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TOWARD FUNCTIONAL RECOVERY IN MULTIPLE SCLEROSIS: ADVANCES IN MYELIN REGENERATION AND NEUROREPAIR RESEARCH

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ABSTRACT

The primary cause of nontraumatic neurological disability in young adults is multiple sclerosis (MS), an autoimmune demyelinating and neurodegenerative disease of the central nervous system. A common condition affecting the central nervous system, multiple sclerosis affects about 1 million young adults worldwide, primarily women. One Episodic neurologic symptomis its hallmark, and over the course of 30 to 40 years, these are frequently followed by fixed neurologic deficits, growing disability, and physical, medical, and socioeconomic decline. Physiological repair mechanisms can aid in the nervous system's recovery from tissue damage in multiple sclerosis. Improving these repair processes is a crucial and becoming more feasible treatment objective for multiple sclerosis. In this review, we provide a comprehensive overview of the recent advances in the understanding and management of multiple sclerosis, with an emphasis on remyelination and neurorepair, signaling pathways, biomarkers of remyelination, and stem cell and regenerative therapies.

KEYWORDS: Multiple sclerosis, remyelination, neurorepair.

1. INTRODUCTION

A long-term autoimmune, inflammatory neurological condition affecting the central nervous system (CNS) is called multiple sclerosis (MS).Myelinated axons in the central nervous

system are attacked by MS, which causes varying degrees of myelin and axon destruction.The majority of patients first experience episodes of reversible neurological deficits, which are frequently followed over time by progressive neurological deterioration [1].The most common cause of non-traumatic neurological disability in young adults is an autoimmune disease.Inflammation with demyelination and astrocyte proliferation (gliosis) and neurodegeneration are the two pathological hallmarks of multiple sclerosis.Peripheral nervous system tissue damage in MS is limited to the CNS. Clinically, there are two possible outcomes for MS: progressive or relapsing.Relapsing multiple sclerosis (RMS) is the most common type of onset, characterized by discrete episodes of neurological dysfunction followed by partial, complete, or no remission [2].

2. EPIDEMIOLOGY

MS is becoming more extremely common worldwide, with distinct regional variations in its incidence.There were 2.3 million MS sufferers in 2013, 2.8 million in 2020, and 2.9 million in 2023.The prevalence of multiple sclerosis varies greatly by region; North America and Europe have the highest rates, while Asia and Africa have lower rates.Global prevalence was 35.9/100,000 people in 2020, up from 30/100,000 in 2013, according to the MS Atlas.The prevalence of MS was 133/100,000 in Europe, 112/100,000 in the Americas, 30/100,000 in the Eastern Mediterranean, 9/100,000 in Southeast Asia, 5/100,000 in Africa, and 5/100,000 in the Western Pacific in 2020[3]. MSIF obtained epidemiologic data from 115 nations, or 87% of the world's population, between September 2019 and March 2020.The incidence and prevalence of MS in adults and children, separately for males and females, the diagnostic criteria used in their nation, and important clinical features like age and MS type at diagnosis were all reported by country coordinators in accordance with earlier Atlas editions.Additionally, coordinators reported the sources of their data, which may have included publications and presentations, registries, administrative data sets, government/health system statistics, electronic medical records, and expert opinions (usually from neurologists, researchers, or MS societies) [4].

3. ETIOLOGY

3.1. Genetic factors

HLA-DRB1*15 and/or other loci in strong linkage disequilibrium with this allele constitute the primary genetic risk for multiple sclerosis.Although the mechanism is still unknown, heterozygotes for HLA-DRB1*15:01 have an odds ratio of MS >3 and homozygotes >

6.26.The protective effects of class 1 alleles, such as HLA-A*02:01, cannot be explained by the theory that HLA-DRB1*15:01 plays a role through antigen presentation [5].

3.2. Immunological factors

The most common theory for the cause of multiple sclerosis is dysimmunity with an autoimmune attack on the central nervous system.Among other suggested mechanisms, CD4+ proinflammatory T cells are involved in the hypothesized "outside-in" mechanism. Researchers speculate that Th1 and Th17 cells are activated and triggered by an unidentified antigen, which causes them to adhere to the endothelium of the central nervous system,pass through the blood-brain barrier, and then trigger an immune attack through cross-reactivity.On the other hand, the "inside-out" theory proposes that tissue damage caused by inflammation is caused by an intrinsic CNS anomaly. The most common theory for the cause of multiple sclerosis is dysimmunity with an autoimmune attack on the central nervous system.Among other hypothesized mechanisms, the "outside-in" mechanism involves CD4+ proinflammatory T cells. Researchers speculate that an unidentified antigen activates and triggers Th1 and Th17 cells, which then attach to the endothelium of the central nervous system, cross the blood-brain barrier, and trigger an immune attack through cross-reactivity. On the other hand, the "inside-out" theory proposes that tissue damage caused by inflammation is caused by an intrinsic CNS anomaly [6].

3.2. Environmental factors

Many studies have been conducted on environmental factors, such as latitudinal gradients found in different countries. The observed multiple sclerosis predispositions of populations in higher latitudes have been linked to vitamin D deficiency. The disease may also be exacerbated by certain infections, such as the Epstein-Barr virus. It is clear that different environmental factors and patient genetics interact in complex ways, and current research attempts to gain a more thorough understanding of these pathways [7].

4. CLINICAL FEATURE

Table 1 lists the more common symptoms of MS that may appear during different courses of the disease [7].

Primary symptoms	More common symptoms	Sensory disturbances (numbness, tingling, itching, burning), Walking difficulties (due to fatigue, weakness, spasticity, loss of balance and tremor, Vision problems

		(diplopia, blurred, and pain on eye movement), Intestinal and urinary system dysfunction (constipation and bladder dysfunction), Cognitive and emotional impairment (inability to learn and depression), Dizziness and vertigo, Sexual problems
	Less common symptoms	Swallowing problems (dysphagia), Speech problems (dysarthria), Breathing problems, Hearing loss, Seizures, Headache
Secondary symptoms	Urinary tract infections, Inactivity, Immobility	
Tertiary symptoms	Social complications, Vocational complications, Psychological complications, Depression	

5. OVERVIEW OF DEMYELINATION AND REMYELINATION

5.1 Mechanism of demyelination in MS

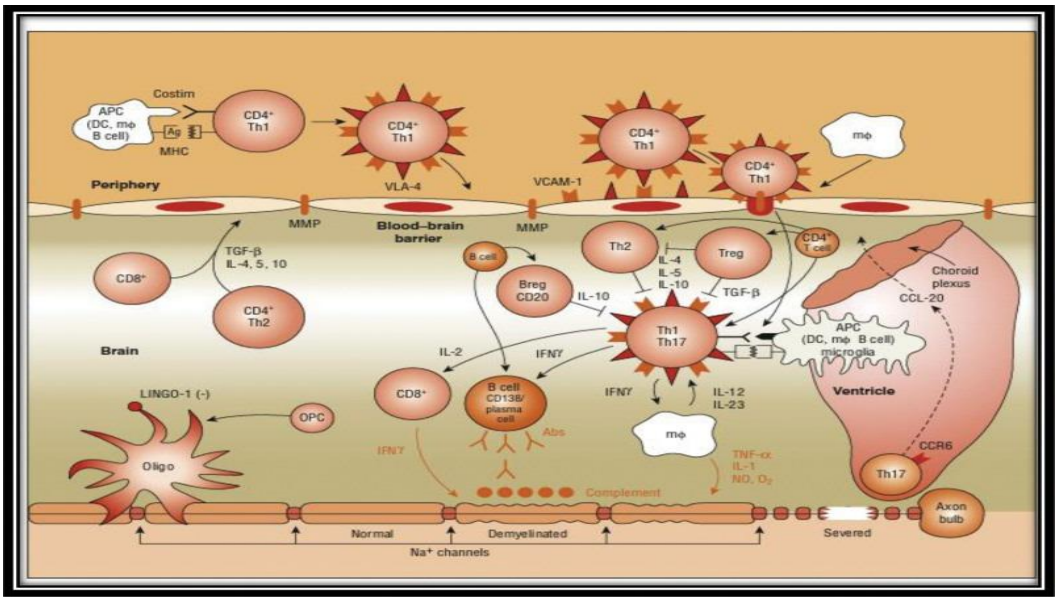
In order to facilitate the effective transmission of a nerve impulse along an axon in the central nervous system, oligodendrocytes are in charge of myelination and maintaining saltatory conduction, which covers the majority of the axons in the central nervous system.The destruction of myelin (demyelination) and nerve fibers (axonal degeneration) in the central nervous system is the ultimate cause of multiple sclerosis (MS), despite the fact that MS is a complicated illness of the central nervous system. Since a variety of immune cell types are implicated in the aberrant immune response, immunological variables have been the primary focus of MS pathology research.Thus, autoimmune reactions against myelin antigens and inflammation that contribute to the pathophysiology of demyelination in the central nervous system are the hallmarks of MS, an immune-mediated illness.As of right now, it is generally acknowledged that immune cells target myelinated axons in the central nervous system, which leads to demyelination and axonal degeneration [8].

T-Cell Development- After passing through the blood-brain barrier and entering the central nervous system, CD4+ T cells (Th1/Th17) identify myelin antigens and release pro-inflammatory cytokines such as IL-17 and IFN-γ.

The Apoptosis of Oligodendrocytes-Independent of T-cells, early oligodendrocyte apoptosis attracts microglia and initiates innate immune activation, which intensifies inflammation [9]. Phagocytosis and Inflammation-M1 astrocytes and activated microglia/macrophages release nitric oxide and cytokines, phagocytizing myelin debris through phosphatidylserine exposure and preventing repair.

Degradation of Myelin-Myelin proteins (such as MBP and PLP) is broken down by complement activation and proteases, revealing immunodominant epitopes and creating membrane assault complexes that lyse cells [10].

Axonal Repercussions-Demyelinated axons experience delayed conduction, neurofilament dephosphorylation, and caliber alterations, which result in neurodegeneration from metabolic support [11].



