited by future generations. The 2018 "CRISPR babies" incident, where CRISPR was used to alter the genomes of embryos, pointed to the need for robust ethical frameworks in genetic research. This event heightened global awareness about the ethical, societal, and medical risks associated with heritable gene editing, emphasizing the necessity for international collaboration and clear ethical guidelines (Cyranoski, 2019).

The Human Genome Organization (HUGO), through its Committee on Ethics, Law, and Society, has been proactive in addressing these issues, advocating for ethical principles that balance scientific progress with social responsibility. HUGO emphasizes the importance of genomic solidarity, prioritizing the well-being of future generations and the public good in gene editing policies. In their detailed review, Mulvihill et al. (2017) discuss the risks of unregulated CRISPR use, advocating for a cautious approach that includes restrictions on germline editing until more is understood about its long-term impacts on human biology and society.

Further advancing the ethical discourse, the Third International Summit on Human Genome Editing convened in London in 2023. This summit brought together leading figures in genetics, bioethics, and policy to address the complex ethical challenges posed by gene editing in medical research. A significant outcome of the summit was the

establishment of the International Commission on the Clinical Use of Human Germline Genome Editing, a body committed to developing global ethical standards for gene editing applications. The commission's mission is to support the responsible integration of gene editing into clinical practice by promoting ethical guidelines that emphasize transparency, patient well-being, and social responsibility. The commission's objectives rest on the recognition that while gene editing holds investigational potential for treating various disorders, it also poses risks that require careful consideration of long-term societal impacts.

8.3.3. Equity and Access to Advanced Therapies

Ensuring equitable access to advanced MS therapies across different healthcare systems and socioeconomic groups remains a significant ethical challenge. Even within high-income health systems, the cost of disease-modifying therapy is a major barrier, and access to MS treatments differs markedly between high-income and low- and middle-income countries.

Multiple sclerosis (MS) disproportionately affects individuals in resource-limited settings, where access to care and treatment remains a significant challenge. To address these disparities, the Multiple Sclerosis International Federation (MSIF) launched a global initiative aimed at improving access to MS care in underserved regions (Multiple Sclerosis International Federation, 2022). This initiative reinforces the importance of equitable healthcare as a fundamental right for MS patients worldwide.

Global Strategies for Improved MS Care

The MSIF initiative integrates several strategic components to bridge the gaps in MS care.

Capacity Building

The initiative focuses on training healthcare providers in underserved regions, ensuring they have the skills and knowledge necessary to diagnose and manage MS effectively. Educational programs and mentorship networks form the backbone of this effort, aiming to create a sustainable impact (Multiple Sclerosis International Federation, 2022).

Technology Transfer

A cornerstone of the initiative is the transfer of technology to enhance diagnostic and therapeutic capabilities in resource-limited settings. This includes providing access to MRI machines, telemedicine platforms, and advanced treatment modalities (World Health Organization, 2022).

Advocacy for Policy Change

MSIF advocates for changes in health policies to prioritise MS care, including the inclusion of MS treatments in national formularies and insurance coverage. Efforts are also directed toward listing MS therapies on the World Health Organization's Essential Medicines List (EML), ensuring global recognition of these treatments as central healthcare necessities (World Health Organization, 2022).

MS Treatment Access Partnership (MSTAP)

A key aspect of this initiative is the MS Treatment Access Partnership (MSTAP), a collaborative effort that unites pharmaceutical companies, healthcare providers, patient organizations, and policymakers. The partnership aims to develop sustainable models for expanding access to MS therapies. These models focus on affordability, local manufacturing, and streamlined distribution channels to ensure the availability of treatments in underserved regions (Multiple Sclerosis International Federation, 2022).

Outcomes and Future Directions

Initial outcomes of this initiative demonstrate improved diagnostic rates and increased availability of MS treatments in targeted regions. However, achieving long-term sustainability requires continuous collaboration and innovation. Future directions include scaling these efforts globally and incorporating patient feedback to refine strategies further (World Health Organization, 2022).

