

Neurobehavioral burden of multiple sclerosis with nanotheranostics

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Abstract: Multiple sclerosis (MS) is a chronic demyelinating neurological disorder affecting people worldwide; women are affected more than men. MS results in serious neurological deficits along with behavioral compromise, the mechanisms of which still remain unclear. Behavioral disturbances such as depression, anxiety, cognitive impairment, psychosis, euphoria, sleep disturbances, and fatigue affect the quality of life in MS patients. Among these, depression and psychosis are more common than any other neurological disorders. In addition, depression is associated with other comorbidities. Although anxiety is often misdiagnosed in MS patients, it can induce suicidal ideation if it coexists with depression. An interrelation between sleep abnormalities and fatigue is also reported among MS patients. In addition, therapeutics for MS is always a challenge because of the presence of the blood–brain barrier, adding to the lack of detailed understanding of the disease pathology. In this review, we tried to summarize various behavioral pathologies and their association with MS, followed by its conventional treatment and nanotheranostics.

Keywords: demyelination, behavioral disorders, behavioral tests

Introduction

Multiple sclerosis (MS) is a chronic neurological autoimmune disease characterized by erosions of myelin, the protective nerve sheath, with a partial preservation or complete loss of axonal activity and nerve transmission.¹ However, remyelination is often possible once inflammation subsides and is mediated by oligodendrocytes, which secrete myelin. Remyelination process can be explained as the phenomenon of newer myelin sheath formation around the damaged axons. It is better explained as more the number of oligodendrocytes around the affected neurons, the faster the process of remyeliantion. Inadequate number of oligodendrocytes may lead to improper or no myelin formation, resulting in abnormal neuronal functioning.² MS has affected more than 1 million people worldwide. The symptoms of MS are weakness, loss of senses, shuffling gait, loss of vision, and cognition.^{3,4} The main driving force involved in the pathology of MS is inflammation, which enhances autoreactivity, followed by demyelination and neuronal damage.⁵ Histopathological studies have also confirmed multicentered inflammatory lesions, which spread throughout the brain and spinal cord.⁶ Autoreactive T-cells are the key players involved in disease generation, breaking down the margin of autoreactivity and self-tolerance, and multiple etiological factors are responsible for this activation. The existence of autoreactive T-cells is evident in both normal and MS patients, but they turn active and seem to be devastating only in MS.⁷ Among all the T-cell clones, CD8 subtype is found to be more associated with the disease, both in number and infiltration when assessed in the brain and spinal cord. CD8 T-cells were also found to be persistent in blood and cerebrospinal fluid (CSF),

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implying the fact that they were activated consistently by an antigen driving the long-lasting autoimmune reactivity.⁸

In brief, a group of activated T-cell subclones specific to the myelin protein will penetrate the blood–brain barrier (BBB), being potentiated by the existing inflammatory cytokines, while the resting T-cells have a restricted access to the BBB.⁹ The coupling of T-cell surface molecules – integrins, selectins, and cadherins – with the corresponding adhesion molecules present in brain capillary endothelial cells facilitates the entry of T-cells. Once the T-cells gain entry, they unleash the autoimmune reactions targeting the myelin antigen. The activation of antibody-producing B-cells further enhances this autoreactivity, thus driving the degeneration of neurons.¹⁰ Figure 1 shows MS pathogenesis. Symptom severity, disability levels, and rate of disease progression vary with each individual and even differs in the same patient with time.¹¹ Significant sex differences were identified among MS patients, with a majority of females displaying the symptoms compared to males.

Also, MS was found to affect women at an early age (18–30 years), while it affected men at a later stage of life (30–40 years). These dissimilarities were possibly due to the protective effects of testosterone in men. Another interesting

aspect observed in MS was the cessation of disease symptoms in pregnant women who were in the third trimester. The elevated levels of estriol were anticipated to be responsible for the protective effect, and the same was observed in mice models as well.¹² The introduction of biomarkers has revolutionized the understanding of the disease pathology and its diagnostics. Some of the recent advancements are the discovery of elevated levels of astrocyte and axonal cytoskeletal proteins, namely, glial fibrillary acidic protein and neurofilament light protein in the CSF corresponding to MS progression.¹³

The elevated levels of the 14-3-3 protein in the CSF corresponded to the disease progression and disability, while a close correlation was established with the downregulation of cystatin-C, a protease inhibitor that neutralizes the actions of lysosomal cathepsins modulating lymphocytic activation.¹⁴ Some other recently found potential biomarkers associated with the disease are osteopontin and pentosidine.^{15,16} Based on the symptoms, MS is categorized into four subtypes. Patients with relapsing remitting form recover with no symptoms following disease onset. Patients with persistent disability symptoms between the cycles of relapses and recoveries are termed as “secondary-progressive” patients.

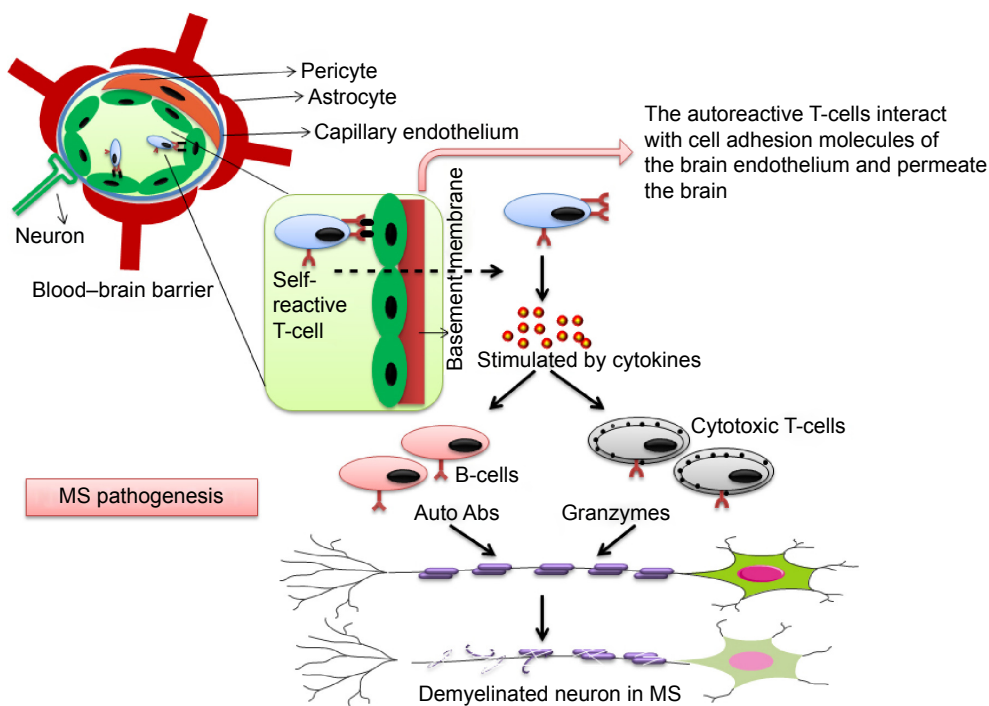


Figure 1 Pathogenesis of MS.