[Fig. 1] Autoimmune theory of the pathogenesis of MS. MS = multiple sclerosis [12].

5.2 Role of oligodendrocyte precursor cells in remyelination:

By their movement to demyelinated lesions, proliferation, differentiation into mature oligodendrocytes, and restoration of myelin sheaths surrounding axons, oligodendrocyte precursor cells (OPCs) play a crucial role in remyelination for multiple sclerosis (MS)

[13].Although oligodendrocyte precursor cells (OPCs) can rebuild myelin, they can potentially exacerbate inflammatory damage. Here, Wang et al. created experimental autoimmune encephalomyelitis (EAE) in a transgenic mouse to replicate the pathogenic characteristic of multiple sclerosis patients—overactivated Wnt signaling in OPCs.These mice displayed worsened EAE pathology in their brains and spinal cords, which was linked to increased T cell recruitment and a subset of cytotoxic macrophages that aided indemyelination.The inflammation surrounding the nerves in MS impairs remyelination, which is insufficient to make up for myelin loss and a failed differentiation of OPCs.Patients with multiple sclerosis, an inflammatory, demyelinating illness, frequently experience neurological impairment as a result of CNS lesions that do not heal [14].The capacity of oligodendrocytes to remyelinate is influenced by a number of molecular variables, including microRNAs (miRNAs), which have been demonstrated to have a significant regulatory function.These short non-coding RNA molecules work by attaching themselves to target mRNAs and either preventing translation or encouraging their breakdown.During the biogenesis process, a larger precursor of miRNA is transcribed from DNA and converted into a short, double-stranded miRNA molecule by an enzyme complex known as Dicer-dependent RNA.The RISC-miRNA complex, which binds to complementary RNA sequences in the cell, is created when miRNA molecules interact with proteins in the RNA-induced silence complex (RISC).This process causes mRNA translation to be inhibited or destabilized, which lowers the amount of protein that mRNA encodes (Gebert and MacRae,2020). The current study has shown that miRNAs play a role in a number of biological processes, including CNS remyelination (Duffy and McCoy, 2019).They function by controlling the expression of genes linked to myelin synthesis, oligodendrocyte proliferation, the elimination of dead or injured cells, and the synthesis of cytokines and chemokines. As demonstrated in vitro and in vivo, OPCs missing mature miRNAs are unable to differentiate correctly, and suppression of miRNAs important in the processing of mature OPCs appears to disrupt normal CNS myelination (Dugas et al., 2010) [15].The OPC differentiation block in chronic MS is probably the result of defective OPC recruitment during earlier stages of the disease, based on neuropathological findings and results obtained using models of demyelination.This is because (i) if the recruitment is too slow, OPCs reach the axons after what we define as a "remyelination-permissive window" and remain undifferentiated; and (ii) if the recruitment is inefficient, OPC density in the lesions stays below the threshold necessary for differentiation [13]. When myelin is damaged, platelets temporarily gather in the vascular niche and move close to OPCs.Long-term exposure to high levels of circulating platelets inhibits the

production of oligodendrocytes during remyelination, but brief interaction with platelets promotes OPC differentiation.These results support platelets' advantageous physiological function in remyelination.Remyelination failure in MS is exacerbated by persistently elevated platelet levels, which also severely impact OPC function [16].

5.3 Differences between acute and chronic lesions

Large confluent demyelinated lesions in the CNS's white and grey matter are the hallmark of multiple sclerosis.Axons are largely retained despite the total loss of myelin, and the degree of axonal damage varies between patients and even within lesions within the same patient.Astrocyte activation during active tissue injury and the development of gliotic scarring in passive lesions are linked to the demyelinating process. Recruitment and development of oligodendrocyte progenitor cells can contribute to the remyelination of MS lesions [17].

Acute lesions:Microglia and macrophages in active MS lesions. Massive microglia activation and the expression of the phagocytosis-associated marker CD68 at the lesion margin and in the surrounding peri-plaque white matter are characteristics of classical active white matter lesions, which are heavily invaded by macrophages. Similarly, meningeal inflammation and the presence of activated Iba1-positive microglia at the site of active myelin damage are linked to slowly growing lesions in the cortex.The demyelinated areas are almost entirely devoid of microglia, just like in chronic white matter lesions in the cortex [18].

Chronic lesions: It is hypothesized that the presence of CALs hinders tissue repair processes, leading to significant intralesional and perilesional tissue damage.8 CAL-associated tissue damage is believed to be mediated by macrophages and microglia through the production of inflammatory mediators, and it also leads to persistent neuroinflammation.A demyelinated core with axonal loss and an inflammatory demyelinating lesion edge are the hallmarks of CALs. Microglia and macrophages are a part of the detrimental inflammatory signature at the CAL edge, along with damaged oligodendrocytes, immune-like oligodendrocyte precursor cells, activated/toxic astrocytes, and smoldering inflammation and centrifugal tissue damage.There are very few lymphocytes at the CAL edge; they are generally found in the perivascular gaps and are mostly composed of T cells, including tissue-resident memory T cells, and plasma blasts [19].

Why chronic plaques fail to remyelinate: Resident OPCs, which make up 5–8% of the adult brain's total cell population, are widely dispersed throughout the grey and white matter and serve as a source for myelin repair after CNS injury [22].Distinct reactions to demyelination and possibly even a distinct vulnerability to age-related functional loss could be explained by this. Both a modified milieu that suppresses oligodendrocyte progenitor cells and a catalyst that intensifies inflammatory reactions within lesions are provided by the extracellular matrix molecules deposited into lesions [21]. Depending on the stage of the lesion, remyelination failure in MS might have several causes. Our results show that whereas loss of oligodendrocytes and a hostile tissue environment impede successful remyelination in mixed lesions, decreased myelin sheath formation in active/demyelinating lesions contributes to remyelination failure despite the presence of mature oligodendrocytes. Therefore, novel animal models that more accurately reflect the various stages of MS lesions are needed for the development of medications that promote remyelination.Depending on the stage of the lesion, remyelination failure in MS might have several causes. Our results show that whereas loss of oligodendrocytes and a hostile tissue environment impede successful remyelination in mixed lesions, decreased myelin sheath formation in active/demyelinating lesions contributes to remyelination failure despite the presence of mature oligodendrocytes. Therefore, novel animal models that more accurately reflect the various stages of MS lesions are needed for the development of medications that promote remyelination [22].

6. CELLULAR AND MOLECULAR DRIVERS:

6.1. Glial interactions:

Astrocytes:Astrocytes, star-shaped glial cells that are widely distributed throughout the central nervous system, have a complicated dual role in multiple sclerosis (MS), an autoimmune demyelinating disease marked by axonal injury, inflammation, and myelin loss. By expressing toll-like receptors (TLRs) and MHC class I/II molecules, they act as early responders in the pathogenesis of multiple sclerosis (MS).This allows them to function as antigen-presenting cells (APCs) that activate T cells, secrete pro-inflammatory cytokines like TNF-α, IL-12, IL-23, and IL-27 to drive Th1/Th17 responses, and disrupt the blood-brain barrier (BBB) to allow peripheral immune cell infiltration.Astrocytes experience reactive astrogliosis at the same time, creating glial scars that prevent remyelination and axonal regeneration while releasing chemokines to prolong inflammation.However, they also provide neuroprotection by encouraging the migration and proliferation of oligodendrocyte precursor cells and releasing anti-inflammatory cytokines (e.g., via TLR3), glutamate

transporter upregulation to reduce excitotoxicity, and NRF2 modulation for cholesterol control and growth factor secretion (GDNF). Astrocytes are positioned as important therapeutic targets because of this functional polarization—pro-inflammatory (A1-like) versus protective (A2-like), with hormones like estrogen improving their anti-inflammatory activities by decreasing MHC II expression and increasing glutamate absorption [23].

Microglia: In multiple sclerosis (MS), a persistent demyelinating illness caused by autoimmune inflammation, microglia—the main immune effectors in the central nervous system—display a complex dual functioning.In response to damage signals, they quickly activate in active lesions, taking on a pro-inflammatory M1-like phenotype in which they phagocytose myelin sheaths, express MHC class I/II molecules to present antigens to infiltrating T cells, and secrete cytokines like TNF-α, IL-1β, IL-6, IL-12, and IL-23.This amplifies Th1 and Th17 responses that prolong demyelination, oligodendrocyte death, and blood-brain barrier leakage.By generating reactive oxygen species (ROS), nitric oxide (NO), and matrix metalloproteinases (MMPs), they also contribute to neurodegeneration by creating toxic microglia nodules that are associated with early lesion expansion and axonal transection, especially in progressive MS phases. On the other hand, under specific circumstances, such as resolution phases or therapeutic modulation, microglia polarize toward an M2-like reparative state, generating neurotrophic factors (BDNF, IGF-1, GDNF), anti-inflammatory mediators (IL-10,TGF-β), and phagocytosing debris, apoptotic cells, and inhibitory myelin remnants to aid in the recruitment, differentiation, and remyelination of oligodendrocyte precursor cells (OPCs).Environmental cues, TREM2/DAP12 signaling for phagocytosis, and PPARγ pathways for resolution all affect this polarization; targeting microglia with CSF1R inhibitors, minocycline, or BTK blockers has therapeutic potential to changethe balance in favor of neuroprotection and stop the progression of MS [24].