The MSIF's global initiative represents a potentially important approach to addressing disparities in MS care. Through capacity building, technology transfer, advocacy, and collaborative partnerships like MSTAP, this initiative sets a benchmark for equitable healthcare delivery. Its success is an argument for unified global efforts to ensure no patient is left behind in the fight against MS.

A composite measure of treatment equity, combining the availability of disease-modifying therapies, access to specialized MS care centres, and out-of-pocket costs, would help to quantify and track disparities in access to MS care worldwide. Available data already indicate inequities both between and within countries, particularly affecting rural and marginalized populations, and addressing them will require targeted programmes to expand MS care infrastructure in underserved areas.

8.3.4. Ethical Considerations in AI and Big Data

The expanding role of artificial intelligence (AI) and big data analytics in Multiple Sclerosis (MS) research and clinical care brings forward investigational potential but also significant ethical challenges. As these technologies become integral to MS management, ethical issues such as data privacy, algorithmic bias, transparency, and the shifting dynamics of the patient-provider relationship must be carefully addressed to ensure trust and fairness in AI-assisted healthcare.

One primary ethical concern in AI applications is data privacy. AI models in MS research often rely on vast amounts of patient data, including genetic information, clinical histories, imaging data, and treatment responses. Given the sensitivity of this data, protecting patient confidentiality and ensuring secure data storage and transfer are of first importance. Effective anonymization techniques and strict data governance policies are necessary to safeguard patient information. Additionally, as data is increasingly shared across multiple platforms and research institutions, the risk of unauthorized access or data misuse grows, making clear the need for robust cybersecurity measures.

Algorithmic bias is another significant ethical issue. Bias in AI systems can arise when the data used to train these models lacks diversity or when the algorithms themselves are not designed to account for variations across patient populations. In MS, where genetic, demographic, and environmental factors influence disease progression and treatment responses, a biased AI model could lead to inaccurate or inequitable recommendations. Addressing algorithmic bias requires training AI models on diverse datasets that represent the broad spectrum of MS patients. Regular audits and testing of AI algorithms for biases can also help ensure that the systems provide fair and accurate predictions.

Transparency in AI-driven decision-making is necessary to maintain patient trust. AI systems often function as "black boxes," where the rationale behind specific recommendations is not easily interpretable by clinicians or patients. In MS care, where personalized treatment decisions are essential, it is important that AI systems are explainable; enabling clinicians to understand and communicate how decisions are made. Developing interpretable models and incorporating explainability tools into AI frameworks are necessary steps toward fostering a transparent AI environment in healthcare.

The evolving nature of the doctor-patient relationship also raises ethical questions. AI has the potential to streamline healthcare delivery, improve diagnostic accuracy, and personalize treatment plans, but it can also disrupt traditional care dynamics. As AI systems take on a more active role in decision-making, clinicians must balance their expertise with AI recommendations, ensuring that technology complements rather than replaces human judgment. Maintaining clear communication with patients about AI's role in their care, along with respecting their autonomy and preferences, is essential to uphold ethical standards in AI-assisted MS care.

As AI and big data become more integrated into MS research and clinical practice, these ethical considerations must be addressed to ensure that technological advancements are harnessed responsibly. By prioritizing data privacy, mitigating algorithmic bias, fostering transparency, and respecting the traditional roles in the patient-provider relationship, healthcare professionals can implement AI in ways that enhance patient trust and improve care quality while adhering to ethical principles.

8.3.5. Informed Consent and Patient Autonomy

As MS treatments become more complex and personalised, there is growing recognition of the need for enhanced informed consent processes that truly empower patients to make autonomous decisions. Patient involvement also matters for research: in a systematic review and meta-analysis, Crocker et al. (2018) found that patient and public involvement modestly increased enrolment in clinical trials. Interactive, patient-tailored consent tools that integrate multimedia elements and personalised information are being developed to help patients make informed and autonomous decisions.

The regulatory and ethical framework around MS treatment is complex and still changing. As new therapies and technologies emerge, it is important to maintain a balance between facilitating innovation and ensuring patient safety and ethical integrity. Ongoing dialogue among stakeholders, including researchers, clinicians, patients, ethicists, and policymakers, will be essential in navigating these challenges and ensuring that advances in MS treatment are developed and implemented in a manner that is both scientifically sound and ethically responsible.

