Recent achievements in stem cell-mediated myelin repair

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Purpose of review
Following the establishment of a number of successful immunomodulatory treatments for multiple sclerosis, current research focuses on the repair of existing damage.

Recent findings
Promotion of regeneration is particularly important for demyelinated areas with degenerated or functionally impaired axons of the central nervous system’s white and gray matter. As the protection and generation of new oligodendrocytes is a key to the re-establishment of functional connections, adult oligodendrogenesis and myelin reconstitution processes are of primary interest. Moreover, understanding, supporting and promoting endogenous repair activities such as mediated by resident oligodendroglial precursor or adult neural stem cells are currently thought to be a promising approach toward the development of novel regenerative therapies.

Summary
This review summarizes recent developments and findings related to pharmacological myelin repair as well as to the modulation/application of stem cells with the aim to restore defective myelin sheaths.

Keywords
multiple sclerosis, pharmacological modulation, regeneration, remyelination, stem cells

INTRODUCTION
Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system (CNS) and is characterized by damage and loss of myelin sheaths and oligodendrocytes. As these axon-glia interactions build the structural base for accelerated nerve conduction and have furthermore been recognized to be important for axonal nutrition [1], their disturbance leads to a variety of symptoms such as visual impairment, loss of sensation and paralysis up to cognitive deficiencies. Pathophysiologically, multiple sclerosis is thought to be driven by autoimmune responses targeting mainly myelinated axons and oligodendrocytes. The underlying reasons and mechanisms are far from being understood, but vigorous neurodegenerative processes are also suspected to contribute, and certainly govern evolving permanent deficits and disability in progressive disease forms [2]. The course of multiple sclerosis varies and has traditionally been subdivided into relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS). A number of therapeutic approaches for multiple sclerosis have been identified and are currently applied mainly in the treatment of RRMS patients. These strategies include general immunomodulation/suppression, modulation of immune cell egress from lymph nodes, their penetration into brain parenchyma up to neutralization and depletion of specific immune cell types [3]. In light of these highly effective treatments currently at disposal to the neurologist, research has focused to unresolved issues such as neuroprotection and repair of demyelinated lesions. Although addressing existing damage is an ultimate therapeutic need, currently no treatments...
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KEY POINTS

- Highly effective treatments for RRMS patients are currently available.
- Unresolved multiple sclerosis issues are neuroprotection and myelin repair, and this limits management options for patients with progressive disease forms.
- Endogenous myelin remyelination activities can be observed; however, they remain inefficient.
- Remyelination therapies either aim at supporting endogenous progenitor and/or stem cell populations in successfully generating new oligodendrocytes or rely on exogenous supply of repair-mediating stem cell types.

are available, and this limits management options for patients with progressive disease forms. Multiple sclerosis brain histopathological analyses [4] and studies on immune-mediated and toxin-mediated animal models of demyelination [5] revealed that in the adult CNS endogenous regeneration activities exist. Nonetheless, particularly upon inflammation, repair efficiencies are low and tend to diminish during disease progression. Therapeutic approaches should, therefore, either address such endogenous cell populations or provide the injured CNS with repair-mediating cells from outside.

PROMOTION OF ENDOGENOUS REPAIR ACTIVITIES

Although the adult CNS is generally regarded as a regeneration incompetent organ, few repair activities can be observed, most notably the replacement of oligodendrocytes and myelin sheaths following demyelination or injury. Mature oligodendrocytes are highly vulnerable and in general degenerate due to primary insult or secondarily as a consequence of oxidative and excitotoxic stress. As these mature cells are unlikely to contribute successfully to myelin repair [6], immature cells such as resident oligodendroglial precursor cells (OPCs) [7] or adult neural stem cells (aNSCs) [8,9] jump in, become activated and are recruited in order to replace lost myelin sheaths and to restore axonal functionality. This regenerative potential is remarkable with the downside that myelin repair is also confronted with a number of limitations and in many instances remains inefficient or even fails – much alike the well-known impairment of axonal regeneration in the adult CNS [12].

