1 Summary

Introduction: Amongst strategies to repair the brain, myelin repair offers genuine cause for optimism. Myelin, which ensheaths most axons in the central nervous system (CNS), is vital for normal neurological function, as demonstrated by the functional deficits that accrue when it is absent in a range of debilitating myelin diseases. Following demyelination, post-mortem and imaging studies have shown that extensive regeneration of myelin is possible in the human brain. Over recent decades preclinical research has given us a strong understanding of the biology of myelin regeneration, opening up several exciting therapeutic opportunities that are on the cusp of clinical translation.

Areas covered: This review discusses diseases that compromise the function of myelin, the endogenous capacity of the CNS to regenerate myelin, and why this sometimes fails. We then outline the extensive progress that has been made towards therapies that promote the regeneration of myelin.

Expert commentary: Finally, we provide a commentary on the first examples of these therapies to reach human patients and the evidence base that supports them, giving our opinion on where attention should be focused going forward.

1.1 Keywords

myelin, regeneration, remyelination, repair, oligodendrocyte progenitor cell, oligodendrocyte, ageing, inflammation, imaging

2 Myelin in health and disease

Myelin is formed from tightly apposed layers of lipid-rich cell membrane produced by specialised glial cells: oligodendrocytes in the central nervous system (CNS), or Schwann cells in the peripheral nervous system (PNS). These compacted layers wrap around segments of axons to act as a dielectric, preventing the radial movement of charge. The result of segmental myelination is that action potentials must jump between unmyelinated nodes of Ranvier by a highly efficient mechanism termed saltatory conduction [1]. Besides their roles in electrical conduction, oligodendrocytes are essential for axonal survival, as evidenced by axonal degeneration when oligodendrocytes are selectively ablated by diphtheria toxin [2] or in human disease where myelin lacks an important protein component [3].

The loss of myelin sheaths around intact axons is known as demyelination and is in contrast to Wallerian degeneration, where myelin degrades as a secondary response to axonal damage. Demyelinated axons have deficits in conduction [4] and are vulnerable to degeneration in the absence of trophic support [5-6]. It is thought that this axonal degeneration that follows unresolved demyelination is the primary cause of disability in progressive multiple sclerosis [7].

The development of therapies to aid myelin regeneration, or remyelination, offers hope for a range of neurological diseases. Some of these diseases result from extrinsic pathologies that damage previously healthy myelin, whilst others are the result of intrinsic deficits in the oligodendrocyte-myelin unit (Fig. 1). These exhibit wide heterogeneity in their outcomes – from complete recovery to rapid progression, secondary axonal loss and disability or death. Though it is difficult to prove causation in human disease, evidence from post-mortem studies and medical imaging, combined

with insights from animal models, suggests that the success or failure of endogenous remyelination plays an important role in the prognosis. Thus, if we can develop therapies to facilitate remyelination where it would otherwise fail, this could dramatically improve the quality of life of afflicted individuals.

2.1 Extrinsic diseases of myelin

The most prevalent causes of demyelination are autoimmune – notably multiple sclerosis (MS), which has a lifetime risk of approximately 1/200 for women and 1/400 for men [8]. In affected people, autoreactive T cells recognising myelin antigens escape tolerance mechanisms and recruit microglia and macrophages to initiate an inflammatory response in the CNS (reviewed: [9]). Based on histopathology, insights from animal models and the success of anti-inflammatory therapies in preventing relapses, we can be fairly confident that this autoimmune process drives the characteristic demyelination with eventual axonal degeneration [10-12]. This occurs in discrete lesions throughout the CNS, with the anatomical location accounting for the patient's associated symptoms and clinical signs [13-14] – often affecting motor systems, sensation, coordination or cognition.

MS can follow different clinical courses, but 85-90% of patients present with relapsing/remitting disease (reviewed: [9]), in which there is functional recovery between clinical episodes. As axonal regeneration is extremely limited within the CNS, recovery will only be possible where axons survive the demyelination and inflammation of the lesion environment. Surviving axons can regain normal conduction as the inflammation resolves, and associated conduction-inhibitory molecules such as nitric oxide diminish [15]. Restored conduction in surviving axons corresponds with remission of the disease [16], whilst axonal degeneration is a strong correlate with persistent functional deficit (reviewed: [17]).

Multiple sclerosis is a heterogeneous condition, and there is debate as to whether a number of similar diseases represent separate entities or should be considered within an MS spectrum of disorders [18]. These are all autoimmune demyelinating disorders but differ in their clinical course, pathology and anatomical involvement. However, a common feature with MS is that axonal preservation is a strong prognostic indicator. In neuromyelitis optica, for example, lesions occur preferentially in the optic nerve and spinal cord, and often with more extensive axonal injury than relapsing remitting MS, manifesting as generally more permanent deficits [19]. Even more extreme, Marburg's variant MS progresses rapidly, with widespread demyelination and axonal damage that completely overwhelm the CNS's capacity to regenerate myelin [18].