8.4. The Role of Technology and AI in Advancing MS Treatment

A 2026 survey of 74 peer-reviewed AI and machine-learning studies in MS monitoring found increasing use of smartphone, wearable, multimodal and registry data, but external validation was reported in only a small minority of studies. The practical implication is that algorithmic performance in development datasets should not be equated with clinical utility until independent validation, calibration, interpretability and workflow integration are demonstrated (Khaled et al., 2026).

Artificial intelligence and machine learning are being studied in MS research and care. Within this book's scope, applications relevant to regenerative therapies include EV characterisation, biomarker identification, patient selection, product quality control, and prediction of treatment response. Clinical benefit from these applications requires prospective validation.

8.4.1. AI in Diagnosis and Prognosis

Yoo et al. (2016) used deep learning of brain lesion patterns to predict future disease activity in patients with early MS. Model performance must be interpreted within the reported dataset and validation design. External validation and assessment of clinical utility are separate requirements.

The study analysed baseline MRI lesion masks from 140 participants using convolutional neural networks and seven-fold cross-validation. For disease activity over two years, the model achieved a mean accuracy of 72.9% (SD 10.3%), compared with 65.0% (SD 14.6%) for lesion load. These internally validated results support further investigation, but do not establish performance in an independent population or clinical utility (Yoo et al., 2016).

Additionally, the integration of serum neurofilament light chain (NfL) data has come to be seen as a promising biomarker for monitoring disease activity in MS. Studies have shown that elevated levels of serum NfL correlate with MRI lesion load and EDSS scores, providing valuable insights into disease progression. The incorporation of sNfL data into AI algorithms has enhanced the ability to assess both disease activity and treatment response, offering a non-invasive tool for monitoring treatment efficacy and guiding personalised treatment decisions (Disanto et al., 2017; Khalil et al., 2018).

8.4.2. AI in Treatment Optimization

Artificial intelligence (AI) has become an important tool in addressing the complexity of treatment optimisation for Multiple Sclerosis (MS), a condition characterised by heterogeneous disease progression and complex clinical manifestations. By leveraging machine learning (ML) models and advanced data analytics, AI offers opportunities to enhance the personalisation, efficacy, and efficiency of MS therapies (Praet et al., 2024).

Individualizing Treatment Strategies

AI models can be investigated for integrating imaging and clinical records in MS prognosis or treatment research. Predictive performance in a development dataset does not establish that an algorithm can select the most effective therapy for an individual patient or improve care. Prospective external validation and impact studies are needed before such systems guide treatment decisions (Aslam et al., 2022; Moazami et al., 2021).

Enhancing Drug Efficacy and Monitoring

AI also plays a critical role in monitoring treatment efficacy. Integrating AI models with real-world data, such as patient-reported outcomes and sensor-based activity monitoring, enables continuous assessment of therapeutic interventions. For example, AI-driven platforms can detect subtle changes in mobility or cognition that may indicate treatment inefficacy, prompting timely intervention (Alouthah et al., 2024).

Additionally, AI models have shown promise in optimising drug selection. Algorithms incorporating genetic, metabolic, and imaging data have been developed to predict individual responses to specific therapies, reducing trial-and-error prescriptions and enhancing patient outcomes (Ontaneda & Prados Carrasco, 2024).

Addressing Challenges in Treatment Optimization

Despite its investigational potential, AI-based treatment optimisation is not without challenges. Limited access to large, high-quality datasets can hinder algorithm training and reliability. Furthermore, the lack of model interpretability and clinician familiarity with AI tools can impede their integration into routine practice (Praet et al., 2024). Collaborative efforts between clinicians, data scientists, and regulatory bodies are essential to overcome these barriers and fully realize the potential of AI in optimising MS treatments.

Looking forward, the integration of federated learning approaches can enhance the scalability and generalizability of AI models by securely training algorithms across multiple datasets while maintaining patient confidentiality.

Furthermore, combining AI with emerging technologies such as digital twins, virtual representations of individual patients, could improve treatment planning by simulating the effects of various therapeutic options (Alouthah et al., 2024).