Oligodendrocytes:The central nervous system's myelin-producing glial cells, known as oligodendrocytes, are crucial to the pathology of multiple sclerosis (MS) because they are directly damaged by the immune system, which results in demyelination, axonal vulnerability, and unsuccessful regeneration. In addition to T-cell cytotoxicity, microglial attacks, oxidative stress from ROS, mitochondrial dysfunction, and unfolded protein response (UPR) activation, which causes cell death when ER homeostasis fails, these cells undergo apoptosis in MS lesions through mechanisms such as IFN-γ signaling through STAT1/IRF-1 pathways, inducing MHC class I/II, Fas, and TNF-α receptor expression. In response,

oligodendrocyte precursor cells (OPCs) proliferate and migrate to lesions for remyelination.However, in chronic/progressive multiple sclerosis (MS), this repair is impeded by inhibitory signals from reactive astrocytes (e.g., ET-1, Jagged1), persistent inflammation, epigenetic barriers to differentiation, deficits in energy metabolism,and age-related OPC pool exhaustion, which causes shadow plaques and neurodegeneration.The dual role of oligodendrocytes as victims and potential therapeutic foci in stopping the progression of multiple sclerosis is highlighted by protective strategies that target OPC maturation through growth factors (PDGF, FGF), anti-inflammatory modulation, or drugs enhancing Wnt/β-catenin signalling[25].

6.2. Signal pathways:

The signaling pathways Notch, Wnt, LINGO-1, and mTOR play crucial roles in the pathophysiology of multiple sclerosis (MS), influencing oligodendrocyte differentiation, remyelination, blood-brain barrier integrity, and neuroinflammation.According to current studies, these pathways provide possible treatment targets [26].

Notch pathway:_Numerous functions, including cell activation, proliferation, cell fate, differentiation, and death, depend on the Notch signaling system.Without any intermediate steps, the Notch signaling pathway receptors undergo three cleavages before being translocated into the nucleus.Notch receptors are heterodimeric, single-span transmembrane glycoproteins that come in four different varieties.Human chromosomes 9, 1, 19, and 6 include the genes that encode Notch-1, -2, -3, and -4.Numerous different tissues contain both Notch-1 and Notch-2. Notch-4 is mostly found in the endothelium, whereas Notch-3 is extensively expressed in pericytes and vascular smooth muscle [27].The Notch intracellular domain (NICD), which trans locates to the nucleus, forms a complex with CSL/RBPJ and MAML, and activates transcription of targets like Hes and Hey genes, is released when ligand binding on neighbouring cells causes successive cleavages of Notch receptors by ADAM proteases and γ-secretase [28].Notch controls astrogliosis, microglial activation, oligodendrocyte precursor (NG2/OPC) differentiation, and remyelination in MS and EAE models; γ-secretase inhibition lessens demyelination and paralysis, whereas context-dependent effects affect neuroinflammation and neuroprotection [29].GSIs show promise in preclinical MS models by reducing inflammation and promoting repair, while notch modulation encourages OPC maturation and myelin repair, but must be precise to prevent impeding positive effects [30].

Wnt/β-Catenin Pathway:_The intracellular signaling cascade known as the wingless and integration site (Wnt) has been largely conserved throughout evolution and is essential for animal growth, development, metabolism, and stem cell maintenance. Extracellular Wnt proteins, Frizzled membrane receptors and their co-receptors, intracellular β-catenin, intranuclear T-cell factors/lymphoid enhancer factors (TCF/LEF), and low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6) all contribute to the complexity of the Wnt pathway. After the Wnt pathway was activated, OPC differentiation was postponed by expressing a dominant-active β-catenin only in OL lineage cells [31].As demonstrated in models with dominant-active β-catenin, canonical Wnt/β-catenin signaling frequently suppresses OPC differentiation and myelination in MS by stabilizing β-catenin, which interacts with TCF7L2 to limit mature oligodendrocyte development. TCF7L2 expression increases in early remyelinating MS plaques; overactivation delays remyelination after demyelination, but regulated levels promote repair. PLP+ oligodendrocytes and myelin repair are improved by antagonists [32].

PI3K/AKT/MTOR Pathway: An intracellular signaling mechanism called the PI3K/AKT/mTOR pathway controls cell activation, proliferation, metabolism, and apoptosis. In MS models such as EAE, hyperactivation of PI3K/AKT/mTOR increases vulnerability to inflammation and disease development by promoting Th17 cell differentiation and autoimmune responses. In preclinical research, the mTOR inhibitor rapamycin suppresses this pathway, lowering immune cell activity and postponing relapses [33].Although the route is crucial for early OPC maturation and myelin protein production (e.g., MBP via mTORC1-SREBP), it is not as involved in adult OPC remyelination after damage; therefore, inhibitors are helpful for stopping the progression of impairment but less effective for repair. PI3K-Akt promotes myelination in Schwann cells by driving mTORC1, but a balance is necessary to prevent feedback inhibition [34].Cannabidiol targeting reduces EAE symptoms by modulating PI3K/AKT/mTOR, indicating the possibility of treating MS by reducing neuroinflammation without compromising basal myelination. Immunomodulatory advantages are prioritized over direct remyelination enhancement in preclinical research [35].

ERK/MAPK Pathway: In multiple sclerosis (MS), the ERK/MAPK pathway promotes oligodendrocyte responses, immune cell activation, and microglial dysfunction, all of which lead to inflammation and poor remyelination. Peripheral blood mononuclear cells from MS patients had higher baseline phosphorylation of MAP2K1 (MEK1) and downstream ERK1

(MAPK3), which promotes B-cell overactivation, T-cell survival, and NF-κB-mediated inflammation via prosurvival signals. The progression of relapsing-remitting multiple sclerosis and dendritic cell priming toward inflammatory phenotypes are correlated with this hyperactivity of the ERK sub-pathway [36].Overactivation of MAPK/ERK in microglia, which may be brought on by the EBV latency protein LMP-1 or HERV-K18, suppresses negative regulators such as DUSP6/1, which causes oligodendrocyte disruption and demyelination in MS lesions. BRAFV600E-induced ERK hyperactivity mimics MS pathophysiology in preclinical models, and inhibitors such as PLX4720 slow the disease's progression [37].By opposing inhibitory signals, ERK/MAPK suppression promotes OPC development and remyelination in demyelinated mice, whereas pathway overactivity impedes myelin regeneration in chronic MS. By activating PP2A, fingolimod may indirectly restore ERK [38].

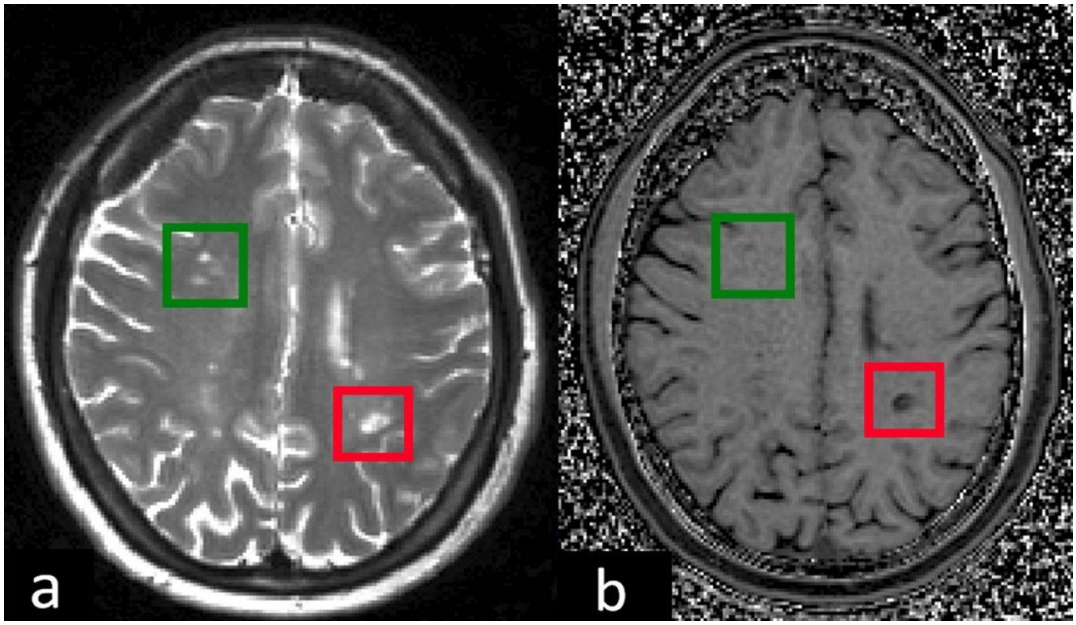
6.3 Axonal protection and neurotrophic support (BDNF/CNTF):

BDNF (brain-derived neurotrophic factor) and CNTF (ciliary neurotrophic factor) provide neurotrophic support and axonal protection in multiple sclerosis (MS), a demyelinating illness where axonal loss causes irreversible impairment. Neurons, immune cells, and glia secrete BDNF, which activates TrkB receptors to improve neuronal survival, prevent axonal transection in active MS lesions, promote synaptic plasticity, dendritic growth, and long-term potentiation, and counteract inflammation-induced neurodegeneration. Research indicates that serum levels of BDNF are lower in MS patients and that its expression is elevated in astrocytes and microglia through treatments like glatiramer acetate, which correlated with less axonal pathology in EAE models [39]. Like cuprizone models, CNTF, which is mostly derived from astrocytes, shows biphasic patterns in demyelination, peaking early to protect oligodendrocytes and axons throughout damage and remyelination. CNTF knockouts worsen EAE by increasing oligodendrocyte death and demyelination. Despite bioavailability issues, combined BDNF/CNTF administration in optic nerve crush models that mimic MS axonal injury promotes rapid, sustained neuritic outgrowth and retinal ganglion cell survival via sustained-release systems like chitosan microspheres, which are frequently enhanced by zinc chelators. This highlights their synergistic glioprotective and remyelination potential as adjuncts to immunomodulators [40].

7.REMYELINATION BIOMARKERS:

7.1 Imaging:

The magnetisation transfer ratio (MTR) is a widely used measurement of the amount of MT exchange occurring between the two proton compartments obtained from two images acquired with and without MT-weighting (by applying a dedicated radiofrequency pulse), allowing a ratio to be estimated from signal intensities. Most commercial scanners can capture MTR sequences, and whole-brain acquisition periods are low enough to accommodate most study methods.Myelin has a substantial influence on MTR, but water content, inflammation, and axonal density may all have a role.5 The MTR in MS brain WM lesions (figure 1) is lower than in normal appearing white matter (NAWM), while NAWM also exhibits a drop in MTR in MS compared to healthy controls.6 Lesion MTR is lower in the presence of demyelination, with significantly higher MTR seen in remyelinated lesions,5 though still lower than in NAWM, which could be due to partial remyelination, structural changes in newly generated myelin, and some degree of axonal loss. Similar results were observed in another postmortem MRI and histological examination of 36 MS patients[41].