Besides these autoimmune disorders, a number of other pathological states can damage myelin. Rapid electrolyte disturbances can cause central pontine myelinolysis, classically with an iatrogenic cause due to rapid correction of hyponatraemia. It is thought that oligodendrocytes in the pons are particularly vulnerable to resultant changes in cell volume, which triggers apoptosis [20]. This is an unusual example of demyelination occurring in the relative absence of an inflammatory infiltrate, and again shows a wide range of prognostic outcomes, which can be difficult to predict. Exposure to various toxins can cause clinical demyelination, presumably by selective toxicity to oligodendrocytes. This includes carbon-monoxide which is thought to kill oligodendrocytes by hypoxia and damage myelin through lipid peroxidation [21]. The disinfectant hexachlorophene, once widely used, became heavily regulated in the 1970s after being shown to alter the ultrastructure of myelin and cause demyelination [22]. Dietary deficiency can also lead to CNS demyelination, for example of vitamin B12, which is an essential cofactor for maintaining a myelin sheath [23]. The JC virus is a direct infective cause of multifocal primary demyelination [24], whilst several other pathogens are possible triggers for autoimmune demyelination through molecular mimicry [25].

As less is known about these rarer causes of demyelination, we lack large-scale studies into the extent of remyelination and how this correlates with clinical progression. However, as for MS, severe and permanent disability is associated with axonal damage rather than demyelination in isolation [26]. Thus, there is reason to believe that enhancing myelin regeneration to improve survival of vulnerable, denuded axons could also be beneficial here.

2.2 Intrinsic diseases of myelin

Whilst all of the above conditions involve the degeneration of healthy myelin by external factors, primary demyelination can also result from genetic disorders that compromise the integrity of oligodendrocytes or the myelin they produce and maintain. Some of these directly affect protein components of the myelin sheath, for example Pelizaeus-Merzbacher disease (PMD), which is caused by a mutant form of the myelin constituent proteolipid protein (PLP) [27]. Other mutations affect cellular lipid turnover, and tend to affect oligodendrocytes due to the careful coordination required in these metabolic pathways to produce and maintain a myelin sheath [28]. Examples include the lysosomal storage disorders (such as Krabbe's disease, Tay-Sachs disease and metachromatic leukodystrophy) as well as extra-lysosomal errors in lipid metabolism (such as adrenoleukodystrophy and Canavan's disease). These generally present in childhood, as myelination is defective throughout development.

As the machinery for producing new myelin is defective in these intrinsic pathologies of the oligodendrocyte-myelin unit, enhancing endogenous remyelination is considered less of a therapeutic goal here. Instead, a cell replacement approach would likely be more beneficial, where genetically functional cells are introduced into the CNS to form new oligodendrocytes and healthy myelin sheaths. Conversely, where demyelination is caused by external factors, transplanting exogenous oligodendrocyte-forming cells may be a fruitless exercise without first confronting the environmental drivers of demyelination and blockades to remyelination [29].

2.3 Current therapies for myelin disease

Supportive therapies can bring about great improvements in quality of life for a patient suffering from a demyelinating disease. These might include, for example, managing pain and urinary symptoms as well as providing appropriate physiotherapy, occupational therapy and social support. However, these measures do nothing to alter the course of the disease, prolong life and prevent further disability. Having established that axonal preservation is vital for a good prognosis, a disease-modifying treatment for demyelinating disease could act through any of the following mechanisms:

- inhibit the pathological processes causing myelin damage
- enhance remyelination to prevent axonal degeneration by either:
  - promoting endogenous remyelination
  - introducing exogenous remyelinating cells
- promote axonal survival by other means.

The past few decades have witnessed major advancements in addressing the first of these approaches. A range of drugs have now shown efficacy in reducing demyelination in relapsing-remitting MS (summary table: [9]). These include interferon-β and glatiramer acetate, which can reduce relapse rate and the development of new lesions in relapsing-remitting MS. More aggressive biological agents have higher efficacy at reducing relapse rate and accumulation of disability, though with associated side-effects [12]. Examples are alemtuzumab, which blocks CD52 to deplete T cells, and natalizumab, which blocks the α-4 integrin necessary for T cell entry to the CNS. As for osmotic

demyelination, the careful management and slow correction of hyponatraemia can prevent central pontine myelinolysis from developing iatrogenically. In the paediatric cases where demyelination arises from intrinsic oligodendrocyte deficits, the possibility of reducing myelin damage is very limited, but may eventually be possible using gene therapy to correct genomic mutations.

However effective some of these treatments are, there are also substantial limitations. Notably, once the damage to myelin has been done, they cannot alter the subsequent neurological outcomes that result from axonal degeneration in the absence of a myelin sheath. Whilst current MS therapies can successfully reduce relapses and even delay conversion to secondary progressive MS [30], little can be done once the progressive phase is reached. Thus, these therapies would ideally be combined with approaches that protect demyelinated axons, preventing entry into a progressive phase in which demyelination-inhibiting drugs become ineffective (Fig. 2).