Notes: Lymphocytes activated due to various insults (inflammation, antigen presentation, free radicals, etc) will invade the blood–brain barrier. Initially, they bind with the cell adhesion molecules present on the capillary endothelium and gain access into the brain. Once inside, the reactive cells attract both the immune cell traffic (T- and B-cells) and mediate the devastating cascade. Cytotoxic T-cells release granzymes, and activated B-cells produce antibodies against the myelin sheath, thus mediating the demyelination process.

Abbreviations: MS, multiple sclerosis; Abs, antibodies.

“Primary-progressive” MS is characterized by symptoms of only progression, but no signs of remission. In “progressive-relapsing” MS, patients display severe symptoms and attacks during the period of remission.¹⁷

MS is not only associated with neurological symptoms but also with a variety of behavioral disturbances such as depression, anxiety, cognitive loss, psychosis, euphoria, sleep disturbances, and fatigue. The impact of these psychological disturbances is such that MS at times is misdiagnosed, supposing it to be a behavioral disorder. In patients with MS, depression is the most common behavioral disorder followed by psychosis.^{18,19} The disease progression is highly variable with a majority of the neurological compromise following the relapses. In particular, RRMS is a critical subtype that always follows a prolonged deterioration and poses a serious threat of progressive disability. The symptoms of depression and anxiety are very common during the early course of the disease, though the correlation is poor toward the lesional load.

Cognitive impairment is often seen as a forerunner of MS, presenting the symptoms of hampered levels of memory, attention, and execution. According to magnetic resonance imaging (MRI) studies, the severity of cognition loss is relative to the cerebral white matter lesions associated with MS progression. Correlations also exist between the white matter lesions and the onset of psychosis, which is seldom seen in MS.²⁰ Hence, early identification of these behavioral abnormalities would certainly help in diagnosing the onset of MS and can be invaluable for the disease management. Behavioral disorders and their association with MS are described as follows:

Depression and MS

Depression is the most common behavioral disorder prevailing among MS patients. The frequency of occurrence and tendency to develop depressive symptoms were higher in patients with MS compared to patients with other chronic neurological disorders. However, depression often remains undiagnosed and untreated in MS patients. Several factors related to genetics, psychology, immunology, and inflammation have a role in mediating the depressive pathology in MS. The higher prevalence of depression in MS could possibly be explained as: both depression and MS are central nervous system disorders and share multiple etiological factors in their pathophysiology. Also, elevated levels of proinflammatory cytokines and imbalances in monoaminergic neurotransmitter metabolism observed in MS were also reflected in depression.²¹ Likewise, a study reported genetic influence,²²

while a brain MRI study showed the evidence of inflammatory markers in the lesions and pleocytosis in the CSF.

The symptoms of depression were found to be associated with the lesions seen in the arcuate fasciculus²³ and medial orbitofrontal regions. They were also associated with poor perfusion rate in the left cerebral hemisphere, while in the right cerebral hemisphere, the frontal lesion load and temporal brain volume were involved.²⁴

Similarities between MS and depression

There are some similarities between the pathologies of MS and depression. The lesions in arcuate fasciculus were found to detach few regions of the cerebral cortex. The lesions were evident in the frontotemporal region, while inflammation had an influence on the hypothalamic endocrine functioning, corroborating the coexistence of depression in MS compared to other neurological disorders.²⁵ The symptoms of depression are not closely related to disease severity and its progression, but they are more prevalent during the relapses and in increased neurological disability.²⁰ Though much progress has been made in identifying the depressive pathological origins, their specificity still remains as a question. When observed in a group of RRMS patients with depression, the proinflammatory cytokine interferon (IFN)- γ was excessively produced and was associated with symptom severity. However, when treated with antidepressive medication, the levels of IFN- γ were lowered, explaining its possible role in depressive pathology.²⁶ Similarly, a case-controlled study observed exacerbations of depression in MS patients undergoing treatment with IFN- β .²⁷ A few studies reported contradictory results revealing the uncertainty of MS medication-induced depression.^{28–31} The major consideration of depression in MS is that it is associated with other behavioral disturbances such as disturbed cognition. Depression may aggravate cognitive dysfunction, which may induce depression, thus worsening the quality of life in all aspects.³²

Observations by Alajbegovic et al³³ revealed some interesting results. They found that depression was common among the MS population studied, with a majority of them being young, educated, unemployed, and single.³³ A longitudinal study also confirmed that the depression associated with MS is chronic and is usually episodic.³⁴ Finally, the most interesting concept to understand is depression and its association with MS relapses. Though there are no substantiating data to support this issue, it definitely seems to pose a fascinating question to be answered. In summary, depression stands as the major behavioral disorder observed in MS, with a potential of aggravating other behavioral abnormalities.

Hence, diagnosis and treatment of depression in MS will significantly improve the quality of life in patients.

Cognition and MS

Cognitive disturbances have been reported in 40%–60% of people with MS. Common manifestations include defects in recent memory and impaired attention, analyzing, visuospatial perception, and executional activities.³⁵ Impairment of cognition can be a potential indicator for the demyelination process³⁶ and is more common and severe in the progressive form of the disease rather than the relapses.³⁷ The results of the study conducted by Deloire et al³⁸ also hypothesized the symptoms of cognitive impairment as a forerunner for MS disability, which follows several years later. The precise pathology of cognitive disturbances in MS has not been identified, but inflammatory lesions, regional degeneration, and repetitive black holes somehow relate them to MS pathology.³⁸

It has been previously reported that inflammatory cytokines tend to cause cognitive disturbances, and the association of MS with inflammation indicates the role of cytokines in correlating MS pathology with that of cognitive impairment.³⁹ Loss of gray matter is quite common among MS patients, and this also leads to the loss of cognitive function. Also, disruptions of the cerebral cortex regions and the thalamus strongly associate memory impairment with that of MS pathology.