8.4.3. AI in Drug Discovery

The landscape of drug discovery has been fundamentally transformed by the emergence of artificial intelligence, particularly in addressing one of the most significant challenges in neuropharmacology: predicting blood-brain barrier (BBB) permeability. Neurological conditions, including Alzheimer's disease, Parkinson's disease, stroke, epilepsy, headaches, and brain tumours, present substantial challenges to human health and well-being, largely due to the complexity of delivering therapeutic agents across the blood-brain barrier (Vatansever et al., 2021).

The blood-brain barrier serves as a highly selective boundary that restricts the passage of most substances into the brain, creating a significant obstacle in developing treatments for neurological disorders. For therapeutic agents to be considered viable candidates for CNS diseases, they must not only demonstrate therapeutic efficacy but also possess the ability to cross the BBB to reach their intended targets (Meng et al., 2021). Traditional drug development approaches have faced considerable challenges in predicting BBB permeability, leading to high failure rates and increased development costs.

Recent advances in artificial intelligence and machine learning have substantially changed this field. The implementation of Large Language Models (LLMs) based on transformer architectures, specifically the MegaMolBART encoder with XGBoost classifier, has shown notable performance in predicting BBB permeability across extensive compound libraries (Huang et al., 2024). These AI systems analyse molecular structures and properties with performance requiring external validation, significantly reducing the time and resources required for initial drug screening.

Experimental validation of AI predictions has become increasingly sophisticated, incorporating multiple approaches to verify computational findings. Research has shown that compounds capable of crossing the BBB typically exhibit specific physicochemical properties, including appropriate lipophilicity, low molecular mass (generally below 400-600 Da), limited plasma protein binding, and stability at physiological pH (Vatansever et al., 2021). The integration of in vitro testing using bEnd.3 brain endothelial cells, FITC-dextran extravasation studies, and advanced analytical techniques such as LC-MS/MS has provided robust validation protocols for AI predictions.

Machine-learning models can estimate selected ADME properties from molecular descriptors. Reliability depends on the training data, applicability domain and external validation. Computational estimates require appropriate experimental confirmation before they inform drug development.

Recent studies have demonstrated the impressive scope of these AI applications. The B3DB dataset, comprising 4,956 BBB-permeable and 2,851 BBB-impermeable compounds, has provided a robust foundation for training AI models. Subsequent validation using the NPRL-CMUH dataset, containing 2,461 BBB-permeable and 2,184 BBB-impermeable compounds, has confirmed the reliability of these predictive models (Meng et al., 2021).

Despite these advances, several problems remain in the field of AI-driven drug discovery. The validation of mechanisms of action, integration of multiple data types, and translation to clinical applications continue to present significant hurdles. Researchers are actively working to enhance model interpretability and improve prediction accuracy for complex molecules. The future of this field lies in the development of more sophisticated neural networks and the ability to adapt to new data in real-time (Wang, 2024).

Computational methods can help prioritise candidates for experimental testing in neurological research. Whether these workflows reduce development time or lead to better treatments has not been established across MS drug-discovery programs. Their contribution to stem cell or EV product development requires experimental confirmation and, ultimately, clinical validation.

This advancement in AI-driven drug discovery represents a significant step forward in pharmaceutical research, offering new hope for patients suffering from neurological conditions. The continued refinement of these technologies, coupled with improved understanding of the blood-brain barrier and ADME properties, promises to accelerate the development of more effective therapeutic agents for CNS disorders.

8.4.4. Natural Language Processing in Clinical Documentation

Natural Language Processing (NLP) has introduced a potentially important approach to managing chronic diseases like Multiple Sclerosis (MS), where detailed, nuanced

information is key for monitoring disease progression, personalizing treatment, and improving patient outcomes. By automating the extraction and analysis of unstructured data within Electronic Health Records (EHRs), NLP empowers clinicians to glean critical insights about a patient's disease course, symptom history, and response to treatment.

Chase et al. (2017) investigated early MS detection using electronic health records. Retrospective discrimination requires external validation and prospective evaluation before earlier detection can be assumed to improve patient outcomes.