The mechanisms by which axons become damaged following demyelination are poorly understood [31], but likely involve a combination of increased energy demands in the absence of saltatory conduction [32], redistribution of ion-channels resulting in neurotoxic levels of calcium [33] and impaired oligodendrocyte-axonal shuttling of metabolites such as lactate [6,34]. Given that we have a built-in mechanism to regenerate myelin and address the root cause of these changes, enhancing this process seems the most obvious approach to maximise axonal survival. Remyelination is the default response to demyelination [35], so discovering what causes it to fail and how to overcome these constraints are key to this line of enquiry.

2.4 Remyelination

Remyelination involves the reinstatement of myelin around intact axons that have lost their sheaths by primary demyelination [29]. In the CNS, this process is performed by newly generated oligodendrocytes that derive from a pool of oligodendrocyte progenitor cells (OPCs) following a demyelinating insult. OPCs are present throughout both grey and white matter in the CNS and exhibit features typical of adult stem cells such as self-renewal and multipotency. In response to demyelination, OPCs proliferate and migrate to the lesion site [36-37] where they differentiate to mature oligodendrocytes, extending processes to remyelinate denuded axons [38]. Consequently, saltatory conduction and function are restored [39-40] and axons are protected from degeneration [41]. The regenerated sheaths never attain the full thickness of those in unlesioned white matter, with expected implications for axonal velocity, though whether this limits the function of neuronal circuits is still unclear. Myelin sheaths generated in adulthood outside an injury context have a similar morphology to remyelination [42], and this could depend in part on the dynamics of the axon being (re)myelinated [35].

Schwann cells, which normally myelinate axons in the peripheral nervous system, can also contribute to remyelination in the CNS. This phenomenon has been observed in human disease [43] as well as animal models [44]. Whilst these Schwann cells were long assumed to migrate from the periphery after blood-brain barrier (BBB) damage [45-46], more recent lineage tracing studies have shown that many CNS-remyelinating Schwann cells in fact differentiate from OPCs [38].

Remyelination can be extensive in MS [47] and is vital for restoring the trophic support to axons to ensure their long-term survival. Whilst axons in lesions that remain chronically demyelinated continue to degenerate, they are spared in lesions where remyelination has taken place [48], though interpretation is difficult as it may instead be that remyelination cannot occur where axons have already begun to degenerate. A more recent study, integrating observations from the cuprizone model and MS lesions, suggested that even acutely damaged axons can be rescued by remyelination [49]. Whilst these findings are important, it is difficult to extrapolate from post-mortem snapshots

into the temporal profiles of demyelination/remyelination in humans. New imaging strategies such as myelin-binding PET ligands can give a lower resolution insight into remyelination in real time, and similarly suggests a correlation with clinical disability [50].

A relapsing-remitting pattern of symptoms is common early in MS, but many of these patients enter a secondary progressive phase in which functional deficits persist and accumulate (reviewed: [9]). Post-mortem tissue has shown that remyelination becomes less extensive later in the disease [51] and the failure of myelin regeneration, with its associated axonal degeneration, are thought to underpin the progressive phase [52].

Remyelination is a complex process, in which failure can occur at different stages. Firstly, there is heterogeneity in the capacity of MS lesions to become repopulated with myelin-producing cells. In an analysis of 56 post mortem cases, two distinct groups were identified based on whether or not oligodendrocyte lineage cells reappeared following demyelination [53]. Furthermore, there was variable expression of the terminally differentiated oligodendrocyte marker MOG (myelin oligodendrocyte glycoprotein), which suggested that, whilst some lesions were unable to recruit OPCs, others contained cells of the oligodendrocyte lineage that were unable to fully differentiate [53]. The degree of ongoing inflammation is an important factor in these different stages of remyelination failure, as active lesions generally have better OPC recruitment than chronic inactive lesions [54].

One of the strongest brakes on remyelination is ageing, a general determinant of regenerative responses throughout the body. Ageing brings about changes in OPCs [55] and their environmental signals [56], both of which negatively impact remyelination. Clinical data has shown that regardless of the initial presentation of MS, each disability milestone is reached at a consistent age, suggesting an age-dependent process is driving progression of the disease [57]. As most situations where we want to enhance endogenous remyelination occur in the adult (and often ageing) CNS, remyelination is likely to be sub-optimal with considerable scope for improvement.

3 Strategies for enhancing myelin regeneration

Prospective therapies to enhance myelin regeneration centre upon one of two strategies, depending on the disease process. For “extrinsic” diseases, in which healthy myelin is damaged by external factors, the focus is on enhancing the capacity for endogenous remyelination. This may be achieved by targeting OPCs themselves or the cues they will sense in the lesion environment. In contrast, for “intrinsic” diseases in which oligodendrocytes cannot produce healthy myelin, the transplant of healthy myelinating cells offers more promise.