Causes of cognitive imbalance in MS

Old age, male sex, *ApoE*, the gene encoding for apolipoprotein E, and variations in brain-derived neurotrophic factor were evaluated as major risk factors associated with cognitive impairment among MS patients.⁴⁰ Some studies reported disability of recognizing emotions in MS patients, which were elusive, and associated it with memory impairment,⁴¹ while some other studies correlated disability of emotion recognition and perception of behavioral aspects and quality of life.⁴² Functional MRI studies have also proved that noticeable differences exist between MS patients and controls in emotion recognition, highlighting the point of disturbed brain function and cognition.^{43–45} Other manifestations that tend to associate disturbed cognition with MS include fatigue and depression, which are more common in MS than in any other neurological diseases. These symptoms are correlated in MS, assuming that they share some commonness in pathology.⁴⁶ Also, the levels of fatigue and depression were noticeably high in cognitively impaired MS patients and were evident from the measures of attention and memory, respectively.⁴⁷ Clinically, an interesting question regarding the correlation between

depression and cognition loss arises: would the treatment for depression in MS alleviate memory impairment?

Currently, no data are available, but in traumatic brain injury, patients have displayed improved cognition when treated for depression.⁴⁸ Other factors that have a correlation toward MS pathology and impaired cognition include dysregulation of the hypothalamopituitary–adrenal axis⁴⁹ and hypercortisolemia. Corticosteroids administered for MS treatment were found to worsen memory, particularly in the relapsing–remitting form of the disease.⁵⁰

Biomarker measurement

Though modest in correlating cognitive impairment in MS with MRI markers, MRI has been invaluable in the diagnosis of cognitive impairment. The identified MRI markers are T2 and T1 lesional loads, with the former showing a limited correlation, while the latter better related brain atrophy with cognitive loss.²⁰ Also, MS patients showed cholinergic imbalances; hence, measuring the levels of choline acetyltransferase and acetylcholinesterase will be ideal for managing cognitive impairment.⁵¹ In conclusion, cognitive loss is identified as an important symptom of MS that is found to significantly hamper daily activities, inclusive of social and emotional functionings. Though the exact pathology remains unclear, the inflammatory and degenerative processes observed in MS can be the major contributors of cognition impairment with a higher impact on executive functions.

Anxiety in MS

Though not much studied in MS patients, anxiety cannot be ignored as it can be an important contributor to disability in them. As per published data, many MS patients exhibit the symptoms of anxiety; in some cases, it is more frequent than depression.^{52,53}

Comparatively, the symptoms of anxiety are more prevalent in MS patients than in controls, and risk factors include female sex, lack of caring, coexisting depression, and ignorance of anxiety symptoms.⁵⁴ Animal studies have also found the association of inflammation, which is default in MS pathology, and anxiety underlying the probable occurrence of anxious symptoms in MS.²¹ Anxiety among MS patients needs strict medical attention as it was found to disrupt executive functioning. Intriguingly, cognition was severely affected than when compared to depression among the patient population studied.⁵⁵ Depression and anxiety run together in a few MS patients and importance should be given to anxiety as it may enhance suicidal tendencies in these patients compared to patients exhibiting little or no anxiety.⁵²

A cross-sectional study also observed symptoms of depression and anxiety occurring twice and thrice, respectively, among MS patients with no sex indifference for depression, while anxiety was reported more in males.⁵⁶ Another interesting feature reported was that some patients presented the symptoms of anxiety well before the diagnosis of MS.^{57,58} Considering the predominance of anxious symptoms among MS patients, attempts had been made to identify the connection of exact brain regions involved in this pathology. But still it has not been fruitful as correlation between anxious symptoms and brain MRI scans lacked correlation.²⁴ The impact of anxiety in different forms of the disease was studied and contradictory results were observed. Some studies had reported that relapses in RRMS were more often associated with anxiety and disclosed higher levels of anxious symptoms,⁵⁹ whereas studies by Noy et al⁵³ and Brown et al⁶⁰ reported the association of anxiety and relapses to be rather conflicting.

The advent of self-administered immunomodulatory drugs for MS has led to the induction of anxiety named “self-injection anxiety” and was reported to affect almost half of the MS population following this practice.⁶¹ Clinical studies have also revealed an increased prevalence of anxiety among the MS patients adding an interesting finding towards the MS associated behavioural compromise. Patients who were included in the study did not show severe neurological compromise but were certainly distressed as they were unable to cope up with the disease and get relief. Other reasons for developing anxiety were anticipated to be strong fear of developing personal and professional lagging, as the patients were emotionally preoccupied and compromised for a complete cure.⁶² In summary, it is quiet natural that people are concerned with health and become anxious, particularly, if affected with a chronic disease or a disease with no cure at all. Thus, MS, being a chronic disorder with incomplete cure, poses the symptoms of anxiety. The question to be answered in this scenario is to identify the pathologies that are common to MS and anxiety and to target them specifically.

Psychosis and its association with MS

Psychosis and MS share a common pathophysiology and the symptoms are displayed due to regional demyelination during the disease progression. The association was also exacerbated due to the medications for MS. IFN- β administered to lower autoimmunity in MS produced psychotic symptoms, while a reduction in these symptoms was noticed on administration of antipsychotic medication.⁶³ Brain MRI has also not shown any significant changes pre- and post-IFN treatment, strengthening the fact of medication-induced psychosis.⁶⁴

In a group of comparative studies among MS patients with psychosis and those devoid of psychosis, lesions were found to be more prominent in the MS psychotic patients than in those without psychosis.^{65,66} These lesions were more specific to the periventricular white matter of the temporal horn regions, and many studies have also associated psychotic symptoms in MS with lesions, substantiating the role of regional demyelination in the pathology of MS psychosis.^{67,68} There are also studies focusing on the involvement of other brain regions such as temporal, frontotemporal, and temporoparietal lesions, associating psychic symptoms in MS patients^{69,70} with temporal and frontotemporal and temporoparietal abnormalities.⁷¹ There is a growing interest to explore the exact relation between psychosis and MS pathology, as several large-scale studies have also pointed out the coexistence of them. It poses a serious challenge in terms of both treatment and diagnosis when these pathologies run together.^{72,73} Clinicians have also highlighted the point that psychic patients who do not respond to standard antipsychotic regimen should be diagnosed for the underlying MS pathology. Hence, preferably, MS needs differential diagnosis both for effective therapeutic outcome and ruling out psychotic symptoms.^{74,75}

Euphoria

Euphoria affects approximately 10% of the MS population and is best described as the phase of cheerfulness without displaying the symptoms of motor hyperactivity. It is related to the frontal lobe pathology, which is common in several other personality disorders, along with the involvement of brainstem, pontine, and periventricular lesions. Interesting correlation was drawn from a study where the association of euphoric symptoms in MS was related to severe cognitive disturbances rather than to disease exacerbations, indicating extensive brain pathology.

