# Glia Disease and Repair—Remyelination

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The inability of the mammalian central nervous system (CNS) to undergo spontaneous regeneration has long been regarded as a central tenet of neurobiology. However, although this is largely true of the neuronal elements of the adult mammalian CNS, save for discrete populations of granular neurons, the same is not true of its glial elements. In particular, the loss of oligodendrocytes, which results in demyelination, triggers a spontaneous and often highly efficient regenerative response, remyelination, in which new oligodendrocytes are generated and myelin sheaths are restored to denuded axons. Yet, remyelination in humans is not without limitation, and a variety of demyelinating conditions are associated with sustained and disabling myelin loss. In this review, we will review the biology of remyelination, including the cells and signals involved; describe when remyelination occurs and when and why it fails and the consequences of its failure; and discuss approaches for therapeutically enhancing remyelination in demyelinating diseases of both children and adults, both by stimulating endogenous oligodendrocyte progenitor cells and by transplanting these cells into demyelinated brain.

## IDENTIFYING REMYELINATION

Remyelination is the process in which new myelin sheaths are restored to axons that have lost their myelin sheaths as a result of primary demyelination. Primary demyelination is the term used to describe the loss of myelin from an otherwise intact axon and should be distinguished from myelin loss secondary to axonal loss—a process called Wallerian degeneration or, misleadingly, secondary demyelination. Remyelination is sometimes referred to as myelin repair. However, this term suggests a damaged but otherwise intact myelin internode being "patched up," a process for which there is no evidence and which does not emphasize the truly regenerative nature of remyelination, in which the prelesion cytoarchitecture is substantially restored. Remyelinated tissue very closely resembles normally myelinated tissue but differs in one important aspect—the newly generated myelin sheaths are typically shorter and thinner than the original myelin sheaths. When myelin is initially formed in the peri- and postnatal period, there is a striking correlation between axon diameter and myelin sheath thickness and length, which is less apparent in remyelination. Instead, myelin sheath thickness and

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length show little increase with increasing axonal diameter, with the result that the myelin is generally thinner and shorter than would be expected for a given diameter of axon (Fig. 1). Although some remodeling of the new myelin internode occurs, the original dimensions are rarely regained (Powers et al. 2013). The relationship between axon diameter and myelin sheath is expressed as the G ratio, which is the fraction of the axonal circumference to the axon plus myelin sheath circumference. The identification of abnormally thin myelin sheaths (greater than normal G ratio) remains the "gold standard" for unequivocally identifying remyelination, and is most reliably identified in resin-embedded tissue, viewed by light microscopy following toluidine blue staining, or by electron microscopy. This effect is obvious when large diameter axons are remyelinated, but is less clear with smaller diameter axons, such as those of the corpus callosum, in which G ratios of remyelinated axons can be difficult to distinguish from those of normally myelinated axons (Stidworthy et al. 2003).

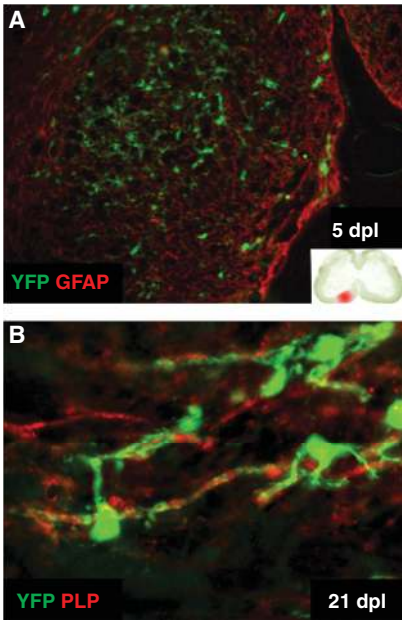


**Figure 1.** Genetic fate mapping of oligodendrocyte precursor cells (OPCs) reveals them to be the principal source of remyelinating oligodendrocytes. Using Cre-lox fate mapping in transgenic mice, it is possible to show that platelet-derived growth factor receptor α (PDGFRA)/NG2-expressing OPCs (green $YFP^+$) in the adult CNS respond to chemically induced focal demyelination of the ventral spinal cord white matter (*inset* in *A*) by proliferation and migration and are abundant within the area of damage, defined here by immunohistochemistry for the astrocyte marker GFAP (red), at 5 d postlesion (dpl) (*A*). At 21 dpl, when the lesion has undergone complete remyelination, green $YFP^+$ OPC-derived remyelinating oligodendrocytes can be seen producing new myelin sheaths around the demyelinated axons, detected by expression of the myelin protein PLP (red) (*B*) (see Zawadzka et al. 2010).