3.1 Enhancing endogenous OPC responses directly

For effective remyelination, OPCs must complete three processes: activation, recruitment (which involves both OPC migration and proliferation) and differentiation. In response to injury, OPCs first become activated from a quiescent to a regenerative phenotype [58]. This is defined by morphological change as well as up regulation of genes such as Olig2, Nkx2.2, Sox2 which are transcription factors associated with the generation of oligodendrocytes during development [59-60]. The stimulus for the switch involves injury-induced changes in surrounding microglia and astrocytes. These cells produce factors such as PDGF and FGF [61] that contribute to the rapid proliferation of OPCs [62].

Once activated, OPCs migrate to the lesion site guided by expression of semaphorins, which are important regulators of migration. Two semaphorins, 3F and 3A, have been identified in developmental studies as providing guidance cues, the former positively accelerating migration and the latter impairing OPC recruitment and remyelination [63].

Finally, the recruited OPCs must differentiate into mature oligodendrocytes. These cells can then establish contact with demyelinated axons, express myelin genes and generate the myelin membrane which wraps and compacts to form a sheath around the axon. There are many similarities between development and repair in the differentiation of OPCs into myelinating oligodendrocytes [64]. This phase of remyelination appears to be particularly vulnerable to failure (discussed: [35]) and there is considerable interest in targeting regulators of OPC differentiation. Some of the approaches closest to translation are inhibition of Lingo1, activation of RXRγ signalling and modulation of the Wnt pathway (Fig. 3). Additionally, the advent of high throughput screening has revealed a wealth of previously unconsidered drugs and mechanisms.

3.1.1 Lingo-1

Lingo-1, a component of the trimolecular Nogo receptor is a negative regulator of differentiation in development [65]. It is up-regulated in CNS diseases and injury, as seen in a number of animal models and in human MS lesions [66]. In a non-immune, toxin-induced model of demyelination in rats, Lingo-1 antagonists promote OPC differentiation and endogenous remyelination [67], and treatment of cultured OPCs with small interference RNAs against Lingo-1 and soluble human Lingo-1 increased differentiation. Mice treated with an antibody against Lingo-1 or deficient in Lingo-1, showed increased remyelination and functional recovery in an experimental autoimmune encephalomyelitis (EAE) model [68], although exactly which cell type within the lesions is primarily affected is not certain [66]. Its specific expression in the CNS makes it a good target for drug mediated inhibition with potential for improving myelin regeneration.

Of the drugs shown to enhance remyelination in animal models, opicinumab, a humanised monoclonal antibody targeting the LINGO-1 protein, has generated the most information in phase II clinical trials. Preliminary results suggest some clinical efficacy in acute optic neuritis (ClinicalTrials.gov Identifier: NCT01721161) but the end points have been missed in the SYNERGY trial for patients with relapsing-remitting MS (ClinicalTrials.gov Identifier: NCT01864148).

3.1.2 Wnt signalling

The canonical Wnt pathway is a negative regulator of oligodendrocyte differentiation in both development and remyelination [66]. Constitutive induction of Wnt signalling in OPCs using transgenic mice that express a dominant active β-catenin gene results in ataxia and tremor within a week after birth due to a delay in oligodendrocyte differentiation and myelination [69]. This effect is, however, transient and CNS myelination is normal in adult mice. Experimental demyelination in these transgenic mice shows delayed differentiation and remyelination without affecting recruitment. However, the effect of Wnt signalling can depend on the differentiation stage of the cell. Fancy et al. [70] subsequently showed that a low Wnt tone was needed for the transition of OPCs to immature oligodendrocytes whilst pathologically high Wnt tone inhibited differentiation.

Wnt pathway inhibitors used in cancer therapy [71] will be a good starting point for developing drugs that modulate this pathway to stimulate OPC differentiation and improve remyelination. However, Wnt is an example of a pathway that is context dependent, with diverse modulatory effects that present challenges for developing therapeutic interventions.

3.1.3 Retinoid X Receptors

Retinoid X receptors (RXRs) are nuclear receptors that have been identified as positive regulators of OPC differentiation from analysis of remyelinating tissue [72]. There are three members in the RXR family, RXRα, RXRβ and RXRγ, all of which are expressed in CNS lesions, but RXRγ was seen to have increased expression during the differentiation phase of remyelination. RXRγ forms homodimers or heterodimers with other nuclear receptors, including retinoic acid receptors, thyroid hormone receptors, vitamin D receptors, peroxisome proliferator activator proteins, and liver X receptors, to control transcription of target genes [73]. A functional role in differentiation was demonstrated by a failure of recruited OPCs in focal demyelination lesions to undergo differentiation in RXRγ knockout mice [72]. Of the nuclear receptors they dimerise with, the vitamin D receptor has recently been shown to be an important factor in the control of OPC differentiation [73].