For successful tissue restoration, precursor and stem cells need to be attracted to lesions where differentiation, interactions with axons as well as myelination must take place. These processes can occur only within a limited window of opportunity and suffer from the impact of numerous inhibitory components [4,13–15,16]. To improve functional recovery therapeutic approaches should, therefore, be devised by either supporting endogenous cell populations to overcome critical inhibitory impacts or by providing the inflamed or injured CNS with repair-mediating cells from outside. We here describe recent developments related to the identification of repair impediments and their biological or pharmacological neutralization. Moreover, an update is provided on recent studies on exogenous stem cell application and on how this could be translated toward more efficient myelin repair in the adult.

Fingolimod, under the trademark Gilenya, was the first oral medication approved for the treatment of RRMS [17]. This compound gained further interest as a number of preclinical studies provided evidence that apart from the effect on lymphocyte trafficking, neural cells might also benefit from sphingosine-1-phosphate receptor modulation – among them oligodendroglial survival and differentiation as well as improved remyelination [18,19]. Such findings were recently supported by the observation that also in the inflamed CNS [experimental autoimmune encephalomyelitis (EAE)], Fingolimod treatment elicited increased OPC proliferation and differentiation responses [20]. Whether this is the underlying mechanism for the observed slowing of brain atrophy in RRMS patients under Gilenya treatment (TRANSFORMS study) [21] is controversially discussed even more so as a similar reduction was not observed in PPMS patients as revealed by the INFOMS study [22].

A completely different mode of action is attributed to the monoclonal antibody BIB03 as it was specifically designed to neutralize the oligodendroglial differentiation inhibitor leucine rich repeat and Immunoglobulin-like domain-containing protein 1 (LINGO-1) [23]. Although blocking or downregulation of LINGO-1 was repetitively shown to boost OPC differentiation and to confer increased remyelination efficiencies in experimental models, it remains to be shown to what extent such an antibody can provide myelin repair in the deep brain parenchyma. Although in a current trial on the effect of BIB035 in acute optic neuritis (Clinical-Trials.gov: NCT01721161) retinal nerve fiber thickness preservation (as primary endpoint) was not affected, improved nerve conduction velocity as measured by visually evoked potential recordings was found. As this secondary outcome probably mirrors functional remyelination, results of a phase
2 study in RRMS (ClinicalTrials.gov: NCT01864148) are eagerly awaited.

GNbAC1 is a humanized antibody directed against the envelope protein (ENV) of the multiple sclerosis-associated retrovirus (MSRV) also known as Human Endogenous Retrovirus Type W (HERV-W) [24–27]. Although evolutionary acquired, this genetic element is thought to act as endogenous genes being mainly silenced but activated upon viral infections and/or in autoimmune conditions [28,29]. Initially discovered in leptomeningeal cells from multiple sclerosis patients [30], reactivated MSRV particles and the ENV protein were then detected in the serum and the cerebrospinal fluid of multiple sclerosis patients [31] and ENV was shown to act as a proinflammatory factor [32]. As the same viral protein was recently shown to induce oligodendroglial stress responses and to inhibit OPC differentiation [33], GNbAC1 can reverse this reaction [34] and raise the possibility that neutralization of HERV-W ENV might constitute yet another approach promoting remyelination. Of note, in a recent phase 2a study, this antibody was found to be well tolerated and safe [35,36,37], and currently a phase 2b clinical trial is initiated.

Semaphorins are a family of molecules initially described in the context of axonal growth cone repulsion and steering [38], but specific members such as Sema4D, Sema-3A and Sema-3F were found in multiple sclerosis tissue where they impact oligodendroglial cell survival, recruitment and differentiation [39–42]. Recent investigations have now shown that anti-Sema4D antibodies can attenuate EAE while preserving blood–brain barrier (BBB) integrity and axonal myelination, and to promote OPCs recruitment to lesion sites [43]. Another member of this family, Sema7A, might turn out to be a biomarker for monitoring multiple sclerosis disease progression based on descriptions on elevated titres in worsening disease courses [44,45]. Two further molecules previously identified in studying axonal guidance mechanisms, namely netrin-1 [46] and ephrinB3 [47], were detected in multiple sclerosis lesions and shown to limit OPC recruitment and their differentiation, respectively. These effects, with repeated demyelinating episodes, contribute to permanent demyelination failure.