How is the relationship between myelin parameters and axon size established in myelination and why is it disengaged in remyelination? In the peripheral nervous system (PNS), axonally expressed neuregulin (NRG)1-type III plays a key role. Reduced expression results in a thinner myelin sheath (increased G ratio), whereas overexpression leads to a thicker than expected myelin sheath (decreased G ratio) (Michailov et al. 2004). In the central nervous system (CNS) however, the role of neuregulins in controlling myelin sheath length and thickness is less clear (Brinkmann et al. 2008), although they may play a role in activity-dependent remyelination. The factors that govern the G ratio in remyelination would seem to be distinct from those operating in developmental myelination, such that an explanation for the increased G ratio in remyelination remains elusive. For example, overexpression of NRG leads to hypermyelination in development but not during remyelination (Brinkmann et al. 2008). Similarly, activation of the Akt pathway, which results in thicker than expected myelin sheaths in development (Flores et al. 2008), does not result in thicker remyelinated sheaths following demyelination in the adult (Harrington et al. 2010). One hypothesis is that, whereas the myelinating oligodendrocyte associates with a dynamically changing axon to achieve its full length and diameter, the remyelinating oligodendrocyte engages an axon that is comparatively static, having already reached its mature size (Franklin and Hinks 1999). As a result, the remyelinating oligodendrocyte is not subjected to the same dynamic stresses encountered

by the developmentally myelinating oligodendrocyte.

## REMYELINATION IS THE NORMAL RESPONSE TO DEMYELINATION

Remyelination shares many common features with regenerative processes occurring in other tissues of the body, and is the expected or default response to demyelination. The evidence for this comes from both experimentally-induced and clinical demyelination. When demyelination is induced by toxins injurious to oligodendrocytes and myelin (e.g., by dietary cuprizone or direct delivery of lysolecithin or ethidium bromide), the remyelination usually proceeds to completion, albeit in an age-dependent manner (Blakemore and Franklin 2008). Similarly, there is evidence that axons undergoing primary demyelination in experimental or clinical traumatic injury undergo complete remyelination, and that the persistence of chronically demyelinated axons is unusual (Lasiene et al. 2008). An exception is when demyelination is induced by, or associated with, the adaptive immune response, such as occurs in the autoimmune-mediated condition multiple sclerosis (MS) and its laboratory animal model, experimental autoimmune encephalomyelitis (EAE). In this context, remyelination occurs in an environment intrinsically hostile to the oligodendrocyte lineage. Thus, remyelination failure in MS (and EAE) is not inevitable, but rather a feature of the specific disease environment of MS. Nevertheless, even in MS—a disease prototypically associated with failed or inadequate remyelination—remyelination can proceed to completion, and can be geographically extensive (Patrikios et al. 2006; Patani et al. 2007; Goldschmidt et al. 2009; Piaton et al. 2009). Similarly, remyelination can be extensive in EAE, and models with significant persistent demyelination are unusual (Linington et al. 1992; Hampton et al. 2008). Remyelination is especially efficient following demyelination of cerebral cortical gray matter, in both experimental models (Merkler et al. 2006) and clinical disease (Albert et al. 2007), although the reasons for this are unclear.

## REMYELINATION RESTORES FUNCTION AND PROTECTS AXONS

Remyelination restores saltatory conduction and reverses functional deficits (Smith et al. 1979; Jeffery et al. 1999; Liebetanz and Merkler 2006). Compelling evidence in support of functional restoration by remyelination is provided by an unusual demyelinating condition in cats, in which the reversal of clinical signs is associated with spontaneous remyelination (Duncan et al. 2009).

An additional and key function of remyelination is the protective effect it has on the underlying axon (Irvine and Blakemore 2008). Axonal and neuronal loss is a major cause of the progressive nature of chronic demyelinating disease, such as occurs in MS (Trapp and Nave 2008), and is primarily a result of the absence of the myelin sheath, rather than the direct damage by inflammation that accounts for axonal loss in acute lesions. Thus, patients on appropriate immunosuppressive therapy and with apparently quiescent disease still show monotonically increasing disability and clinical progression, as these patients manifest persistent demyelination regardless of their lack of active disease. Indeed, remyelination is not the principal reason for the resolution of clinical signs following an acute relapse, which rather likely results from the resolution of inflammation, paired with adaptive responses by affected axons that serve to restore conduction.