Chemical agonists and antagonists of RXR signalling or retinoids are widely available and are used in the treatment of cancer and metabolic disorders [74]. These have been used to modulate OPC differentiation *in vitro*, and the agonist 9-cis-retinoic acid (9cRA) can accelerate remyelination in aged rats [72]. This is complemented by a beneficial effect of 9cRA on macrophage responses through RXRα [75], meaning rexinoids show much promise as myelin regenerative therapeutics. Bexarotene, an RXR agonist is currently in a phase IIa clinical trial (ISRCTN14265371).

3.1.4 High-throughput screening candidates

The development of high-throughput screens has identified several OPC-modulating candidates in recent years. Many of these are already FDA-approved for other uses, which would accelerate any subsequent clinical trials. Commonly, immunopositivity of myelin proteins, such as MBP, is quantified to find drugs that promote OPC differentiation. A primary OPC culture model was used to identify benztropine in this manner, which was subsequently shown to enhance remyelination *in vivo*, likely via muscarinic receptor antagonism [76]. Another approach involved screening a library of molecules on pluripotent epiblast stem cell-derived OPCs, yielding miconazole and clobetasol as possible remyelinating agents, with additional validation *in vivo* [77].

To further interrogate OPC-modulating compounds, more functional assays have been developed. These include the micropillar array [78], in which mature oligodendrocytes wrap conical pillars with concentric rings of myelin. This assay led to clemastine, a drug with both antimuscarinic and antihistamine properties approved primarily for symptomatic treatment of allergies. It has been shown to promote remyelination *in vivo* after lysolecithin-induced demyelination [79]. Experiments suggest clemastine has this effect by inhibiting the M1 muscarinic receptor, and oligodendroglial-specific genetic ablation of M1 receptors showed accelerated remyelination and functional recovery in EAE [79]. Clemastine is also in a phase II clinical trial for use in MS (ClinicalTrials.gov Identifier: NCT02040298), from which preliminary results have shown an increase in the measured optic nerve conduction, but without a significant improvement in vision [80].

3.2 Enhancing OPC responses by optimising the lesion environment

The activity in many of these intracellular OPC pathways will be directed by the chemical and physical properties of the lesion environment. Like all stem cells, OPCs are maintained in a niche, which will be drastically altered by disease [81-82]. Following demyelination, this coordinated response involves other glial cells, blood vessels, pericytes, the extracellular matrix and infiltrating cells and molecules from the periphery.

3.2.1 The innate immune system

Whilst the immune system can drive demyelination through autoimmune disease (reviewed: [9]), components of the immune response are also necessary to prepare the lesion environment for remyelination. In particular, microglia (the CNS-resident macrophages) and infiltrating monocyte-derived macrophages can phagocytose debris from the disintegrating myelin sheaths and secrete pro-regenerative factors to drive the OPC response [83]. Myelin debris inhibits OPC differentiation *in vitro* [84] and *in vivo* it was shown that remyelination is impaired if a lesion is supplemented with additional myelin debris [85]. As human monocytes from older subjects (>55) are less capable of phagocytosing myelin debris, and compounds such as 9cRA can restore phagocytosis in aged murine macrophages [75], this is a means by which the lesion environment could eventually be optimised therapeutically.

Macrophages also secrete a range of molecular signals into the lesion environment, and this is disturbed with ageing [56,86]. Experiments using microglia-conditioned media have shown that these secreted factors can modulate the OPC response, and this depends on the phenotype of the conditioning microglia [87]. Some of these molecules are classical growth factors, including insulin-like growth factor (IGF-1) and transforming growth factor (TGF-β). Both have long been known to promote OPC differentiation *in vitro* [88-89] and their expression is delayed in the slow remyelination of old rats [86]. More recently, transcriptional profiling of the OPC retinal transplant model identified endothelin-2 as a factor promoting remyelination [90-91], and activin-A was found to be essential for the pro-differentiation effects of microglia-conditioned media [87].

The ability of circulating immune cells to influence the lesion environment is exciting from a clinical perspective, as these cells are peripherally accessible and hone to sites of injury, easily passing across the BBB. Heterochronic parabiosis experiments have shown that remyelination in an old mouse can be rejuvenated by exposure to, *inter alia*, the monocytes of a young parabiotic partner [92]. Follow-up studies have shown that relatively few monocytes from a young partner are required to direct a more pro-regenerative response amongst microglia and macrophages in an aged CNS [87,92]. There is a growing appreciation that other peripherally-derived immune cells can also contribute to remyelination, notably Treg cells, which can enhance OPC differentiation by secretion of the growth-regulatory protein CCN3 [93]. This involvement of the adaptive immune system exemplifies how myelin repair strategies must consider how the immune system can both drive myelin pathology and contribute to its repair.