Automated extraction of disability information from clinical notes requires validation against appropriate clinical assessments. Missing observations, documentation practices and differences between institutions can affect performance.

Lefkovitz et al. (2024) conducted a scoping review of clinical NLP applications in neurological disorders. The review describes a heterogeneous evidence base whose methods, validation settings and outcomes require separate appraisal.

Social and behavioural information may be relevant to clinical NLP research, but benefits for MS outcomes or health equity require direct evaluation in the intended setting.

Electronic health-record algorithms can support research case ascertainment. Identifying MS cases or phenotypic information should be distinguished from demonstrating that algorithm-guided care improves long-term outcomes.

NLP can support structured extraction from clinical records. Its clinical value depends on accuracy, transportability, clinician oversight and demonstrated benefit in prospective implementation studies.

8.4.5. AI in Personalized Rehabilitation

AI-assisted rehabilitation is being studied as a potential means of adapting exercises and monitoring functions in MS. A model's predictive performance or usability does not by itself demonstrate improved clinical outcomes. Comparative, prospective validation is needed before these tools are treated as effective rehabilitation interventions.

Challenges in MS Rehabilitation

MS is characterised by diverse clinical phenotypes and subclinical disease progression, leading to varying disability levels among patients (Praet et al., 2024). Traditional rehabilitation approaches often rely on standardised treatment protocols, which may not address the unique needs of individuals. This has led to inconsistent outcomes in reducing disability progression and improving the quality of life.

AI-Driven Approaches

Potential research uses of AI in MS include:

Predictive Analytics: Using AI to analyse patient data, such as MRI biomarkers, motor function, and cognitive assessments, to predict disease trajectories. This allows clinicians to anticipate potential complications and adapt treatment plans accordingly (Praet et al., 2024). **Precision Medicine**: AI facilitates the identification of specific rehabilitation needs by analysing real-time data from wearable devices and patient-reported outcomes (PROs). This enables a personalised approach to therapy, addressing both physical and cognitive challenges associated with MS (Van Wijmeersch et al., 2022). **Optimising Treatment Selection**: AI models help in selecting the most effective disease-modifying therapies (DMTs) for patients by incorporating biomarkers and historical data, thus reducing therapeutic inertia and improving long-term outcomes (Gasperini et al., 2019).

Current Innovations

Projects like the CLAIMS initiative exemplify AI's application in MS rehabilitation. The CLAIMS platform integrates clinical and subclinical data to create full prognostic models, helping clinicians make informed decisions (Praet et al., 2024). These models leverage advanced machine learning algorithms to analyse heterogeneous patient data, addressing the complexity of MS.

Future Directions

While the integration of AI in MS care warrants further investigation, several obstacles persist.

Data Standardisation: There is a need for harmonized datasets to improve AI model accuracy and reliability. **Patient Engagement**: Ensuring patients' trust and adherence to AI-based tools is critical for successful implementation. **Regulatory Approval**: AI-driven platforms require rigorous validation to meet clinical standards and gain regulatory acceptance (Van Wijmeersch et al., 2022).

AI's role in personalised MS rehabilitation is still evolving. However, its potential to improve patient outcomes by addressing the heterogeneous nature of MS makes it a central component of future healthcare strategies.

8.4.6. AI in Predicting and Managing Comorbidities

Patients with Multiple Sclerosis (MS) frequently experience various comorbidities that can complicate treatment, lower quality of life, and affect overall health outcomes. Comorbidities like depression, cardiovascular disease, and osteoporosis are common among MS patients, and managing these alongside the primary disease requires a comprehensive approach. Artificial intelligence (AI) offers promising solutions for both predicting and managing these comorbid conditions by analysing extensive data to identify at-risk patients early and provide individualized management plans.

AI systems designed for predicting comorbidities in MS analyse a range of factors, including genetic information, clinical history, lifestyle habits, and environmental influences. This holistic data approach allows AI to identify patterns and risk factors that may signal the likelihood of developing a comorbidity. For example, indicators from routine health data and behavioural patterns can provide early warning signs for depression, enabling preemptive mental health support. Similarly, factors such as blood pressure, cholesterol levels, and lifestyle choices can be assessed to predict cardiovascular risks, while bone density markers and family history might indicate susceptibility to osteoporosis.