On the basis of results of a clinical trial in which simvastatin was shown to reduce brain atrophy and disability in SPMS [48], statins might constitute further promising drugs in regard to the development of remyelination therapies. These findings may mechanistically reflect previous preclinical observations on statin-improved OPC survival, differentiation and remyelination [49,50]. However, despite the encouraging result of the clinical trial, it may appear awkward to use an inhibitor of cholesterol synthesis to reconstitute a membrane, whose major constituent is cholesterol.

Apart from these compounds, which had been studied for quite some time in the context of remyelination, additional pharmacological substances have recently been investigated. High-throughput screenings were conducted and revealed that, for example antimuscarinic compounds [51,52], the antifungal agent miconazole and the glucocorticoid clobetasol [53] or benztrapine [54] act as oligodendroglialogenic agents. Moreover, the NSAID indomethacin [55], histamine receptor blockers [56,57], choline metabolites [58] and the estrogen receptor β agonist indazole-Cl [59] were similarly found to exert beneficial effects to the oligodendroglial precursor cell compartment. Finally and most notably, Gli-antagonist 61 (GANT61), a specific blocker of the transcriptional regulator Gli1, was applied to mice with experimental demyelination and shown to boost adult neural stem cell-mediated remyelination [60]. In light of recent descriptions of a substantial contribution of such adult stem cells to myelin reconstitution [9], a pharmacological modulation of stem cell niche activities could open additional therapeutic avenues. A more general description of current compounds with suspected remyelination activities has been provided in a current overview article [61]. Moreover, innovative in-vitro, ex-vivo and in-vivo screening tools, which are described in the article by Stankoff et al. (pp. 286–292) in the same issue of Current Opinion of Neurology, have allowed identifying promising candidates with remyelinating efficacy.

EXOGENOUS CELL-BASED APPROACHES

Although various stem cell types are investigated in regard of multiple sclerosis tissue restoration [62], pharmacological modulation of neural stem cell activities, such as described above, is a new approach and probably because of increasing knowledge of stem cell inhibitory pathways [60,63,64]. So far, stem cells have mainly been considered in the context of transplantation and for providing either exogenous cell replacement or myelin repair via immunomodulatory or trophic activities (Fig. 1). Related to such bystander processes, the influence of mesenchymal stem cells [(MSCs); Fig. 1] on adult NSCs as well as on resident OPCs has gained much interest. Naturally, bone marrow-derived MSCs have the ability to differentiate into osteoblasts, chondrocytes and adipocytes [65] and are responsible for tissue renewal in the aged body or upon damage. Notwithstanding, MSCs can also secrete factors fostering oligodendroglial differentiation [66,67].

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and enhance remyelination in EAE animals [68]. Human umbilical cord-derived MSCs can also promote remyelination [69], and multiple intrathecal injections of autologous bone marrow MSCs into the EAE animals improved the disease score, increased the number of progenitors, diminished immune cell infiltration and reduced the area of demyelination [70]. Such observations are currently challenged in a phase 1 clinical trial assessing the intrathecal administration of autologous MSC-derived neural progenitors in multiple sclerosis patients (ClinicalTrials.gov: NCT01933802). Furthermore, a phase 2a proof-of-concept study showed an improvement of visual acuity and shortening of delayed visual-evoked response latency after intravenous infusion of autologous bone marrow MSCs in SPMS patients (ClinicalTrials.gov: NCT00395200) [71]. Both clinical trials are based on preclinical data showing immunomodulatory as well as neuroprotective effects in EAE [72,73] and are particularly important for safety reasons as available data also demonstrated possible disease worsening in CD8+ T-cell-driven myelin oligodendrocyte glycoprotein-EAE (MOG-EAE) [74]. However, it cannot be ignored that MSCs mainly exert positive immunomodulatory effects such as an impairment of T-cell trafficking across the BBB [75] and induction of neuroprotective microglia phenotypes [76]. Likewise, the influence of MSCs on oligodendroglial dynamics under non-inflammatory conditions has been controversially discussed. MSCs transplanted upon cuprizone-mediated demyelination activated oligodendrogenesis and remyelination [77,78], whereas intravenously or intranasally applied cells did not affect the CNS [79,80]. These observations clearly emphasize the need for further investigations. An overview of multiple sclerosis-related clinical trials with MSCs is provided in Table 1 [81–88].