This study also concluded that euphoric symptoms are predisposed regardless of the sex and are mostly associated with the progressive form of MS and its severity.⁷⁶

Sleep and fatigue

As MS is associated with behavioral symptoms, it strongly influences sleep patterns and the associated disturbances. Sleep disturbances in MS patients were related to the degenerated regions involving the basal ganglia, thalamus, and limbic system.⁷⁷ It has been reported that a majority of the MS patients display symptoms of insomnia, narcolepsy, sleep-related breathing abnormalities, and rapid eye movement sleep behavior disorder, thus significantly influencing quality of

Table 1 Association of MS with behavioral disorders

Serial number	Behavioral disorder	Pathology of MS involved	Regions involved	Significance	References
1	Depression	Inflammation, pleocytosis	Arcuate fasciculus, frontotemporal region	Major behavioral disorder	Harel et al ¹⁹ McDonald and Ron ²⁰
2	Cognition	Inflammatory lesions, regional degeneration, gray matter loss, repetitive black holes	Cerebral cortex regions and thalamus	Forerunner of demyelination	Feinstein ³²
3	Anxiety	Inflammation	–	Symptoms are a predispose for MS diagnosis	Feinstein et al ⁵²
4	Psychosis	Regional demyelination	Periventricular white matter of the temporal horn region	Lesions more predominant in MS psychotic patients	Feinstein et al ⁶⁴ Honer et al ⁶⁶
5	Euphoria	Inflammatory lesions	Frontal lobe	Extensive brain pathology	Kosmidis et al ⁷⁶
6	Sleep	Neuronal loss	Basal ganglia, thalamus, limbic system	Sleep abnormalities and fatigue strongly correlate with each other	Kanyak et al ⁸⁰ Tachibana et al ⁸²
7	Fatigue	Lesions	Parietotemporal and frontal regions		

Abbreviations: MS, multiple sclerosis; -, not reported.

life.^{78,79} Clinical studies also revealed sleep disturbances in terms of onset and duration of sleep among MS patients.⁸⁰

Fatigue in MS

The other major manifestation of MS is the perception of fatigue, and multiple factors are involved in its origin, which include elevated body temperature and cytokine levels, hyperactive hypothalamic–pituitary–adrenal axis, and some medications for associated disorders like depression and spasticity.^{78,81,82} Though fatigue is quiet common among MS patients, it is incompletely understood. However, two subtypes of fatigue have been reported. These are motor fatigue, which develops due to excess muscular stress, and mental fatigue, which develops due to the intense level of tiredness. The etiology of fatigue overlaps with that of other associated disorders and hence can be considered as both primary fatigue, which is purely associated with MS, and secondary fatigue, which is related to the comorbidity.

The symptoms of fatigue mainly arise due to neuronal demyelination, which severely hampers neurotransmission. In the recent past, fatigue in MS had been related to parietotemporal and frontal region lesions.⁸³ Neurotransmitter imbalances, excessive muscular stress, lesions in other brain regions, coexisting disturbances, and medications for MS are other possibilities of fatigue generation.⁸⁴

Sleep–fatigue correlation

Interesting correlations also exist between symptoms of sleep disturbances and fatigue in MS patients. A comparative study of healthy controls and MS patients concluded that sleep abnormalities were common among MS patients and showed

a strong correlation with fatigue. However, this correlation was not observed in the healthy controls.⁸⁵ Altogether, a precise relationship has not been established between sleep and fatigue; the published data suggest an interrelation between them. Thus, sleep disturbances and fatigue either independently or collectively influence the quality of life in MS patients. The association of pathology of all the behavioral disorders with that of MS is shown in Table 1.

Behavioral screening studies for MS

Although several animal models exist for studying behavioral changes in MS, the experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein is the most widely accepted mouse model that mimics MS in humans.⁸⁶ As MS is associated with several behavioral abnormalities, animal models serve as extremely useful tools for assessing and developing novel therapeutic approaches. For example, analyzing the locomotor activities in these animal models provide useful information on the involvement of specific pathologies. A brief introduction of them is as follows:

Open field test

This test screens for spontaneous locomotor activity. In this test, animals are allowed to move and explore freely in an enclosed square area while being monitored for their locomotor activity.²¹

Grid walk test

This test provides valuable information on the formation of lesions in the dorsal column and descending motor control abnormalities by analyzing an animal's ability to place its

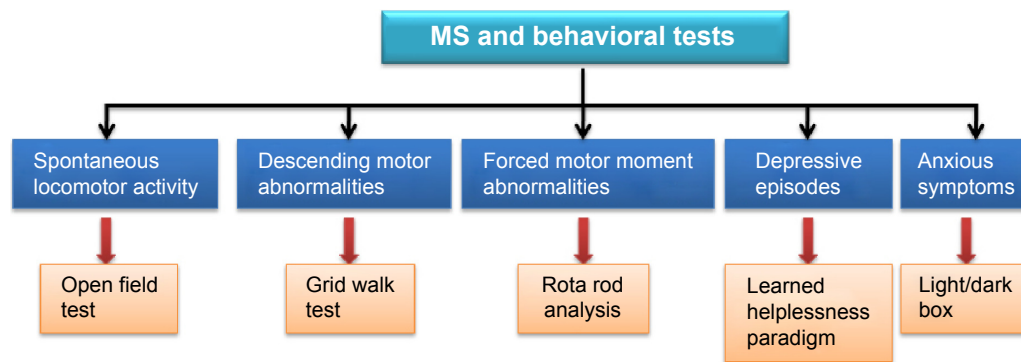


Figure 2 MS and its associated behavioral tests.