Axial view of brain slices from a patient with multiple sclerosis showing appearance on (A) T2w imaging and (B) the corresponding slice's magnetization transfer ratio (MTR) map. The lesion in the red box has a low MTR value and is consistent with demyelination because it is noticeably hypointense when compared to normal appearing white matter (NAWM). Three lesions in the green box appear isointense or only slightly hypointense when compared to

NAWM; this corresponds to a higher lesion MTR (similar to or slightly less than NAWM), indicating potential remyelination. fig1[41].

Myelin Water Fraction (MWF), commonly abbreviated as "MWS," is an important MRI-based biomarker for measuring remyelination in multiple sclerosis (MS) by calculating the fraction of water trapped within myelin sheaths. Open-access papers emphasize its relationship with myelin content and healing processes in lesions and normal-appearing white matter (NAWM). [42] MWF compares short T2 relaxation periods of water in myelin to longer times in other compartments, resulting in a direct myelin content proxy confirmed against histology, such as Luxol fast blue staining. In MS, higher baseline MWF in fresh lesions predicts better myelin regeneration at 6 months, showing less edema and improved repair. Studies reveal that MWF increases in the corpus callosum NAWM following clemastine treatment, demonstrating drug-induced remyelination beyond localized lesions [43].

PET imaging using amyloid tracers, such as [^11^C] PiB, indicates lower uptake in demyelinated MS lesions relative to normal-appearing white matter, indicating myelin breakdown. Remyelinated shadow plaques show restored binding. Fluorinated tracers, such as [^18^F] florbetaben and [^18^F] florbetapir, have longer half-lives for therapeutic application. They correlate lower white matter uptake with disability severity in relapsing-remitting and progressive MS. These tracers bind to beta-sheet structures in myelin basic protein, allowing assessment of dynamic remyelination in longitudinal studies of newly created lesions[44]. ^18^F]FDG-PET reveals variable glucose metabolism in MS lesions, with hypermetabolism in acute inflammatory plaques and hypometabolism in chronic ones, but the high brain background restricts specificity. Emerging 4-AP derivatives target exposed potassium channels on demyelinated axons, resulting in positive signals in demyelinated locations and improved detection of tiny lesions. PET/MRI hybrids improve co-registration by reducing partial volume effects and accurately mapping myelin dynamics and inflammation in cortical gray matter and the spinal cord[45].

Current tracers encounter obstacles such as high lipophilicity, resulting in nonspecific binding, while newer ones, such as [18F]-3F4AP, which targets exposed axonal channels, offer promise for detecting demyelination/remyelination. PET voxel maps from these tracers stratify lesions by repair potential, allowing for individualized therapy[46].

7.2. Fluid:

Neurofilament light chain (NfL)is a sensitive biomarker of axonal damage in multiple sclerosis (MS). It may be detected in both cerebrospinal fluid (CSF) and serum, with substantial correlations between the two compartments indicating progressive neurodegeneration. Elevated blood NfL levels near MS onset predict long-term clinical outcomes, including disability progression after 15 years, surpassing standard predictive markers. In progressive MS, longitudinal fluctuations in serum NfL track subclinical disease activity, correlate with brain shrinkage and MRI lesion burden, and decline with effective disease-modifying treatments (DMTs) [47].Serum NfL concentrations rise during relapses and the development of new gadolinium-enhancing lesions in both relapsing-remitting and progressive MS subtypes, allowing for non-invasive therapy response monitoring. It stratifies patients for treatment trials based on their risk of future disability, with higher baseline levels associated with worse recovery and longer-term impairment. Unlike imaging, serum NfL detects diffuse axonal damage in normal-appearing white matter, providing a dynamic, blood-based tool for individualized MS therapy [48].

According to a meta-analysis, CSF NfL was doubled in relapsing MS (3080.6 ng/L) compared to remission (1541.7 ng/L, $p<0.0001$) and significantly higher in 746 MS patients compared to 435 controls (effect size 0.61, $p<0.00001$). CSF NfL is higher in relapsing-remitting MS patients (2124.8 ng/L) compared to progressive MS patients (1121.4 ng/L, $p=0.0108$). When combined with neurofilament heavy chain, baseline CSF NfL accurately classifies 93% of active cases in CIS and relapsing-remitting MS over a two-year period, with an overall accuracy of 87%. Serum NfL, which has a 42-fold lower level in blood but is still clinically significant, predicts relapses and the worsening of EDSS and has a strong correlation with CSF ($r=0.62$) [49].

Myelin basic protein (MBP) fragments play an important role in the pathogenesis of multiple sclerosis (MS) by serving as immunogenic peptides that cause autoimmune reactions and demyelination. Matrix metalloproteinases (MMPs), particularly MT6-MMP, efficiently cleave MBP isoforms and splice variants such as Golli-MBP BG21 and J37, resulting in fragments like the N-terminal 1-15 peptide. This fragment promotes the proliferation of T cell clones specific to it in experimental autoimmune encephalomyelitis (EAE), a model for MS, demonstrating MT6-MMP's superior effectiveness among investigated MMPs in creating these immunogenic products. MBP fragments from immunodominant epitopes like 83-99

have been studied as altered peptide ligands for MS therapy; however, trials experienced problems, including as hypersensitivity and cross-reactivity[50].

MBP's structural flexibility as an intrinsically disordered protein (IDP) makes it vulnerable in MS, as post-translational alterations affect its isoforms and membrane contacts. In MS brain tissue, charge isoforms such as C8 exhibit enhanced deimination, lowering MBP's positive charge and compacting ability, loosening myelin structure, and promoting breakdown into pieces detectable in cerebrospinal fluid. These alterations are associated with disease severity, as more widespread deimination in aggressive MS cases further destabilizes myelin, potentially exposing fragments as autoantigens [51].

Specific MBP segments differ in size from those found in cerebrovascular episodes, potentially providing diagnostic specificity for MS flares; nevertheless, therapeutic value awaits validation. These degradome products are essential myelin breakdown signals that have been catalogued in extensive atlases for MS research.Lower blood and saliva MBP levels (not fragments) in MS patients indicate total myelin degradation, with serum cutoffs of 1055 ng/mL (91% sensitivity, 100% specificity) differentiating cases. CSF IgGs from MS patients hydrolyse MBP into fragments with strong catalytic activity, which may drive neuropathology[52].

8. PHARMACOLOGICAL AND REGENERATIVE STRATEGIES:

8.1 Current medications used in remyelination:

8.1.1 Clemastine:

Clemastine fumarate, an over-the-counter antihistamine, has garnered attention for its potential remyelinating effects in multiple sclerosis (MS) by promoting oligodendrocyte precursor cell (OPC) differentiation. In multiple sclerosis (MS), clemastine fumarate primarily stimulates remyelination by antagonistically binding to muscarinic acetylcholine receptors on oligodendrocyte precursor cells (OPCs), specifically the M1 subtype, CHRM1, which drives OPC differentiation into mature myelinating oligodendrocytes without direct immunomodulation.

Main Mechanism:

Blockade of CHRM1Clemastine inhibits inhibitory signalling that prevents maturation by binding to M1 muscarinic receptors on OPCs. Myelin basic protein (MBP) expression, wrapping of demyelinated axons, and OPC process extension are all improved by this

activation of downstream ERK1/2 pathways. Using high-throughput micropillar screens that demonstrated dose-dependent OPC differentiation in rat cultures and cuprizone mice with restored g-ratios, Mei et al. (2014, cited in the ReBUILD trial) discovered this[53].Secondary Routes: ERK Activation and Gsta4/4-HNEFurther research demonstrates that clemastine increases the detoxification of glutathione S-transferase alpha 4 (Gsta4)/4-hydroxynonenal (4-HNE), lowering oxidative stress on OPCs and encouraging remyelination in EAE models. According to Carlström et al. (2020), Gsta4 overexpression improves clinical scores and spinal cord myelin through decreased lipid peroxidation, mimicking the effects of clemastine[54].

Preclinical Remyelination Evidence

Preclinical studies in animal models of demyelination, such as cuprizone-fed mice and lysolecithin-induced lesions, demonstrate that clemastine enhances OPC maturation into myelinating oligodendrocytes via muscarinic receptor antagonism, particularly M1 subtype. This leads to increased myelin basic protein expression and g-ratios closer to normal, indicating thicker myelin sheaths around axons. These findings establish a mechanistic basis for remyelination without direct immunomodulation [54].

Preclinical Verification for All Models

Electron microscopy revealed thicker sheaths, confirming that clemastine accelerated remyelination (30–50% faster OPC-to-oligodendrocyte transition) in LPC spinal cord lesions via ERK signaling. Yamazaki et al. (2023) linked CHRM1 blockade to corpus callosum repair independent of inflammation, reporting functional motor recovery in mice with internal capsule demyelination [55].

Results of the ReBUILD Clinical Trial

Clemastine (5.36 mg twice daily) decreased visual evoked potential (VEP) latency by 1.7 ms per eye ($p=0.0048$), a biomarker of optic nerve remyelination following chronic damage, according to the seminal ReBUILD phase II trial (NCT02040298), a randomized double-blind crossover study in 50 MS patients. Increased myelin water fraction in the corpus callosum's normal-looking white matter was found in MRI sub studies, supporting healing in non-lesional regions. Fatigue was common, but no significant adverse events happened [56].

Encouraging Mechanistic Research

Clemastine activates ERK signalling and Gsta4/4-HNE pathways to drive OPC differentiation, according to additional open-access research. EAE mouse models exhibit improved clinical scores, decreased axonal loss, and increased remyelination in the corpus callosum and spinal cord. Human OPC cultures bridge the gap between preclinical and clinical translation by confirming dose-dependent myelination promotion[55].