3.2.2 Astrocytes

Astrocytes are another important component of the OPC's environment. This glial population is not traditionally considered part of the immune system, but does have important roles in responding to myelin damage and coordinating the OPC response. Like microglia, astrocytes express innate pattern-recognition receptors that allows them to sense and respond to injury [94] and secrete factors that influence OPCs [95-96]. Inappropriate astrocyte responses can also contribute to damage in autoimmune demyelination [95]. One intriguing role of astrocytes seems to be in determining fate choice of OPCs, which tend to form Schwann cells rather than oligodendrocytes in regions devoid of astrocytes [43-44,97]. The clinical relevance of this is not yet clear, as it remains to be seen whether Schwann cells can meet the complex trophic needs of a CNS axon to prevent degeneration [29].

3.2.3 Other environmental cues

There are a number of other cues in the lesion environment that are important to consider. The extracellular matrix (ECM) undergoes changes after injury that are counterproductive to remyelination [98], making the ECM a potential therapeutic target. OPCs are sensitive to mechanical as well as chemical properties of the ECM [99,100]. Another cue comes from the axons themselves, which can modulate OPC responses both through electrical currents [101] and activity-dependent release of neurotransmitters [102-103]. Finally, angiogenesis is an important step in any regenerative process, allowing delivery of nutrients and peripherally derived cells and signals [104]. Lesions of aged mice exhibit reduced angiogenesis, which is restored by heterochronic parabiosis [92]. Associated with the vasculature are pericytes, which have recently been identified as another important cellular source of factors to promote OPC differentiation [105].

Thus, OPCs respond to demyelination in the context of a complex environment of cellular, molecular and physical cues. As we expand our comprehension of these interactions, we are beginning to understand the important determinants of successful remyelination. In addition to knowing which cues are most important, it is likely that the timing of onset and offset will also be vital to consider. A cause for remyelination failure may well be discoordination in the timing of these influences on OPCs [106].

3.3 Cell transplant therapies

Where genetic abnormalities impair oligodendrocyte function, replacing these from the defective host OPC pool will not be a useful strategy for myelin regeneration, be it by targeting OPCs directly or indirectly through the lesion environment. For these diseases, cell transplant therapies offer more promise, and to offer any success, large numbers of progenitors biased to oligodendrocyte differentiation and myelinogenesis are required.

Several different approaches have been taken to generate human OPCs. These have employed a range of sources, including foetal and adult human tissue [107-110], embryonic stem cells [111-112], and induced pluripotential stem cells (iPSCs)[113], with varying degrees of success. Whilst each of these sources have demonstrated the potential to generate myelinogenic oligodendrocytes, each has its own strengths and weaknesses as a cellular therapeutic [113]. More recent developments in the generation of iPSC-derived OPCs have demonstrated that the induction of three transcription factors (Sox10, Olig2, Nkx6.2) is sufficient to rapidly generate O4+ oligodendrocytes with an efficiency of up to 70% in 28 days and a transcriptome comparable to primary human oligodendrocytes [114]. In addition, OPCs have been recently generated directly from murine fibroblasts [115,116]. Whilst this success has yet to be replicated in humans, it demonstrates promise of another potential therapeutic source [117].

Regardless of their derivation, to be practical, safe, and effective, OPCs must be deliverable with a uniform myelinogenic phenotype, in both reliable purity and significant quantity [29]. This is a particular concern for oligodendrocytes derived from human ES cells (ESCs). Whilst these demonstrate similar regeneration properties to those described above [118], the persistence of undifferentiated ES cells in the donor pool introduces a risk of teratomas or neuroepithelial tumors after implantation [119,120]. With this in mind, more recent advances in optimising the methods for generating OPCs from human iPSCs and ESCs have led to the production of highly enriched populations of human OPCs that appear efficient at myelinogenesis *in vivo*, while manifesting no evidence of tumorigenic potential [113].

One exception to the concept that vast numbers of transplanted cells are required to induce a clinical effect is the lysosomal storage disorders [121]. Because wild-type lysosomal enzymes may be released by integrated donor cells, and taken up by deficient host cells through the mannose-6-

phosphate receptor pathway [122] a relatively small number are required to induce a correction [123-124]. The intracerebral delivery of OPCs would thus seem an especially attractive method for treating those demyelinating diseases associated with enzyme deficiencies specific to brain.
In addition to these approaches, the transplant of non myelinogenic cells has had some success in treating genetic myelin disorders. For over 30 years allogenic haematopoietic stem cell transplantation (HSCT) has been used to slow the progression of metachromatic leukodystrophy (MLD) [125]. The mechanistic basis for this is thought to involve introduction of the deficient enzyme arylsulfatase-A into the CNS by bone-marrow derived mononuclear phagocytes, thus slowing the toxic accumulation of sulfatide substrates in oligodendrocytes [126]. Remyelination by endogenous OPCs may thus have a role to play if this key metabolic function is successfully compensated by other cell types.