Notes: MS is associated with several behavioral abnormalities, and the studies of these disorders involve the development of animal models mimicking them. These behavioral tests can serve as useful tools for evaluating and developing effective therapeutics against common behavioral disorders in MS.

Abbreviation: MS, multiple sclerosis.

hindlimbs on bars. Animals are trained to walk on a runway consisting of bars placed at an elevation of 1 m above the ground. Comparatively, the number of falls were higher in the MS-induced animals than in the controls when they tried placing their hindlimbs firmly on the bars.⁸⁷ Other tests for assessing locomotor activities include BBB open field locomotion score and narrow beam test.⁸⁸

Rota rod analysis

This is the highly accepted test for assessing forced motor moments in rodents. Initially, animals are trained on a rotating rod at a speed of 4–40 rotations per minute and then the grip strength is analyzed by observing the animals' latency fall from the rotating rod.⁸⁶

Learned helplessness paradigm

This test mainly aims at studying the depressive episodes in animal models conducted in a customized shuttle box equipped with a shock generator and the software for controlling, processing, and recording. Initially, the animal is conditioned with a phase of inescapable foot shocks and then placed in a testing chamber, from where it can escape to a shock-free chamber.

After sufficient training, the animal is subjected to the same escapable foot shocks, but this time, a light stimulus strikes before the shock is applied. The ability of the animal to avoid the shock with each light stimulus and to escape into the shock-free chamber is tested. If the animal fails to do so, it is assumed as learned helplessness behavior.⁸⁹

Light/dark box

The light/dark box model measures anxiousness. In this model, animals (mice) are placed in a light and dark compartment, and exploratory behavior is monitored. The

analysis is done based on the length of time spent by the animals and number of crossings they make into the lighted compartment, assuming these activities to be reflective of anxious behavior.²¹ A summary of behavioral screening in MS is shown in Figure 2.

MS therapeutics and applications of nanotechnology

The strategic treatment for MS is mainly concerned to alleviate its symptoms and associated disease progression. The first-line drugs considered for the treatment of MS are IFNs, particularly type 1 and include IFN- β 1a and IFN- β 1b, along with glatiramer acetate. Their mechanism is mainly targeted against the reactive T-cell activity with a strong inhibition of T-cell proliferation, migration, and antigen presentation.⁹⁰ Interesting outcomes were seen when the first-line drugs were combined with a natural metabolite, methyl thioadenosine, that possessed potent immunomodulatory activity preclinically.⁹¹ Corticosteroids with a mechanistic activity of immune suppression were also effective against MS and its progression. Dexamethasone, prednisone, prednisolone, and methylprednisolone are some of the corticosteroids used to treat MS. However, indiscriminate and long-term use of these drugs is strictly discouraged on account of their severe side effects.⁹²

Chemotherapeutics like cyclophosphamide and mitoxantrone with a background history of immune suppression were found to be efficacious in treating MS. With respect to the mechanism of action, cyclophosphamide showed activity against T and B lymphocytes, while mitoxantrone was found to be cytotoxic.^{93,94}

Monoclonal antibodies

Monoclonal antibodies (mAbs) have made significant contributions toward MS therapeutics. For instance, natalizumab,

a humanized mAb, when tried in a patient population resulted in significant reduction in disease recurrence with an action against α -integrins (cell surface molecules essential for T-cell binding and entry). However, long-term use poses the risk of progressive multifocal leukoencephalopathy. mAbs under evaluation include alemtuzumab (Campath) and rituximab, which were observed to have a potential activity against the expression of CD52 and CD20 on leucocytes. When administered to a patient population, they resulted in a considerable reduction in the levels of inflammatory monocytes, lymphocytes, and dendritic cells.^{95,96} Daclizumab is a humanized mAb that acts against CD25 expression, thereby limiting the proliferation of reactive T-cell. This drug has recently finished its Phase II trial.⁹⁷

Drug trials

The drug in trials with a prime motto of ameliorating disease progression and severity is the oral formulation BG00012. Its active principle is fumarate. It has the characteristic mechanisms of neuroprotection, relieving inflammatory cascade and oxidative stress.

Fingolimod is one of the class of MS therapeutics with an activity of lymphocyte complexatoin. BG00012 is in Phase II clinical trials,⁹⁸ and fingolimod has recently received approval for human use.⁹⁹ Finategrast, an antagonist for the very late antigen-4 (a cell adhesion molecule expressed on brain capillary cells) has finished its Phase II clinical trial and acts by inhibiting the entry of T-lymphocytes into the BBB. Laquinimod and teriflunomide are the representatives for drugs in Phase III trials, and their mechanisms include the immune response shift toward Th2 type and inhibition of lymphocytic expansion.^{16,100} A proprietary recombinant human IFN- β 1b (NU 100) and an extended-release polyethylene glycol coated IFN- β 1a (BII-B017) are in Phase III trials for the treatment of RRMS.¹⁰¹

Applications of nanotechnology

Nanotechnology has always been fascinating as it deals with ultrasmall particles with a dimension range of 1–100 nm. Specific advantages attributed to these nanoparticles (NPs) considering their size and durability are that they offer huge surface area, improving bioavailability and absorption, and enhanced physicochemical stability of theranostics (therapeutic + diagnostics). A unique feature of this technology is that NPs can be tailor-made for specific targets, thus nullifying unwanted biodistribution and achieving the maximum efficacy at a reduced drug dose.⁹¹ The same application would also benefit drug permeation through the complex BBB, which is viewed