Recent Trial Findings and Restrictions

Multi-parametric MRI data from the ongoing TRAP-MS trial (NCT05359653) revealed myelin repair signals but stopped the clemastine arm because of an unanticipated worsening of disability in progressive non-lesional MS, highlighting difficulties in patient selection. Despite this, clemastine is positioned as a repurposed candidate requiring optimized protocols due to its safety profile and biomarker evidence [57].

8.1.2 Opicinumab

By preventing LINGO-1 from inhibiting oligodendrocyte precursor cell (OPC) differentiation and myelination in multiple sclerosis (MS), opicinumab, a humanized monoclonal antibody that targets LINGO-1 (leucine-rich repeat and Ig domain-containing Nogo receptor-interacting protein 1), promotes remyelination.

Mechanism of LINGO-1 Inhibition

On OPCs, LINGO-1 forms a ternary complex with Nogo-66 receptor (NgR1) and p75 neurotrophin receptor (p75NTR). This complex activates RhoA GTPase signalling, which inhibits OPC maturation into myelin-producing oligodendrocytes. By neutralizing LINGO-1, opicinumab breaks up this complex and prevents the activation of the RhoA/ROCK pathway, which permits Fyn kinase-mediated myelin gene expression (such as MBP and PLP) and process extension for remyelination. Without immunomodulation, preclinical cuprizone and EAE rodent models demonstrated improved myelin sheath thickness, axonal protection, and restored thinner g-ratios [58].

Promotion of OPC Differentiation

Opicinumab increases ERK/MAPK signaling downstream by inhibiting LINGO-1, which promotes OPC proliferation and differentiation while lowering apoptosis in demyelinated lesions. Ex vivo cerebellar slice assays and in vitro rat OPC cultures showed dose-dependent increases in MBP+ oligodendrocytes and myelinated axonal segments (up to 50% more).

This mechanism was positioned for progressive multiple sclerosis (MS), where chronic demyelination predominates, because it was shown to be independent of inflammation [59].

Support for Axonal Integrity

Additionally, LINGO-1 prevents neuronal regeneration; opicinumab's blockade supports the effectiveness of remyelination by preserving axonal cytoskeletons through decreased RhoA-mediated collapse. Electron microscopy verified that the 30–40% improvements in locomotor scores in MOG-EAE mice treated with anti-LINGO-1 were associated with spinal cord remyelination and neurofilament preservation.

Unlike immunomodulatory treatments, opicinumab (anti-LINGO-1 monoclonal antibody) targets the LINGO-1 protein to promote oligodendrocyte precursor cell (OPC) differentiation and remyelination in multiple sclerosis (MS).

Basis for Preclinical Remyelination

Opicinumab improved OPC maturation into myelinating oligodendrocytes, increased myelin basic protein expression, and improved axonal integrity with thinner g-ratios indicating repair in preclinical rodent models of demyelination, such as cuprizone and EAE. Mi et al. (2007) established the mechanism without immunosuppression by showing that LINGO-1 antagonism restored spinal cord remyelination and locomotor function in MOG-EAE mice [60].

Phase II of the SYNERGY Trial (NCT01864148)

Four opicinumab doses (3–100 mg/kg) were tested in this 72-week randomized trial involving 813 relapsing MS patients on interferon-β1a. Although the primary endpoint (confirmed disability improvement) failed overall ($p=0.53$), the Overall Response Score ($p=0.0022$ at 24 weeks) indicated trends in younger patients with lower disease burden at 10 mg/kg. Direct evidence was limited because there were no primary remyelination biomarkers, such as VEP or MR [61].

Phase II RENEW Trial (NCT01721161)

Six 100 mg/kg post-steroid doses in 82 patients with acute optic neuritis did not significantly improve full-field VEP latency recovery in intent-to-treat ($p>0.05$), but the per-protocol subgroup ($n=44$) recovered more quickly (mean difference favoringopicinumab). Sustained trends were observed in the follow-up RENEWED, but the sample size was small and there were no benefits to clinical function [62].

Limitations of Clinical Translation

Human trials like RENEW and AFFINITY failed primary endpoints despite strong preclinical remyelination (e.g., SYNERGY trial rationale), indicating incomplete pathway translation or biomarker gaps (no direct MWF/VEP primacy). However, the safety profile supported additional investigation prior to program termination [61].

8.1.3 BTK inhibitors:

MOA:By inhibiting Bruton's tyrosine kinase in microglia and macrophages, BTK inhibitors reduce neuroinflammation and maintain oligodendrocyte function and myelin integrity, thereby promoting remyelination in multiple sclerosis (MS).

Myelin Protection and Microglial Inhibition

BTK inhibitors, such as tolebrutinib, block pro-inflammatory activation that causes demyelination in CNS myeloid cells by penetrating the blood-brain barrier. By preventing microglial ROS production and oligodendrocyte phagocytosis, tolebrutinib decreased myelin loss in cuprizone models while maintaining PLP and MBP expression. BTKs differ from pure immunomodulators due to their dual peripheral (B-cell suppression) and central (microglial modulation) actions [64].

Dual Action Preclinical

By preventing microglial pro-inflammatory differentiation and encouraging anti-inflammatory M2-like states, evobrutinib maintained PLP/MBP expression in EAE and cuprizone models. By lowering g-ratio abnormalities and axonal loss without relying solely on B-cell depletion, this maintains endogenous repair capacity [65].Evidence of Preclinical RemyelinationEvobrutinib improved g-ratios and axonal conduction in EAE mice by reducing leptomeningeal inflammation and B-cell infiltration while promoting remyelination through decreased NLRP3 inflammasome activity in microglia. By preventing Fc-receptor-mediated myelin debris clearance and promoting endogenous OPC differentiation, fenebrutinib similarly reduced demyelination in toxic models [66].Links in Clinical Neuroprotection Slowed brain atrophy and NfL reduction in phase II HERCULES are correlated with tolebrutinib's CNS penetration (>90% BTK occupancy), indicating remyelination contributions beyond peripheral B-cell effects. Microglia are important BTK targets in smoldering lesions, according to reviews Clinical Translation and Neuroprotection Phase II HERCULES trial data for tolebrutinib demonstrated a 0.5-point EDSS reduction in non-relapsing secondary progressive multiple sclerosis, with MRI evidence of stabilized T2

lesions and slowed brain atrophy associated with microglial BTK occupancy (>90% at therapeutic doses). BTK is target compartmentalized inflammation while sparing regulatory B-cells, in contrast to B-cell depleters [67].

Current Phase III Perspectives

Neurofilament light chain reduction and other remyelination biomarkers are correlated with tolebrutinib's CNS accumulation as opposed to evobrutinib's lower penetration. Although liver safety monitoring is still crucial, reviews highlight the potential of BTKis in progressive MS where smoldering lesions predominate [66].

8.2 Stem cells:

8.2.1 Mesenchymal stem cells:

By regulating immune responses and fostering neuroprotection, mesenchymal stem cells (MSCs) present a promising treatment option for multiple sclerosis (MS). Their safety and varying effectiveness in raising Expanded Disability Status Scale (EDSS) scores are demonstrated by clinical trials. Open-access reviews demonstrate how MSCs can promote remyelination and reduce inflammation from a variety of sources and delivery methods. Effectiveness of Meta-Analysis Based on EDSS changes, a systematic review and meta-analysis of 22 clinical studies involving 285 MS patients revealed that 40.4% (95% CI: 30.6–50.2%) improved, 32.8% (95% CI: 25.5–40.1%) remained stable, and 18.1% (95% CI: 12.0–24.2%) worsened after MSC therapy. MSCs derived from the placenta or umbilical cord performed better than those derived from bone marrow, with improvement rates of 56.7%; intravenous administration produced better results (57.6% improvement) than intrathecal (32.8%). The safety profile of MSCs was confirmed in both short-term and long-term follow-ups, with minor adverse events such as headaches (57.6%) and fever (53.1%) occurring, but no significant complications[68].

Overview of Clinical Trials Intravenous or intrathecal infusions of autologous bone marrow-derived MSCs (BM-MSCs), adipose-derived MSCs (AD-MSCs), and umbilical cord MSCs (UCMSCs) reduce gadolinium-enhancing lesions and stabilize EDSS in progressive MS subtypes such as SPMS and PPMS. Numerous phase I/II trials have reported safety and feasibility. For example, while UCMSC studies in China and Panama reported symptom relief, fewer MRI foci, and improvements in quality of life without significant side effects, BM-MSC trials in Israel and Iran demonstrated EDSS declines in 53–71% of patients over 14–48 months. Because of their high proliferation, low immunogenicity, easy non-invasive

collection, and strong Treg induction, UCMSCs are unique in that they can repair immune defects related to multiple sclerosis in vitro[69].

Mesenchymal stem cells (MSCs) reduce Th1/Th17-driven inflammation that results in demyelination in multiple sclerosis (MS) by suppressing pro-inflammatory T cells and promoting regulatory T cells (Tregs). They accomplish this through direct cell-cell interactions, paracrine secretion of factors like hepatocyte growth factor (HGF), and cytokine modulation, which increases TGF-β and IL-10 while decreasing IL-17 and IL-6[70].

Regulation of Th17/Treg Balance Under inflammatory conditions such as LPS stimulation, MSCs convert differentiated Th17 cells (CD4+CD3+RORγt+) into functional Tregs (CD4+CD25+Foxp3+), dramatically reducing the Th17/Treg ratio in CD4+ T cells. HGF secreted by MSCs contributes to this change because blocking HGF with antibodies reverses the effect, increasing Treg differentiation and inhibiting Th17 markers. As a result, MSCs restore immune balance related to MS pathogenesis by decreasing pro-inflammatory cytokine mRNA (IL-17, IL-6) and protein levels while increasing anti-inflammatory ones (IL-10, TGF-β)[71].