4 Expert commentary

With new therapies in the pipeline that target OPCs directly, modulate the lesion environment or transplant myelinating cells, we are certainly entering a very exciting era for remyelination. Half a century on from the first descriptions of remyelination [127], we are beginning to see clinical trials of remyelination therapies for demyelinating disease.

However, there is still much work to be done. Preliminary results from the first clinical trials of opicinumab and clemastine have been somewhat disappointing (ClinicalTrials.gov Identifiers: NCT01721161 and NCT02040298). Whilst both these drugs have enhanced the speed of optic nerve conduction (a functional readout of myelination), they have done little symptomatically for patients. These negative results are likely a necessary step towards developing successful therapies for remyelination. We know that MS is a heterogeneous condition, and early trials like this will help to identify the hallmarks of patients who will respond best to a certain type of therapy. Even with a patient-targeted therapy, there are many more degrees of freedom: at what stage of the disease should a remyelination-promoting drug be initiated, for how long should it be given, and how does it combine with other MS drugs? The early clinical trials are a chance to ask these important questions.

In parallel with drugs to promote endogenous remyelination, cell transplant therapies for myelin regeneration have now progressed into early clinical trials. Neural stem cells were engrafted into the forebrain white matter of four young patients with PMD, with magnetic resonance imaging (MRI) changes suggesting the donor cells successfully contributed to myelination after one year [128]. A longer-term follow-up of this cohort (ClinicalTrials.gov Identifier: NCT01391637) will soon tell us more about the efficacy of this treatment and the prospects for larger-scale trials, which to an extent are limited by the scarcity of patients with this disease [129].

The ability to monitor the success of these novel therapies in humans depend upon simultaneous advances in medical imaging. MRI has long been used for diagnostic purposes in MS, where the number and location of lesions and dissemination with time form the commonly used McDonald diagnostic criteria. However, the prognostic value of traditional MRI is limited, particularly for progressive MS, as subtle changes in myelin are difficult to distinguish from other tissue changes such as oedema or inflammation [130]. Thus, monitoring demyelinating disease in humans is also an important area to address.

The combination of clinical trials with advances in CNS imaging techniques should soon give us an answer to the most fundamental question of all: does remyelination benefit patients? Whilst this is a widespread assumption, the evidence to date is arguably rather circumstantial:

- **Demyelination predisposes axons to degeneration, which correlates with disability.** In MS lesions, demyelination is accompanied by substantial axonal degeneration [131]. In mice with genetically-ablated oligodendrocytes, axonal degeneration is a consequence of demyelination independent of the extensive inflammation that confounds many other models [2]. Axonal degeneration is also a feature of the paediatric disease PMD, in which myelin lacks the protein PLP [3]. Disability is associated with the extent of axonal loss in post-mortem studies of MS patients [7].

- **Remyelination protects axons from degeneration.** When cuprizone-treated mice were depleted of endogenous OPCs, prompt remyelination by transplanted neural progenitor cell-derived oligodendrocytes protected axons from degeneration [41]. In post-mortem MS lesions, areas of remyelination are associated with axonal preservation [48].

- **Successful remyelination is associated with good neurological outcome.** Remyelination leads to neurological recovery in pregnant cats, that suffer demyelination following administration of an irradiated diet [40]. A novel PET technique to image myelin in living people suggests remyelination inversely correlates with clinical disability [50].

The current evidence base in favour of remyelination therefore consists largely of interventional studies in animal models of human disease, alongside correlations drawn from human post-mortem tissue and more recently imaging. Unequivocal evidence that remyelination benefits humans has proved elusive with these observational studies, and some argue that other approaches could yield more effective therapies. For example, inflammation in the CNS can cause direct damage to axons, besides removing their myelin – including direct T cell-mediated cytotoxicity [132-133] and the production of reactive oxygen and nitrogen species [134]. This direct neurotoxic effect of inflammation will likely be compounded by the loss of a protective myelin sheath. An argument has also been made that MS could be a primary neurodegenerative disease with inflammatory demyelination being a secondary feature [17].

Thus alongside efforts to enhance remyelination, there is much interest in strategies that could limit the initial damage and in other means to promote axonal survival (Fig. 2). The gaps in the current evidence base are becoming ever smaller as we obtain functional data from patients treated with remyelination-promoting therapies and develop high-resolution imaging tools to monitor this in real time. Consequently, we will soon have a clearer insight into how remyelination can benefit people with these diseases, and how to take these therapies forward.

5 Five-year view

The next five years will see the drugs of today's early clinical trials tested in different clinical applications, whilst some promising pre-clinical candidates get closer to therapeutic use. We can also expect to see an expansion of imaging techniques and other clinical parameters used to monitor their efficacy in human patients. Meanwhile, as basic science research continues, we will learn of more nuanced approaches to modulates OPCs and their environment, with implications for myelin regeneration and broader CNS pathology.