as a major hurdle for conventional drugs to pass through. When applied, this technology would certainly benefit the nervous system, promoting the viability and integrity of neurons that have a limited regenerative capacity. Hence, nanotechnology holds a strong future potential for theranostic applications against a variety of neurological disorders and thus overcome the limitations of existing therapeutics. The following is a brief description of NP application in MS. It is evident that matrix metalloproteinase-9 (MMP-9) plays a pivotal role in disrupting the BBB, thereby attracting peripheral T-cells and thus initiating the neuroinflammation cascade. A study has shown that the quantum dot complexed with MMP-9–small interfering RNA (nanoplex) has significantly blocked the expression of MMP-9 in brain microvascular endothelial cells and leukocytes. In addition, upregulation of extracellular matrix proteins like collagen types I, IV, and V was observed, indicating the successful delivery and efficacy of small interfering RNA nanoplex in vitro.¹⁰² Tissue inhibitor of matrix metalloproteinase-1 is a potent inhibitor of MMP-9, which is one of the main culprits associated with neuroinflammation. A study reported encapsulation of tissue inhibitor of matrix metalloproteinase-1 in poly(lactic-*co*-glycolic) acid NPs and strong neuroprotective activity in vitro by inhibiting the gelatinase-mediated MMP-9 activity.¹⁰³ Interesting results were reported showing the antioxidant activity of fullerene (C60), where C60 suspension on administration showed strong protective activity against carbon tetrachloride intoxication in rats.¹⁰⁴ This could be a potential application if tested against disorders such as MS that are associated with free radical toxicity. In an EAE model induced by the proteolipid protein PLP_{139–151} administration, significant improved tolerance was observed in mice that were administered in prior with the PLP_{139–151}-coupled poly(lactic-*co*-glycolic) acid NPs. Antigen-presenting cells internalized these NPs via scavenger receptors and induced both T-cell anergy and enhanced T-reg activity. The disease improvement was also confirmed with the vascular (AngioSense 750EX) and protease-activatable cathepsin B (Cat B 680 FAST) near-infrared fluorescence imaging agents, which showed a respective decrease in the BBB breakdown and inflammation.¹⁰⁵

The T-cell regulatory activity was also improved in an autoimmune model, wherein the authors fabricated disease-relevant self-peptides and the class I peptide–major histocompatibility complex-coated iron oxide NPs.¹⁰⁶ Thus, the antigen-specific T-reg cell expansion can be a potential approach if modified using the MS specific antigen. Similar results were also reported where gold NPs carrying 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester

and myelin oligodendrocyte glycoprotein showed significant disease remission. NP-treated dendritic cells showed a tolerogenic phenotype and induced the differentiation of T-reg cells, which significantly abrogated disease generation.¹⁰⁷ The monolayer mannoseylated liposomes packed with various immunodominant myelin basic protein (MBP) fragments showed significant reduction in the level of anti-MBP antibodies. This nanoformulation showed a synergetic therapeutic effect, wherein disease scores were reduced showing a first EAE wave inhibition followed by subsequent remission *in vivo*.¹⁰⁸ Gold NPs encapsulated with potential antioxidant compounds epigallocatechin gallate, and α -lipoic acid were found to have strong wound healing and migration along with an increase in the antioxidant activity *in vivo*. This could be a future application for evaluating against MS pathology.¹⁰⁹ Cerium oxide (CeO) NPs are regarded as potent antioxidants on account of their ability to either donate or receive electrons. It was also reported that CeO NPs showed an extremely small size of 2.9 nm with enhanced brain uptake and reduced reactive oxygen species *in vivo*. The authors claim that custom-made CeO NPs provide protection against autoimmune diseases associated with free radical toxicity.¹¹⁰ Further to this, a novel pH-sensitive IFN- β microparticle was synthesized using trimethyl-chitosan, poly(ethylene glycol)-D-methacrylate, and methacrylic acid. These microparticles showed effective drug release over 24 hours both *in vitro* and *in vivo*.¹¹¹

Neuroprotection was also made possible with the implication of fullereneol (polyhydroxylated C60), which mitigated neuronal excitotoxicity antagonizing the glutamate receptors in amyotrophic lateral sclerosis model.¹¹² This function of fullereneol also holds potential application for MS too. The implication of nanosurgery is underway and is at the threshold of revolutionizing neurological disorders. Earlier, nanosurgery was only a dream, but now the current nano tools have made the dream come true. The introduction of quantum dots for imaging, multipartite NPs for neuromodulations, and nano manipulations mediated by atomic force microscopy cantilever have all made it possible.¹¹³ A study reported the use of atomic force microscopy tip as nanoscissors for indenting the cell wall by only 1 μm , which otherwise is inevitably difficult employing conventional techniques.¹¹⁴ This kind of applications opens up new avenues for single-cell and chromosomal surgeries operating at submicron levels and would highly benefit the MS brain where damaged neurons can be successfully treated without affecting the healthy ones. NPs not only have a role in therapeutic application, but have also improvised the diagnostic strategy with enhanced sensitivity,

enabling early detection of the disease. Likewise, the titanium dioxide nanocomposite film modified with alginate coat has been immobilized with MBP, and when tested for antigen detection, it showed an astounding sensitivity of 0.18 ng/mL. Thus, NPs have improved MS diagnosis, wherein a blood sample is sufficient instead of CSF samples.¹¹⁵ MS diagnosis was even improved with the application of very small superparamagnetic iron oxide particles, which showed a characteristic accumulation in the inflammatory lesions. The very small superparamagnetic iron oxide particles were also successful in discriminating inflammatory events between control and established EAE, with clear demarcation of the central nervous system alterations preceding immune cell infiltration.¹¹⁶

Similarly, detection sensitivity was enhanced using the streptavidin antibody-conjugated superparamagnetic iron oxide particles, as they are superior contrast agents because of their T_2^* relaxation properties. These target-specific NPs were able to visualize CD3⁺ T-cell infiltrates in a murine MS model, confirming the diagnostic potential of NPs.¹¹⁷ Some recent advancements were made with the introduction of gadolinium–diethylene triamine pentaacetic acid and ultrasmall superparamagnetic particles of iron oxide (USPIO). Gadolinium–diethylene triamine pentaacetic acid is a routinely employed inflammatory marker,¹¹⁸ while USPIO can detect cellular infiltration and inflammation progression in MS.¹¹⁹ The application of stem cell therapeutics has also garnered significant attention; it was found that patients administered with autologous hematopoietic stem cells showed much improved outcome in Phase II trials.^{120,121} Further, coupling nanotechnology platforms would improve the stem cell-based theranostics. For instance, carbon nanotube substrates regulate growth and neurite regeneration, which can find potential application for neural regeneration.¹²² Intriguingly, USPIOs can label stem cells and help in tracking the fate of these cells using MRI.¹²³ Table 2 summarizes the list of nanotechnology applications in MS.

What is in the pipeline?