When considering exogenous cell replacement, alternative cell types such as aNSCs or even OPCs appear to be logical sources when to be engrafted into different CNS regions (Fig. 1). Apart from subventricular zone-derived adult NSCs, which were repetitively shown to contribute to the formation of new oligodendrocytes [8,9,10], targeting hippocampal NSCs and programming them into oligodendrocytes was also described [11]. Moreover,

**Table 1.** Multiple sclerosis-related clinical trials with mesenchymal stem cells

<table>
<thead>
<tr>
<th>MSC source</th>
<th>Administration</th>
<th>Indications</th>
<th>Patients (n)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous freshly cultured bone marrow MSCs</td>
<td>IV</td>
<td>RRMS</td>
<td>9</td>
<td>[81]</td>
</tr>
<tr>
<td>Autologous culture-expanded bone marrow MSCs</td>
<td>IV and IT</td>
<td>SPMS</td>
<td>10</td>
<td>[82]</td>
</tr>
<tr>
<td>Autologous culture-expanded bone marrow MSCs</td>
<td>IV and IT</td>
<td>RRMS, SPMS and PPMS</td>
<td>15</td>
<td>[83]</td>
</tr>
<tr>
<td>Allogeneic umbilical cord MSCs</td>
<td>IV and IT (following preconditioning with CTX)</td>
<td>PPMS</td>
<td>1</td>
<td>[84]</td>
</tr>
<tr>
<td>Autologous culture-expanded bone marrow MSCs</td>
<td>IT</td>
<td>Treatment-refractory MS</td>
<td>10</td>
<td>[85]</td>
</tr>
<tr>
<td>Fresh bone marrow cells enriched for MSCs</td>
<td>IV</td>
<td>Chronic MS</td>
<td>6</td>
<td>[86]</td>
</tr>
<tr>
<td>Autologous nonexpanded adipose MSCs</td>
<td>IV</td>
<td>Treatment-refractory MS</td>
<td>3</td>
<td>[87]</td>
</tr>
<tr>
<td>Autologous culture-expanded bone marrow MSCs</td>
<td>IT</td>
<td>SPMS</td>
<td>10</td>
<td>[88]</td>
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CTX, cyclophosphamide; IT, intrathecally administered; IV, intravenously administered; MSCs, mesenchymal stem cells; PPMS, primary progressive MS; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS.
neural precursor cells are also able to modulate the immune system in EAE models when transplanted subcutaneously [89]. Not only cell differentiation must be controlled, but grafted cells need to be also successfully recruited to lesion sites. It is, therefore, of interest to note that population of inflammatory demyelinating lesions by transplanted OPCs was found to depend on cell-surface glycoprotein CD44 expression [90]. Further limitations consist of adverse astroglial differentiation of aNSCs and of the immune response directed against grafts. For instance, the modulation of chordin, microRNA-153 and Hes6 may support transplantation efficiencies of cells as they were found to regulate astrogligenic differentiation of NSCs [63,64,91–93].

For a long time, pluripotent embryonic stem cells (ESCs), with their potential to differentiate into cells of all three germ layers, were thought to be ‘saviours’ among all regenerating cell types (Fig. 1). And despite the safety and ethical issues, they are still in focus when it comes to the development of new therapies for conditions with ineffective or deficient endogenous cell repair mechanisms. It is, indeed, possible to generate OPCs from human embryonic stem cells [94] and to transplant human ESC-derived OPCs into irradiated brains [95] or spinal cords [96] for functional myelin restoration. Although this requires a deep knowledge of the mechanisms involved in pluripotent stem cell differentiation, it is still unclear to what degree such processes can be controlled properly. Moreover, ethical issues remain, basically related to their origin, that is the inner cell mass of human blastocysts.