Suppression of T cells and effectors As seen in MS clinical trials with fewer gadolinium-enhancing lesions, MSCs suppress effector T-cell proliferation, especially CD4+ and CD8+ T cells, and dampen Th1 responses by lowering IFN-γ-producing cells and IL-17 intensity. Additionally, they inhibit the maturation of B cells, natural killer cells, and dendritic cells while using soluble factors and cell-contact mechanisms to change macrophages into an anti-inflammatory M2 phenotype. Without the need for neural differentiation, this results in peripheral tolerance, decreased CNS infiltration, and stopped autoimmune attacks in MS models such as EAE [72].

Increased Immune Modulation Through IFN-γ licensing, MSCs promote tolerogenic environments, increase PD-L1 expression on monocytes, and improve immunosuppressive capacity via pathways like JAK-STAT1. By reducing neutrophil phagocytosis/oxidative burst and monocyte activation (downregulating CD40, CD80, TLR2, and MHC-II) through modulated CD4+ T cells, they further regulate innate immunity. By promoting neuroprotection in addition to immunosuppression, these paracrine and bystander effects position MSCs as effective for MS, with safety verified in trials [73].

8.2.2 Neural progenitor cells (NPCs):

Particularly, mesenchymal stem cell-derived neural progenitors (MSC-NPs) represent an advanced approach in stem cell therapy for multiple sclerosis (MS) by combining immunomodulation with neuroregenerative potential. Derived from bone marrow MSCs through ex vivo manipulation to express neural markers like Nestin, neurofilament medium (NFM), and GFAP, MSC-NPs do not typically differentiate into mature neural lineages in vivo but exert therapeutic effects via paracrine signaling. In preclinical MS models like experimental autoimmune encephalomyelitis (EAE), intrathecal MSC-NP injections reduce neurological deficits, enhance spinal cord myelination, decrease CNS immune infiltration, and recruit endogenous neural progenitors, as evidenced by increased Nestin-positive cells near transplant sites[74].

The effectiveness of intrathecal MSC-NP therapy in progressive MS patients with EDSS 3.0–6.5 is demonstrated by phase II clinical trials, such as the randomized, placebo-controlled study (NCT03355365). Particularly in ambulatory-impaired subgroups (EDSS 6.0-6.5), patients who received six bimonthly autologous MSC-NP injections showed trends toward improved Timed 25-Foot Walk (T25FW) and 6-Minute Walk Test (6MWT), with 3.7% improvement in T25FW compared to -54.4% worsening in placebo ($p=0.030$). Better bladder function, slower MRI grey matter atrophy, and changes in CSF biomarkers such as elevated MMP9 and decreased CCL2, which indicate decreased inflammation and neurodegeneration, were further advantages[75].

The mechanism of MSC-NPs in MS therapy emphasizes bystander effects over cell replacement, releasing trophic factors (BDNF, NGF, VEGF) that support endogenous repair, promote oligodendrocyte progenitor survival, and foster remyelination without tumorigenic risks associated with embryonic stem cell-derived NPCs. Unlike pure neural stem cells (NSCs), which migrate to demyelinated areas and differentiate into neurons, astrocytes, or oligodendrocytes while modulating Tregs and reducing ICAM-1/LFA-1 expression, MSC-NPs offer easier autologous production from adult bone marrow sources. This positions them as safer for progressive MS, addressing both inflammation and axonal loss unmet by disease-modifying therapies[74]. MSC-NPs' transition from MSC enhancement of ESC-derived oligodendrocyte precursor cells (OPCs) in dysmyelinated models—boosting OPC engraftment and myelination—to standalone therapy in human trials is exemplified by their ongoing evolution from concept to clinic. Although there have been no significant adverse

events, there are still issues with patient stratification, long-term efficacy monitoring, and dosage optimization. Future research attempts to improve these for primary and secondary progressive multiple sclerosis, which could transform regenerative neuroimmunology [76].MSC-NPs' transition from MSC enhancement of ESC-derived oligodendrocyte precursor cells (OPCs) in dysmyelinated models—boosting OPC engraftment and myelination—to standalone therapy in human trials is exemplified by their ongoing evolution from concept to clinic. Although there have been no significant adverse events, there are still issues with patient stratification, long-term efficacy monitoring, and dosage optimisation. Future research attempts to improve these for primary and secondary progressive multiple sclerosis, which could transform regenerative neuroimmunology[74].

8.2.3 IPCS:

Because they allow for patient-specific cell replacement and disease modelling, induced pluripotent stem cells (iPSCs) have great potential for treating multiple sclerosis (MS) by addressing both demyelination and neurodegeneration. iPSCs, which are derived from somatic cells such as fibroblasts through reprogramming factors (Oct4, Sox2, Klf4, c-Myc), differentiate into neural stem cells (NSCs), oligodendrocyte progenitor cells (OPCs), or neurons, thereby promoting remyelination in MS models without raising ethical concerns regarding embryonic sources[77].

Effects of Preclinical Remyelination By secreting leukemia inhibitory factor (LIF), which promotes endogenous OPC survival and blood-brain barrier integrity, iPSC-derived NSCs and OPCs improve mobility, decrease inflammation, and improve myelin repair in experimental autoimmune encephalomyelitis (EAE) models of multiple sclerosis. Because of trophic support and immunomodulation, transplanting iPSC-OPCs into demyelinated shiverer mice results in robust engraftment and myelination, outperforming other progenitors. By using retinoic acid and SMAD inhibition to accelerate differentiation, direct reprogramming to induced OPCs (iOPCs) avoids the risks associated with pluripotency[76].

Modeling the Pathophysiology of MS In vitro, iPSCs from MS patients replicate disease phenotypes, exposing genetic and epigenetic changes in neurons and leukocytes that cause axonal loss and Th17 inflammation. Drug screening for customized treatments is made possible by patient-derived iPSC-NSCs, which exhibit hypersensitivity to oxidative stress and mimic MS neurodegeneration. Unlike allogeneic transplants, this autologous method prevents

rejection, putting iPSCs in a position to study relapsing-remitting versus progressive MS subtypes[77].

Difficulties in Clinical Translation The dual neurotrophic and immunosuppressive effects of iPSC therapies are supported by preclinical data, but tumorigenicity risks, differentiation efficiency (44–70% for OPCs after 75 days), and scalability cause human trials to lag. iPSC-OPCs improve engraftment in dysmyelinated models when combined with MSCs, indicating hybrid approaches for progressive MS. Phase I trials in the future might concentrate on intrathecal iPSC-NSC delivery for efficacy and safety[78].

8.3 Drug repurposing:

8.3.1 Metformin:

A synthetic guanidine derivative, metformin, is frequently used as an oral antidiabetic medication and is regarded as a multi-vector application agent for the treatment of several inflammatory conditions. Metformin's positive effects on immune cells have been validated by recent research, with a focus on immunological mechanisms [78].Based on preclinical and early clinical studies, metformin offers promise as a repurposed medication for multiple sclerosis (MS), especially in boosting remyelination and neuroprotection through mitochondrial regulation. Its add-on use in patients with progressive multiple sclerosis is being evaluated in ongoing trials.There are a number of active clinical trials. Pediatric and young adult MS patients are being recruited for a single-centre, phase I, randomized clinical research in Canada to examine the safety, viability, and visual outcome measures of metformin add-on medication. Another single-centre, phase IIa, randomized placebo-controlled clinical trial will assess the impact of metformin 500 mg prolonged release tablets and clemastine 1,34 mg prolonged release tablets on safety, visual evoked potentials, and MRI outcome measures in patients with relapsing multiple sclerosis who are receiving a disease-modifying treatment. sufferers are being recruited for a single-centre, phase I, placebo-controlled, randomised clinical trial in PMS sufferers in the United States [79].

Mechanism of action:Through neuroprotective and remyelination-promoting effects seen in preclinical models and early clinical research, metformin shows promise in the treatment of multiple sclerosis (MS) [80].

AMPK Activation: AMP-activated protein kinase (AMPK), which regulates energy homeostasis and promotes the development of oligodendrocyte precursor cells (OPCs) into

myelin-producing oligodendrocytes, is primarily stimulated by metformin. In models of toxin-induced and autoimmune demyelination, this route promotes remyelination, lowers oxidative stress, and improves mitochondrial function [81].

Anti-Inflammatory Effects: By modifying T helper 17 (Th17) and regulatory T cells (Treg), downregulating pro-inflammatory microglia/macrophage markers such as Mac-3 mRNA, and preventing chronic smouldering inflammation in MS lesions, metformin reduces neuroinflammation. It preserves myelin integrity by reducing perivascular cuffing and immune cell infiltration in demyelination caused by lysophosphatidylcholine (LPC) [82].

Neuroprotective Mechanisms: In addition to upregulating brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 21 (FGF21) to prevent neurodegeneration, metformin crosses the blood-brain barrier to have anti-apoptotic, antioxidant, and anti-excitotoxic properties. By enhancing OPC bioenergetics, decreasing the degree of demyelination (for example, using Luxol fast blue staining), and preserving visual evoked potential delay in optic chiasm models, it guards against axonal loss [83].

Ongoing Clinical Trials:Metformin (850 mg oral tablet) being evaluated as an add-on in non-active progressive MS patients aged 18–70 in a multicenter, randomised, placebo-controlled trial (MACSiMiSE-BRAIN, NCT05893225), which measures brain remyelination and neurodegeneration using MRI and clinical outcomes over 104 weeks. Another study (NCT06463743) examines the safety and effectiveness of metformin monotherapy or add-on in MS. Metformin and interferon beta-1a were evaluated in a phase II open-label trial (NCT05298670) for relapsing-remitting multiple sclerosis, reporting effects on outcomes such as relapses [80].

Preliminary Clinical Evidence: Metformin (850–1500 mg/day) decreased new T2 hyperintense brain lesions on MRI in a pilot study of 50 obese MS patients with metabolic syndrome without causing any notable side effects. Metformin and clemastine combination therapy demonstrated remyelination in MS patients, indicating the possibility of myelin repair. Metformin use may enhance outcomes for interferon beta-1a-treated relapsing-remitting multiple sclerosis patients, according to retrospective research [84].