Agents possessing neuroprotective activity are worth considering as treatment for MS, and histone deacetylase inhibitors is one such class possessing strong anti-immune activity.¹²⁴ Inflammatory insults play a major role in the onset and progression of MS, and hence therapeutics acting against it can be invaluable. Likewise, inhibitors of midkine (a cytokine that drives T-cell traffic followed by inflammatory cascade) are under development, as they have shown a remission preclinically in an MS model.¹²⁵

Table 2 Various nanotechnology applications in MS

Serial number	Nano application	Composition	Remarks	References
1	Therapeutic	Quantum dot complexed with the MMP-9-siRNA (nanoplex)	Reduced MMP-9 expression in brain microvascular endothelial cells and leukocytes	Bonoiu et al ¹⁰²
2		Tissue inhibitor of MMP-1-loaded PLGA NPs	Inhibition of MMP-9 and inflammation	Chaturvedi et al ¹⁰³
3		PLP ₁₃₉₋₁₅₁ -coupled PLGA NPs	Disease abrogation via T-cell energy and enhanced T-reg activity	Eaton et al ¹⁰⁵
4		pMHC-coated iron oxide NPs	Disease abrogation via T-reg cell expansion	Tsai et al ¹⁰⁶
5		ITE- and MOG-loaded gold NPs	Dendritic cell-induced T-reg cell differentiation	Yeste et al ¹⁰⁷
6		Mannosylated liposomes carrying MBP fragments	Nullified MBP antibodies showing EAE abrogation	Stepanov et al ¹⁰⁸
7		Epigallocatechin gallate- and α -lipoic acid-loaded gold NPs	Strong antioxidant activity	Leu et al ¹⁰⁹
8		CeO NPs	Strong antioxidant activity	Heckman et al ¹¹⁰
9		Fullerene (C60) suspension	Strong antioxidant activity	Gharbi et al ¹⁰⁴
10		Nano-encapsulated <i>H. pylori</i>	Hypothesized for future MS therapy	Pezeshki et al ¹³⁰
11		Trimethyl chitosan-loaded IFN- β microparticles	Sustained drug release	Kondiah et al ¹¹¹
12		Fullerenol (polyhydroxylated C60)	Strong neuroprotection antagonizing glutamate receptors	Dugan et al ¹¹²
1	Surgery	Quantum dots	Best for imaging	Jeffries et al ¹¹³
2		Multipartite NPs	Best suited for neuromodulations	
3		Atomic force microscopy	Suitable for submicron level operations involving single-cell and chromosomal surgeries	Obataya et al ¹¹⁴
1	Diagnosis	MBP-coated TiO ₂ nanocomposite films	Enhanced detection sensitivity of 0.18 ng/mL	Derkus et al ¹¹⁵
		VSOP NPs	Specific accumulation and detection of inflammatory lesions	Millward et al ¹¹⁶
2		Streptavidinated CD3 ⁺ antibody-conjugated SPIO NPs	Visualization of CD3 ⁺ T-cell infiltrations	Luchetti et al ¹¹⁷
3		Gadolinium-DTPA	Routinely employed inflammatory marker	Heta et al ¹¹⁸
4	USPIO	USPIO	Detects cellular infiltration and inflammation progression	Richards et al ¹¹⁹

Abbreviations: CeO, cerium oxide; DTPA, diethylene triamine pentaacetic acid; EAE, encephalomyelitis; *H. pylori*, *Helicobacter pylori*; IFN, interferon; ITE, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester; MBP, myelin basic protein; MMP, matrix metalloproteinase; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NPs, nanoparticles; PLGA, poly(lactic-co-glycolic) acid; PLP, proteolipid protein; pMHC, class I peptide-major histocompatibility complex; siRNA, small interfering RNA; SPIO, superparamagnetic iron oxide; TiO₂, titanium dioxide; USPIO, ultrasmall superparamagnetic particles of iron oxide; VSOP, very small superparamagnetic iron oxide particles.

Physiologically, carbon monoxide has some anti-inflammatory and cytoprotective activities. Interestingly, when carbon monoxide-releasing molecules were tested preclinically in the EAE model, they were found to alleviate the pathologic disease scores, driving the need for clinical evaluation.¹²⁶ The potential immunomodulatory role of vitamin D is also being evaluated in a Phase II trial.¹²⁷ Table 3 gives a summary of the drugs included.

Future trends

As per the data published, there is commonness in the pathologies of MS and some behavioral disorders. Hence, selectively identifying and targeting the events of MS will also prove to be potential for the alleviation of comorbidities. Some of our own results are discussed here. We have adopted the principle of neuroprotection and observed anti-inflammatory effects of anti-mucosal addressin cell adhesion molecule-1 (MAdCAM-1) mAb, which protected