In this context, induced pluripotent stem cells (iPSCs) [97] could represent a valuable alternative cell model (Fig. 1). Corresponding autologous transplantation schemes come with less ethical concerns and furthermore diminish rejection reactions and the need to immunosuppress recipients. However, it remains to be shown whether the potential of iPSCs is similar to the one of ESCs and whether they could fully replace them. Furthermore, important technical issues need to be solved, among them the establishment of efficient protocols for the generation of oligodendroglial cells. iPSC-dependent oligodendrogenesis was shown to include a radial glia intermediate step as revealed by the expression of paired box protein 6 followed by a transition into OLIG2-positive and NKX2.2-positive cells, massive cell proliferation and subsequent expression of myelin proteins [98]. However, the generation of human iPSC-derived oligodendroglia is time-consuming and first protocols employed 200 days of culturing before myelin markers were expressed [99]. Despite this technical drawback, such cells were nevertheless shown to generate myelin-forming oligodendrocytes upon transplantation into demyelinated lesions [100,101]. In practice, though, even a direct conversion of fibroblasts into OPCs was sufficient to allow myelin generation after engraftment into shiverer mutant mice [102]. Moreover, human iPSC-derived OPCs are also able to myelinate axons within 24 h after transplantation into injured spinal cords [103]. When comparing reprogrammed mouse skin-derived fibroblasts to mouse CNS-derived neural precursors, both cell types revealed similar differentiation capacities, tissue integration and myelin formation properties [104] highlighting the great potential of reprogrammed cells. Importantly, a substantial step toward multiple sclerosis repair was made by a proof-of-concept study demonstrating that autologous transplantation of iPSC-derived OPCs from PPMS patients might be practicable as such cells myelinated axons of shiverer mice [105]. Nonetheless, improved protocols need to be developed that assure a shorter time course. A recently published procedure indeed described oligodendroglial differentiation within 75 days [106]. Another important question to be solved is to what degree such cells retain an increased tumorigenic potential based on their modulation via oncogenic factors. Although Wang et al. [100] did not detect any tumors within a 9-month period post grafting of human iPSC-derived OPCs into myelin-deficient shiverer mice, further studies explored the underlying mechanisms of carcinoma formation [107–110]. In a similar vein, migration of transplanted cells appears to be limited but was found to be stimulated following overexpression of the polysialylated neural cell adhesion molecule concomitantly with the generation of myelin in demyelinated corpus callosum [111]. Finally, yet another advantage of engrafting reprogrammed cells has surfaced through the observation that they can participate in remyelination by promoting survival, differentiation and remyelination of endogenous oligodendroglial cells [112]. However, intracerebral transplantation raises the question of the production of a sufficient large number of cells with good manufacturing practice quality, and the neurosurgical risk.

CONCLUSION

It has been recognized that while ever more effective immunomodulatory treatments of patients with RRMS provide significant benefit, long-term improvement will depend on the generation of neuroprotective and repair therapies. A number of novel aspects related to endogenous as well as exogenous regeneration mechanisms are currently explored in detail that hold promise in reversing disability and improving patient’s quality of life.
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However, it remains to be shown to what degree the modulation of intrinsic mechanisms can be successfully applied, whether all multiple sclerosis lesions retain responsiveness to repair attempts and whether the application of exogenous repairmediating cells turns out to be superior and how they can efficiently be delivered to demyelinated areas. Moreover, in times of first clinical studies, assessing repair activities efforts must be undertaken to improve and refine imaging techniques and search for suitable biomarkers in order not to miss out on glioprotective and regenerative effects.

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Conflicts of interest
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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


In this recent article, a significant contribution of adult NSCs from the subventricular zone to remyelination was demonstrated.


A study highlighting the importance of intracellular protein shuttling and distribution in regulating oligodendroglial differentiation.


In patients with primary progressive multiple sclerosis Fingolimod/Gilenya, treatment did not slow brain atrophy.


Recent achievements in stem cell-mediated myelin repair - Jadasz et al.


35. First experimental evidence that neutralization of HERV-W/MSRV envelope proteins not only affects infection but could also rescue myelin repair processes.


37. Results on the outcome of a clinical trial for the HERV-W ENV protein neutralizing antibody GNbAC1.


45. A strong experimental example on how molecules previously identified in the Sema7A family and this occurs via Toll-like receptor 4: inference for neuroAIDS.

46. Rimondini A, Zehntner SP, Kuhmann T, et al. Statin therapy inhibits remyelina-


50. Miron VE, Rajasekharan S, Tran JP, et al. Simvastatin regulates oligoden-


55. A strong experimental example on how molecules previously identified in the Sema7A family and this occurs via Toll-like receptor 4: inference for neuroAIDS.


58. Recent achievements in stem cell-mediated myelin repair - Jadasz et al.


60. Most recent update on all current approaches investigated clinically and preclinically in the context of myelin repair. Moreover, a discussion of further diseases with white matter impact is provided.


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