Preclinical Support for Use:Metformin reduces CNS autoimmunity, demyelination, and inflammation through AMPK activation in animal models such as experimental autoimmune

encephalomyelitis (EAE). Myelin protection and functional recovery are confirmed by research in models of toxin-induced demyelination [85].

8.3.2 Benztropine:

Benztropine is a synthetic anticholinergic drug that shares molecular similarities with atropine and diphenhydramine. It is sometimes referred to as benztropine mesylate or Cogentin. Balancing cholinergic overactivity in the basal ganglia, it lessens tremors, rigidity, and muscle spasms. It was approved by the FDA in 1984, mostly for Parkinson's disease and drug-induced extrapyramidal symptoms [86].One synthetic medication that belongs to the class of muscarinic receptor antagonists, or anticholinergic medicines, is benztropine. As a result, the medication is structurally similar to atropine and diphenhydramine. By successfully treating drug-induced extrapyramidal symptoms and being essential in the prevention and treatment of acute dystonic responses, this medicine broadens its therapeutic use beyond Parkinson's disease [87].

Mechanism of action: By competitively inhibiting muscarinic acetylcholine receptors, particularly M1 and M3 subtypes, in the central nervous system (CNS) and smooth muscle, benztropine mesylate (Cogentin) predominantly operates as a centrally acting anticholinergic drug [88].By competitively inhibiting muscarinic acetylcholine receptors, particularly M1 and M3 subtypes, in the central nervous system (CNS) and smooth muscle, benztropine mesylate (Cogentin) predominantly operates as a centrally acting anticholinergic drug [89].

Primary Anticholinergic Effects:_It reduces excessive cholinergic activity in comparison to dopamine deficit in Parkinson's disease or antipsychotic-induced Parkinsonism by binding to muscarinic receptors in the basal ganglia. At therapeutic levels, this blockade reduces tremors, stiffness, bradykinesia, and dystonia by blocking acetylcholine-mediated signalling and re-establishing neurotransmitter balance without significantly disrupting the peripheral parasympathetic nervous system [87].

Dopaminergic Modulation:_By binding to the dopamine transporter (DAT), benztropine prolongs dopamine availability at synapses and enhances dopaminergic transmission in the striatum, thereby mildly inhibiting dopamine reuptake. Its unusual effect, which has a later onset and less euphoric potential than normal reuptake inhibitors, contributes to the antiparkinsonian advantages [90].

Preclinical Evidence:Research shows that by boosting mature oligodendrocytes and myelin regeneration, benztropine reduces the severity of EAE in preventative and therapeutic regimens, on par with or superior to medications like FTY720 or interferon-β. It speeds up remyelination in the corpus callosum in cuprizone demyelination models. Immunosuppressive combinations provide additive advantages that enable dose reductions [91].

Clinical status:The data supporting benztropine's use in treating multiple sclerosis (MS) is limited to preclinical research conducted around 2013. As of late 2025, no human clinical trials had progressed to the approval stages, despite encouraging remyelination outcomes in animal models such as EAE and cuprizone-induced demyelination [91].Ongoing trials for remyelinating drugs such as Bruton's tyrosine kinase inhibitors (e.g., tolebrutinib, fenebrutinib) are highlighted in recent evaluations of novel MS therapeutics; however, benztropine is not included in phase 2 or 3 listings. Given that anticholinergic adverse effects include cognitive impairment, articles highlight the necessity for human research to evaluate tolerance [92].

9. ENVIRONMENTAL AND LIFESTYLE MODIFIERS:

9.1. Dietary impacts:

In individuals with multiple sclerosis (MS), ketogenic diets have the potential to improve quality of life and mobility while lowering inflammation, fatigue, and depression. Supplementing with vitamin D is associated with a decreased risk of multiple sclerosis and a slower rate of advancement, especially when adequate serum levels are maintained. Numerous exercises improve physical function and lessen MS symptoms. The ketogenic diet has drawn interest as a possible therapeutic strategy for reducing inflammation and enhancing clinical outcomes in individuals with multiple sclerosis because of its anti-inflammatory and neuroprotective properties [93].In MS patients reaching ketosis, ketogenic diets increase adiponectin, decrease leptin, and stabilise neurodegeneration indicators such as neurofilament light chain. After six months, studies show improvements in walking distance and Expanded Disability Status Scale ratings, as well as approximately 50% decreases in fatigue and depression levels. Without exacerbating neurodegeneration, these modifications promote improved body composition and decreased adipose inflammation [94].

Vitamin D Impacts:Low vitamin D levels raise the likelihood of MS onset; every 20 ng/mL increase above 24 ng/mL is associated with a 41% reduced incidence, particularly in women

who take more than 400 IU per day. In several trials, supplementation lowers T1 and T2 lesions, relapse rates, and the progression of disability; nevertheless, the effects of high dosages on annualised relapses are inconsistent. By affecting T cell and oligodendrocyte activity, maintaining serum levels between 30 and 60 ng/mL promotes immunomodulation [95]. One can get vitamin D from food. There are two types of vitamin D found in food: vitamin D2 and vitamin D3. Animals provide vitamin D3, while plants provide vitamin D2. But the most significant source of vitamin D in a person is their skin, where UV-B radiation converts 7-dehydrocholesterol into vitamin D.Despite having a safe profile, numerous randomised, placebo-controlled clinical trials of high-dose native vitamin D supplementation have not yet shown consistent efficacy. The efficacy of low calcemic 1,25(OH)2D analogues in the treatment of MS and EAE has not been proven. The discovery that 1,25(OH)2D levels in the central nervous system (CNS), but not in the serum, inversely correlate with disease activity in mice generated for EAE, is one warning against vitamin D administration for the treatment of multiple sclerosis. This discovery offers a compelling case for tissue-specific 1,25(OH)2D targeting [96].

Exercise Benefits: Resistance training is the most effective way to reduce tiredness in MS patients, whereas aerobic exercise is the most effective way to improve quality of life. Walking ability (MSWS-12) and endurance (6MWT) are both improved by multicomponent training. Resistance training combined with two to three days a week of moderate-intensity aerobic exercise (10–40 minutes) improves strength, balance, and lessens tiredness [97].One novel way to address the issue of physical inactivity in MS patients is to encourage exercise through patient-provider interactions. Improvements in physiological capability (e.g., aerobic endurance, muscular strength/endurance, walking ability), symptoms (e.g., weariness, depression), and quality of life are common advantages of exercise involvement [98].

9.2 Gut- Brain Axis and Circadian Rhythm Influences:

The pathogenesis of multiple sclerosis (MS) is significantly influenced by the gut-brain axis and abnormalities in circadian rhythm. This is mainly due to gut dysbiosis, which modifies metabolites originating from the microbiota, such as butyrate, and increases permeability, which causes inflammation and mitochondrial dysfunction in CNS cells. By affecting glial function, immunological control, and remyelination, these mechanisms worsen the symptoms of multiple sclerosis[99].The gastrointestinal tract (GIT) contains more than 100 trillion microorganisms, primarily in the colon. Bacteria, fungi, eukaryotes, viruses, and archaea

make up this combination of microbial communities, also referred to as the GIT or GUT microbiota. One of the environmental elements influencing health is the gut microbiota, and any changes to its makeup either enhance or decrease susceptibility to chronic diseases. It has recently been noted that bidirectional contact between the gut microbiome and the central nervous system can impact CNS processes. The final products or intermediates of the microbiota's metabolism are known as gut metabolites. Some of these metabolites can cross the blood–brain barrier (BBB) and enter the bloodstream, where they can impact CNS pathways and regulatory processes [100].

Gut Dysbiosis Mechanisms:Changes in the gut microbiota in multiple sclerosis (MS) lower ceramide, a pro-apoptotic lipid raised by lipopolysaccharide (LPS) from leaky gut, and decrease short-chain fatty acids (SCFAs) like butyrate, which typically maximize mitochondrial activity. Toll-like receptor (TLR) activation, peroxynitrite production, and pro-inflammatory cytokines like TNF-α are all heightened by this dysbiosis, which encourages the collapse of the blood-brain barrier and T-cell infiltration into the central nervous system. Additionally, butyrate deprivation exacerbates demyelination by failing to counteract ceramide's suppression of oligodendrocyte differentiation [101].

Circadian Rhythm Dysregulation: The risk of multiple sclerosis is influenced by circadian genes such as ARNTL and CLOCK. Shift work and sleep disturbances are associated with increased gut permeability and microbiome changes that intensify mitochondrial oxidative stress. Both daytime orexin and nighttime melatonin, which are both decreased in multiple sclerosis, typically increase mitochondrial oxidative phosphorylation through the melatonergic pathway; ceramide and inflammation inhibit both, upsetting the daily metabolic cycles in glia and immune cells. By encouraging the formation of acetyl-CoA for melatonin synthesis, gut-derived butyrate maintains this rhythm; its removal is associated with weariness and worsening symptoms [102].MS susceptibility is increased by polymorphisms in the ARNTL (rs3789327 CC genotype, OR 1.67) and CLOCK (rs6811520 CC genotype, OR 1.40) genes, most likely as a result of altered transcription of circadian regulators regulating immune responses and latitude-dependent prevalence. Through increased IL-1β from monocytes and T-cell polarization, myeloid-specific BMAL1 deletion in mice aggravates experimental autoimmune encephalomyelitis (EAE), demonstrating time-of-day influences in illness induction [103]. Circadian rhythm sleep abnormalities, such as delayed sleep phase and irregular patterns, are more common in MS patients and are associated with

severe fatigue (prevalence 3-fold higher than in controls). Actigraphy shows subgroup changes in sleep latency but no overall circadian shifts; exhaustion is linked to altered sleep architecture rather than obvious rhythm disruption [104].Melatonin suppression and pro-inflammatory changes are two ways that shift work increases the incidence of multiple sclerosis in young people. Clock abnormalities in immunity and metabolism are linked to diurnal symptom variations, such as spring/summer relapses. Restoring rhythms by chrono nutrition, such as time-restricted eating, may counteract the immunological and metabolic dysregulations seen in multiple sclerosis [105].