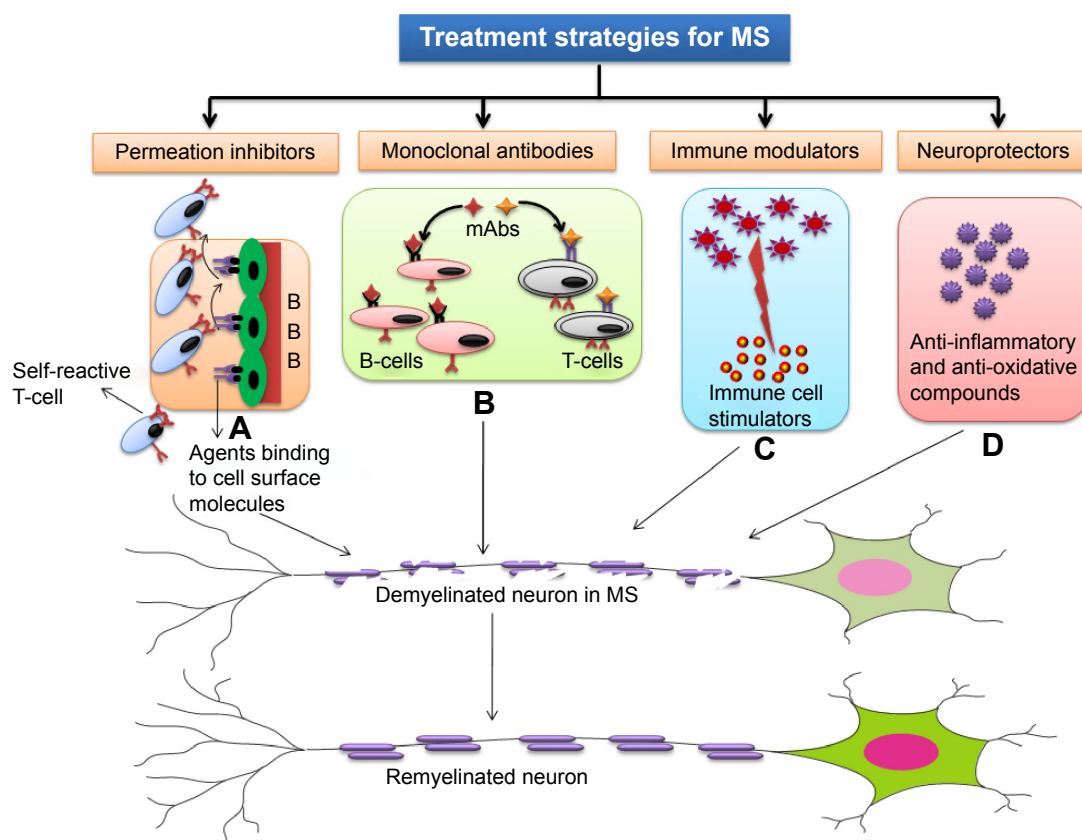
oligodendrocytes. Also, simultaneous administration of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoline and the neuroprotector glycine-proline-glutamic acid (N-terminal tripeptide of insulin-like growth factor) resulted in strong neuroprotector activity, confirmed with the significant reduction in impaired neurological scores in vivo.⁴ More rapid disease remittance was observed in mice when a combination of anti-MAdCAM-1, vascular cell adhesion molecule-1, intercellular cell adhesion molecule-1, and ligand for lymphocyte function-associated antigen integrin mAbs was administered.¹²⁸ Also, the contribution of integrin (α 4 β 7 and α E β 7)-mediated inflammation in MS was significantly remitted, administering antibodies against them.¹²⁹

Thus, after the analysis of the results, we assume that the antibodies targeting the inflammatory ligands involved in MS may also impede the inflammatory component of the depressive episodes and cognitive impairment followed by

Table 3 Current drugs in trials for MS treatment

Serial number	Drug	Mechanism of action	Level of study	References
1	IFN- β 1a, IFN- β 1b, and glatiramer acetate	Inhibition of T-cell proliferation, migration, and antigen presentation	Completed	Chourbaji et al ⁹⁰
2	Corticosteroids	Immune suppression	Completed	Moreno et al ⁹²
3	Cyclophosphamide and mitoxantrone	Immune suppression	Completed	Chan et al ⁹³ Fauci et al ⁹⁴
4	Methyl thioadenosine	Immunomodulatory activity	Preclinical	Moreno et al ⁹²
5	Natalizumab	Inhibition of T-cell migration	Phase III	Buck and Hemmer ⁹⁵
6	Alemtuzumab	Anti-CD52 and depletion of lymphocytes	Phase III	Buck and Hemmer ⁹⁵
7	Rituximab	Anti-CD20 and depletion of B-lymphocytes	Phase III	Buck and Hemmer ⁹⁵
8	Daclizumab	Anti-CD25 and depletion of reactive T-lymphocytes	Phase II completed	Hauser et al ⁹⁷
9	BG00012	Inhibition of inflammation and oxidative stress	Phase II	Saidha et al ⁹⁸
10	Fingolimod	Inhibition of T-cell entry into the brain	FDA approved	Lutterotti ⁹⁹
11	Firatergrast		Phase II completed	Buck and Hemmer ⁹⁵
12	Laquinimod	Immune response shift toward Th2 type	Phase III	Yang et al ¹⁰⁰
13	Teriflunomide	Inhibition of the lymphocytic expansion		Warnke et al ¹³¹
14	Histone deacetylase inhibitors	Anti-inflammatory and neuroprotective	Preclinical	Faraco et al ¹²⁴
15	Midkine inhibitors			Muramatsu ¹²⁵
16	Carbon monoxide-releasing molecules			Fagone et al ¹²⁶
17	Vitamin D	Immunomodulatory actions	Phase II	Dorr et al ¹²⁷

Abbreviations: FDA, US Food and Drug Administration; IFN, interferon; MS, multiple sclerosis.

**Figure 3** Strategic treatment for MS.

Notes: (A) Permeation inhibitors bind either with cell adhesion molecules present on the capillary endothelium or cell surface molecules of the reactive T-cells and prevent them from gaining access into the brain (eg, IFN- β 1a, natalizumab). (B) mAbs particularly target the T- and B-cells based on their surface markers and eventually deplete them (eg, daclizumab and rituximab target CD25 and CD20 expressed on T- and B-cells, respectively, and deplete them). (C) Immunomodulators such as corticosteroids, chemotherapeutics, and the lead compound laquinimod act against immunity by suppressing it or shift the immune response to Th2 type. (D) Neuroprotectors such as BG00012 and histone deacetylase inhibitors (under evaluation) and protect neurons from the insults of inflammation and free radical stress.

Abbreviations: IFN, interferon; mAbs, monoclonal antibodies; MS, multiple sclerosis; BBB, blood-brain barrier.

their remission. Hence, it is postulated that identifying and targeting the pathological events specific to MS can definitely prove to be beneficial for relieving the comorbid behavioral symptoms as well. This is because of the findings of commonness in the pathologies of MS and behavioral disorders. Future also holds an interesting question to answer: shall the treatments initiated for behavioral symptoms improve MS too? Figure 3 shows strategic treatment options for MS.

Conclusion

Behavioral disturbances are found to be common among MS patients, irrespective of the severity and the course of disease progression. Depression seems to be the most common neuropsychiatric abnormality observed in MS compared to any other neurological disorders. It is also associated with cognitive impairment in few of the MS patients, raising the question of commonness in their pathology. It is worthwhile to identify the early symptoms of this memory loss, as it is considered to be a forerunner of MS in a group of patients. Psychosis, euphoric symptoms, sleep disturbances, and fatigue are other influential comorbidities associated with MS that will drastically affect the quality of a patient's life. Hence, identifying disease-specific targets and coupling them with NPs will result in the effective diagnosis and treatment of the disease. This would also positively affect and help overcome the associated behavioral symptoms in MS. "The better the quality of life, the better the recovery".

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Disclosure

The authors report no conflicts of interest in this work.

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