Implementing AI to manage comorbidities in MS patients not only helps predict the onset of conditions but also assists in creating tailored preventive and management strategies. By integrating patient-specific information, including current MS treatments and potential drug interactions, AI systems can support clinicians in crafting personalised recommendations. This individualized approach is especially beneficial in complex cases where treatments for MS and comorbid conditions may interact. AI-driven recommendations can thus optimise treatment strategies, reducing the likelihood of adverse interactions and supporting a balanced approach to patient care.

In clinical practice, the adoption of AI in comorbidity management has the potential to improve patient outcomes significantly. Early prediction of comorbidities allows healthcare providers to take preventive actions, which can reduce the frequency and severity of these conditions. This proactive approach not only enhances patient quality of life but can also reduce hospitalizations and other healthcare costs related to comorbidity complications. By focusing on both prediction and personalised management, AI has the potential to transform how comorbidities are handled in MS care, fostering a more thorough, preventative approach to treatment.

8.4.7. Challenges and Future Directions

While the potential for AI in advancing MS treatment is enormous, several challenges need to be addressed.

The performance of AI algorithms heavily depends on the quality and consistency of the data they are trained on. Efforts are needed to standardize data collection and reporting across different healthcare systems and research institutions. As AI systems become more complex, ensuring that their decision-making processes are transparent and interpretable to healthcare providers and patients becomes essential. Issues of data privacy, consent, and potential biases in AI algorithms need to be carefully addressed to ensure equitable and ethical implementation of AI in MS care. For AI tools to be adopted, they need to be seamlessly integrated into existing clinical workflows without adding to the cognitive burden of healthcare providers. Rigorous validation studies and clear regulatory pathways for AI-based medical tools are needed to ensure their safety and efficacy.

Future directions in AI for MS treatment include:

Multi-modal AI platforms that integrate imaging, molecular biomarkers, clinical assessments and patient-reported outcomes could give a broader picture of MS pathophysiology and treatment response, and federated learning would allow such models to be trained on decentralized data, addressing privacy concerns while drawing on larger and more diverse datasets. AI could also support clinical trials themselves, in patient selection, monitoring and data analysis , and underpin digital therapeutics that adapt to the individual for symptom management and rehabilitation. Applying these methods to genomics and other -omics data is a further step towards individualized treatment.

AI and digital technologies are expanding the research toolkit available for MS diagnosis, monitoring, trial design, and decision-support development. Their clinical value depends on external validation, prospective impact studies, transparent performance across populations, clinician oversight, and appropriate regulatory governance (Khaled et al., 2026).

8.5. Concluding Remarks

The evidence reviewed in this book leaves two tasks: controlling inflammatory activity and limiting or repairing accumulated tissue damage. Established treatment addresses the first more reliably than the second. Cellular and EV

strategies need to demonstrate durable effects on disability, function, or quality of life using defined products and suitable comparators.

A broad 2026 review of MS research reached a similar practical conclusion: major advances are occurring in biomarkers, imaging, disease-progression biology, and trials directed at compartmentalised inflammation, but prevention or reversal of established progression remains an unresolved therapeutic problem (Klotz et al., 2026). The recent literature therefore strengthens the rationale for regenerative research while also sharpening the distinction between biological activity, surrogate-marker change, and demonstrated patient benefit.

Inflammatory control and tissue repair are distinct therapeutic goals. B-cell depletion reduces relapses and new lesions (Hauser et al., 2017; Steinman et al., 2022), and ocrelizumab can slow disability progression in selected patients with primary progressive MS (Montalban et al., 2017). These effects do not establish regeneration of previously damaged tissue. Repair-related claims for cellular and EV products therefore require endpoints that can distinguish them from suppression of new inflammatory injury.

Remyelination failure varies between lesions. OPC recruitment and differentiation, inflammatory conditions, and metabolic constraints can all limit repair (Chang et al., 2000; Dawson et al., 2003; Heß et al., 2020; Hughes et al., 2013). Progressive-MS CSF can reduce progenitor differentiation in culture, implicating the lesion environment (Zveik et al., 2022, 2024). Cholesterol availability is another constraint in experimental remyelination (Berghoff et al., 2021). A treatment directed at one mechanism may therefore have different effects across disease stages and lesion types.