5.1 New treatments for myelin regeneration

Whilst opicinumab, clemastine and bexarotene have progressed furthest in terms of clinical translation, a number of other therapies are close behind, some of which will advance through clinical trials themselves in the next five years. Progress will be most rapid where compounds licensed for other clinical uses are found to have efficacy in enhancing remyelination, exploiting

similarities between OPC signalling and the pathways involved in applications such as cancer or the immune system. This has been seen already in the accelerated progression of bexarotene, previously licensed for T cell lymphoma [135], and clemastine, which can be purchased over-the-counter as an antihistamine. Similarly, licensed compounds have been identified that enhance the pro-remyelination functions of macrophages, such as amphotericin B and macrophage colony-stimulating factor, which in combination promote remyelination in mice [136]. The development of high throughput functional assays such as the micropillar array [78] has revolutionised our ability to give old drugs a new lease of life in myelin regeneration.

5.2 New techniques to monitor myelin regeneration in humans

Newer MRI techniques show promise for more precise monitoring of myelin diseases than traditional approaches. These include using the magnetisation transfer ratio (MTR), a parameter that describes interactions between water and macromolecules of myelin [137]. The MTR has been shown in animal models to decrease with acute demyelination and to increase in remyelination [138]. This has proved a more sensitive and specific means to observe myelin, with some correlation to remyelination as seen in the brain of a patient who died shortly after imaging [139]. However, it is still not considered a reliable metric for remyelination in human patients [140].

Another promising approach is positron emission tomography (PET), which can be used with specific radioligand compounds to visualise myelin. 1,4-bis(p-aminostyryl)-2-methoxy benzene (BMB) and Pittsburgh compound B (PiB) are two such compounds that cross the BBB and bind to myelin in a dose dependent and reversible manner [141-142]. PiB is being used to study Alzheimer's disease in humans [143] and has potential as a brain myelin imaging technique. However, the technology remains limited as an imaging modality by cost, access and low resolution [143]. Improvements in both MRI and PET techniques over the next five years will be a necessary part of taking myelin repair strategies from preclinical models to clinical trials.

The anatomical, functional and histopathological heterogeneity of lesions in MS can also complicate comparisons in clinical studies. One of the most useful sites for monitoring remyelination is the optic nerve, which becomes demyelinated in optic neuritis, a common presentation of relapsing-remitting MS. These lesions uniquely allow *in vivo* correlation between clinical signs and symptoms (acuity, visual fields and colour vision), electrophysiological consequences (measured through visual evoked potentials) and imaging (using optic nerve MTR). Together, these parameters have formed some of the key metrics of the early myelin repair clinical trials for opicinumab (ClinicalTrials.gov Identifier: NCT01864148) and clemastine (NCT02040298). Future advances in myelin imaging and electrophysiology may facilitate similar comparisons elsewhere in the CNS for a more holistic approach to monitoring therapies.

5.3 New targets for myelin regeneration

Although diseases of primary demyelination are the obvious clinical settings for myelin regeneration, these therapies may find wider application in other CNS pathologies. Myelin dysfunction has now been implicated as a component of various diseases long assumed to be limited to neurons and their circuits [144,145]. Windrem et al. [146] provided compelling evidence that glia have a primary role to play in schizophrenia. They transplanted schizophrenia-derived glial progenitors from humans into newborn mice, which subsequently developed with normal mouse neurons surrounded by "schizophrenic" glia. These exhibited decreased myelination and abnormally shaped astrocytes, as revealing signs of "schizophrenic" behavior. Myelin dysfunction is also seen in neurodegenerative diseases such as amyotrophic lateral sclerosis [147], Huntington's disease [148] and Alzheimer's disease [149], which may eventually benefit from myelin regenerative strategies, albeit more likely a longer-term goal.

6 Key issues

To progress towards this “five-year view”, there are several key areas to address:

- **Studying myelin pathology.** Furthering our knowledge of the natural history and heterogeneity of lesions in MS and other diseases will help us to target appropriate therapies to patients who will benefit most.

- **Medical imaging techniques.** This will be necessary to help stratify patients for therapies, as well as monitor therapeutic success in clinical trials.

- **Understanding OPC biology.** Whilst our understanding of OPC biology has expanded rapidly in recent years, further fundamental research is necessary to bring more variety of therapies to clinical trials. OPCs are a relatively recently discovered population of stem cell and, compared to the intestinal crypt or the bone marrow, there is much to be learned about their intracellular pathways, their niche during health and disease, and how they are influenced by ageing and other systemic phenomena.

Addressing these issues will require a concerted effort by clinicians, biologists and medical imaging scientists. However, as the first clinical trials begin, and with several other therapies close behind, we are in sight of myelin regeneration therapies becoming a clinical reality.

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