10.RESEARCH MODELS &FUTURE FRONTIERS.

10.1 Models:

Cuprizone: The loss of oligodendrocytes and myelin sheaths in the white matter tracts is a gradual (and ultimately irreversible) feature of demyelinating illnesses of the central nervous system.Peripheral nerve polyneuropathy, Guillain-Barré syndrome, and multiple sclerosis are a few examples of myelin diseases [106].Animal models of cuprizone oral intoxication are frequently used to test myelin regeneration treatments for the treatment of conditions like multiple sclerosis. which, within a few days, causes oligodendrocyte apoptosis. This is soon followed by the activation of the brain's innate immune cells, such as astrocytes and microglia, which ultimately results in the demyelination of certain white and grey matter brain regions. Adaptive immune system cells, especially T and B cells, are thought to have a non-dominant role during cuprizone-induced demyelination, despite the fact that this model has shown slight disruption to the blood–brain barrier [107].Thus, this model captures a number of significant aspects of the progressive course of MS. The cuprizone model can be used to study two primary elements of MS pathology: remyelination of the demyelinated axons and the mechanisms behind innate immune cell-driven myelin and axonal degradation.Cuprizone is administered orally to young adult mice for five to six weeks in order to cause acute demyelination. In our experience, a five-week poisoning strategy (0.25% cuprizone put into pulverised rodent chow) results in continuous and severe demyelination. If animals are offered normal chow after week 5 (i.e., acute demyelination), spontaneous endogenous remyelination occurs. This endogenous regenerating process is seriously disrupted if the duration of cuprizone intoxication is extended (i.e., chronic demyelination). In order to obtain persistently demyelinated lesions, most labs, including ours, use a 12–13-week cuprizone intoxication period. Myelin repair is more slower, even if remyelination follows chronic cuprizone-induced demyelination as well [108].

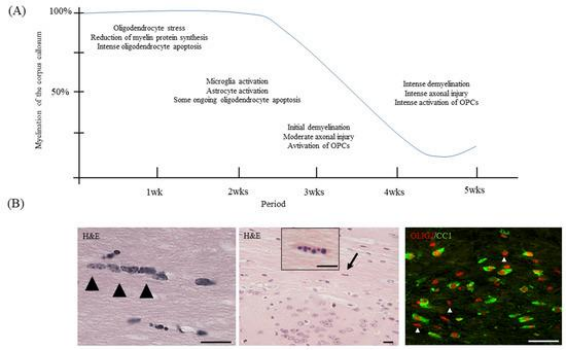


Figure 1: Cuprizone model hallmarks. (A) Diagrammatic representation of pathogenic features during cuprizone-induced demyelination. The myelination levels are shown by the blue line. (B) Interfascicular oligodendrocytes (arrowheads) in a control mouse's corpus callosum are shown in the left image. An apoptotic cell (arrow) appears in the centre image following a week of cuprizone intoxication. Mature OLIG2+/CC1+ oligodendrocytes and immature OLIG2+/CC1− oligodendrocytes (white arrowheads) are shown in the image on the right. 10 µm is the scale bar. Adenomatous polyposis coli gene clone CC1 (CC1) and oligodendrocyte transcription factor 2 (OLIG2) are abbreviations [108].

3D culture system:systems for three-dimensional (3D) cell culture to improve the therapeutic value and physiological significance of cells, cell-derived products, and tests. More biomimetic settings are made possible by 3D culture technologies, which enhance cell-cell and cell-matrix interactions and better preserve cellular traits and functions. The development of functional biomaterials, the building of scalable bioreactor systems, and the use of 3D models for disease modelling and medication discovery are some of the major issues in the field that are addressed by the papers included in this Special Issue. When taken as a whole, the contributions show how sophisticated 3D culture formats can enhance the quality and usefulness of in vitro systems for both basic research and treatment development [109].Animal use can be decreased using in vitro 2D or 3D cell culture. Compared to an in vivo model, cell culture offers the benefit of allowing for more independent research of cellular systems. Compared to three-dimensional cell culture, two-dimensional cell culture is more straightforward and is highly beneficial for researching how cells interact or behave in response to different stimuli [110].

10.2 Emerging Tech:

Gene therapy:Gene therapy protocols aimed to deliver therapeutic molecules into the central nervous system may represent an alternative therapeutic strategy in patients affected by

inflammatory demyelinating diseases of the central nervous system, where systemic therapies have shown limited therapeutic efficacy, possibly owing to the blood-brain barrier, a major obstacle for the entry of therapeutic molecules into the central nervous system [111].Recombinant adeno-associated viral vectors (AAVs) are the most popular option for gene therapy due to their favourable safety profile and wide cell tropism, which have been shown in both human and animal research.However, the majority of AAV serotypes cannot effectively reach the central nervous system due to the BBB. Only little access into the adult central nervous system is demonstrated by natural AAV serotypes that do pass the blood-brain barrier, such as AAV9 and F-clade derivatives. AAV.PHP.eB, a capsid with an improved ability to penetrate the BBB, was discovered as a result of site-directed evolution of the AAV9 capsid.Due to its effective BBB penetration, wide CNS cell tropism, and decreased tropism for peripheral organs in comparison to its mother AAV9 capsid, AAV.PHP.eB is currently the most widely utilized vector for systemic delivery to the mouse CNS [112].

Nanotechnology:By facilitating targeted immunomodulation, improving drug distribution across the blood-brain barrier (BBB), and encouraging neuroprotection or remyelination, nanotechnology offers novel therapy options for multiple sclerosis [113].Tumour cell surfaces' active signalling pathways are targeted and disrupted by magnetic nanoparticles. The blood-brain barrier (BBB) and reticuloendothelial system (RES) interactions involving endothelial cells, astrocytes, and pericytes make it difficult to administer traditional medicines to brain/CNS illnesses. Through receptor- or absorptive-mediated transcytosis, active targeting through surface nanoengineering and site-specific ligands improves NP penetration, pharmacokinetics, and bioavailability across BBB/RES. PEGylation of liposomes (less than 100 nm in diameter) resolves problems such as quick RES clearance, low BBB transit, short half-life, and aggregation.Therapeutic genes can be delivered by coating the liposome surface with monoclonal antibodies to glial fibrillary acidic proteins, transferrin receptors, or human insulin receptors (nanoliposome), which further aids in bypassing RES and the BBB [114].

10.3 Translational Hurdles:

Animal-to-human scaling and regulatory barriers: The limited predictive power of animal models, such as experimental autoimmune encephalomyelitis (EAE), which fail to fully capture human disease heterogeneity, creates translational challenges in multiple sclerosis (MS) research, especially animal-to-human scaling and regulatory barriers. This results in

high failure rates in clinical translation, with only approximately 5% of animal-tested therapies receiving regulatory approval. Animal studies frequently employ young, healthy rats in artificial environments that do not replicate the intricate, multimorbid characteristics of MS patients. This leads to disparities such as 50% progression to human research but only 40% to randomized controlled trials (RCTs), with median delays of five to ten years Regulatory obstacles exacerbate this by imposing strict FDA/EMA requirements for safety, efficacy, and ethical approvals; lengthy review procedures; a lack of funding; and flaws in preclinical design, such as inadequaterandomization, blinding, and generalizability, which exclude promising candidates despite an 86% concordance in favorable outcomes between animal and early human data. [115]. Regulatory barriers in multiple sclerosis (MS) drug development significantly impede progress by imposing prolonged ethical and regulatory approval processes that can span years, often delaying the transition from promising preclinical animal studies to human trials due to rigorous requirements from agencies like the FDA and EMA, which demand comprehensive evidence of safety, efficacy, and manufacturing quality under frameworks such as the Common Technical Document (CTD) and Good Clinical Practice (GCP) guidelines[116].

11. CONCLUSION :

The "final frontier" in multiple sclerosis research is the pursuit of efficient remyelination and neurorepair, which moves the emphasis from merely slowing the disease's progression to actively repairing neurological damage. The underlying neurodegeneration and chronic demyelination that cause long-term disability are largely unaddressed by current disease-modifying therapies (DMTs), even though they have mastered the management of the peripheral immune response. The data in this review show that effective repair necessitates a multifaceted strategy: we must alter the hostile, pro-inflammatory microenvironment of the MS lesion in addition to promoting the differentiation of oligodendrocyte precursor cells (OPCs) via pathways like Notch and Wnt. Additionally, incorporating cutting-edge imaging modalities like PET-based radioligands and Myelin Water Fraction (MWF) is crucial for delivering real-time. As pharmacological interventions, including BTK inhibitors and metformin, synergise with regenerative medicine, such as mesenchymal stem cell therapy, they present a promising avenue for restoring saltatory conduction and safeguarding axonal integrity in patients.Although there are many obstacles to overcome before these laboratory discoveries can be applied to clinical settings, the field's future is still cautiously optimistic. A better understanding of why endogenous repair fails in chronic "smouldering" lesions is

necessary to move from rodent models to human applications, especially with regard to the inhibitory roles of astrocytes and the metabolic exhaustion of ageing OPCs. Personalised treatment plans are poised for a revolution thanks to emerging technologies, including AI-driven predictive modelling for lesion recovery and drug delivery systems based on nanotechnology. Furthermore, it is impossible to ignore the complementary roles that lifestyle modifications and the gut-brain axis play in boosting the body's natural ability to regenerate itself. The creation of "next-generation" neurotherapeutics that combine immunomodulation with strong neuroregenerative qualities must ultimately be the top priority for the next ten years of MS research. By bridging the gap between benchside molecular discovery and bedside clinical application, the scientific community advances the objective of helping MS patients achieve a true functional recovery in addition to treating the disease.

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