For MSC approaches, experimental findings often support transient immunomodulatory and trophic effects rather than direct replacement of lost oligodendrocytes (Pluchino et al., 2020; Scolding et al., 2017). Neural and oligodendrocyte-lineage products pursue different repair mechanisms. Trials should measure the relevant host response and tissue effect, as well as the fate of the administered cells.

Early neural-cell trials in progressive MS established feasibility and supplied initial safety observations, without controlled evidence of efficacy (Genchi et al., 2023; Leone et al., 2023). MSC studies have produced heterogeneous results and have not established a reproducible disability benefit (Nahayati et al., 2025; Sheikhi et al., 2025). SMART-MS did not meet its primary endpoint and identified route-specific safety concerns (Kvistad et al., 2026). A published EV treatment case now adds a human observation, but concomitant laser therapy and the absence of a control prevent attribution of benefit (Mohammad Jafari et al., 2025). Controlled evidence of therapeutic EV efficacy in MS remains lacking.

Adjacent remyelination trials show why intermediate biological effects need clinical confirmation. Opicinumab followed mechanistic and preclinical work but did not meet its primary phase II endpoint (Cadavid et al., 2019; Mei et al., 2016; Mi et al., 2005). Clemastine improved an optic-nerve conduction measure in one setting (Green et al., 2017), whereas a subsequent progressive-MS study reported accelerated disability accumulation and investigated pyroptosis as a possible mechanism (Kocot et al., 2025). Cell and vesicle trials need the same attention to disease stage, endpoint selection, and harm.

The next studies need better product definition, validated potency assays, consistent release criteria, and designs suited to slowly evolving disability. Biomarkers such as serum NfL can inform disease-activity assessment but do not replace a clinical endpoint (Disanto et al., 2017; Khalil et al., 2018). Lesion-level stratification and comparable manufacturing methods would make both positive and negative results more interpretable.

Engineered immune-cell approaches pursue depletion or antigen-specific tolerance rather than tissue replacement. Early CAR-T observations in neuroinflammation (Samadzadeh et al., 2026) and experimental MOG-specific CAR-Treg work (Frikeche et al., 2024) illustrate these different aims. Their manufacturing experience may inform other cell programmes, but efficacy and safety remain specific to the product and indication.

Experimental work has identified potentially modifiable barriers to remyelination, including the lesion environment, stage-dependent cellular responses, and metabolic requirements. These findings justify testing defined interventions. They do not predict which candidate will produce meaningful repair in patients.

Clinical claims should follow evidence appropriate to the product and indication. ISSCR guidance opposes the use of unproven cell interventions outside properly regulated research or an established evidence-based pathway (Lovell-Badge et al., 2021). This distinction is especially relevant in progressive MS, where unmet need can encourage

premature use of interventions whose benefit and long-term risks are uncertain.

Patient involvement can improve trial relevance and feasibility (Crocker et al., 2018; Gray et al., 2023). For cell and EV therapies, outcomes should capture changes in daily function and participation alongside biological measures. Reproducible benefit on those outcomes is the evidence needed to change practice.

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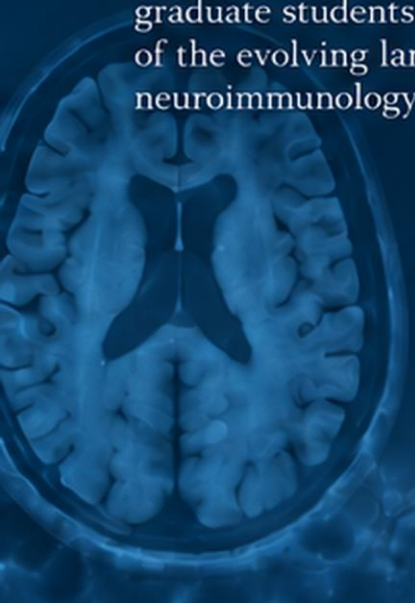
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