Evidence that myelin is required for axon survival is based on observations in genetic mouse models, as well as in studies of human pathology (Nave and Trapp 2008). Transgenic mice lacking 2′-3′ cyclic nucleotide phosphodiesterase (CNP) or proteolipid protein (PLP) show long-term axonal degeneration, even in the presence of myelin sheaths that are either ultrastructurally normal or show only minor abnormalities (Griffiths et al. 1998; Lappe-Siefke et al. 2003). Further analysis of the PLP mutant mice has revealed a disturbance in axoplasmic transport in the absence of PLP (Edgar et al. 2004) and has led to the identification of myelin-associated sirtuin 2 as a potential mediator of long-term axonal stability (Werner

et al. 2007). Myelin is also important for axon survival in humans, as patients with Pelizaeus–Merzbacher disease (PMD) caused by mutations in PLP show axon loss (Garbern et al. 2002), and studies of MS autopsy tissue show that axon preservation is seen in those areas in which remyelination has occurred (Kornek et al. 2000). Axon degeneration has recently been observed as a consequence of genetically induced oligodendrocyte-specific ablation, even in Rag 1–deficient mice that have no functional lymphocytes (Pohl et al. 2011). These observations offer compelling evidence that axonal survival is dependent on intact oligodendrocytes, and that axonal degeneration in chronically demyelinated lesions can occur independently of inflammation. The nature of the trophic exchange from oligodendrocyte to axon remains to be fully elucidated. However, recent data suggesting that transfer of energy metabolites from oligodendroglia to axons through monocarboxylate transporter 1 (MCT1) may contribute to the survival of axons (Lee et al. 2012; Morrison et al. 2013)

## MECHANISMS OF REMYELINATION

### Oligodendrocyte Precursor Cells (OPCs) Are the Principal Source of New Myelin-Forming Oligodendrocytes

Remyelination involves the generation of new mature oligodendrocytes because (1) there is a greater number of oligodendrocytes within an area of remyelination compared with the equivalent area before myelination (Prayoonwiwat and Rodriguez 1993); and (2) remyelination occurs within areas depleted of oligodendrocytes (Sim et al. 2002b). In a vast majority of cases, the new oligodendrocytes that mediate remyelination are derived from a population of adult CNS stem/progenitor cells, most often referred to as adult oligodendrocyte progenitor cells, and sometimes called NG2 cells (in this article, we will generally refer to these cells as OPCs). These multiprocessed proliferating cells are widespread throughout the CNS, occurring in both white matter and gray matter (3%–8% of the cell population) (Horner et al. 2000; Dawson et al. 2003; Richardson et al. 2011). Adult OPCs are derived from their developmental forebears, and the two cells share many similarities, although the adult cell has a longer basal cell cycle time and migrate less rapidly (Wolswijk and Noble 1989), traits that characterize adult human as well as murine OPCs (Windrem et al. 2004). However, adult OPCs can be induced to proliferate and migrate like perinatal cells in vitro by the growth factors platelet-derived growth factor (PDGF) and fibroblast growth factors (FGF) (Wolswijk and Noble 1992), both of which are significantly up-regulated during remyelination (Hinks and Franklin 1999).

Evidence obtained using Cre-lox fate mapping in transgenic mice following experimental demyelination has shown that OPCs produce the vast majority of remyelinating oligodendrocytes (Fig. 1) (Tripathi et al. 2010; Zawadzka et al. 2010). Remyelinating oligodendrocytes may also come from the stem and progenitor cells of the adult subventricular zone (SVZ), either from the progenitor cells contributing to the rostral migratory stream (RMS) (Nait-Oumesmar et al. 1999) or from the GFAP-expressing neural stem cells of the SVZ per se (Menn et al. 2006). However, the contribution that SVZ-derived cells make relative to that from local OPCs may be relatively small, especially so in larger brains, such as human, and their acute contribution to repair beyond the periventricular white matter is likely negligible.

### Remyelination Requires the Activation, Recruitment, and Differentiation of Adult OPCs

In response to injury, local OPCs undergo a switch from an essentially quiescent state to a regenerative phenotype. This activation is the first step in the remyelination process and involves not only changes in morphology, but also up-regulation of several genes, many associated with the generation of oligodendrocytes during development, such as the transcription factors Olig2, Nkx2.2, MyT1, and Sox2 (Fancy et al. 2004; Watanabe et al. 2004; Shen et al. 2008). The activation of OPCs is likely to be in response to acute injury-induced changes in microglia and astrocytes, two cell types exquisitely

sensitive to disturbance in tissue homeostasis (Glezer et al. 2006; Rhodes et al. 2006). These two cell types themselves activated by injury are the major source of factors that induce the rapid proliferative response of OPCs to demyelinating injury (Fig. 1). This response is modulated by levels of the cell cycle regulatory proteins p27Kip-1 and Cdk2 (Crockett et al. 2005; Caillava et al. 2011), and is promoted by the growth factors (PDGF and FGF) (Woodruff et al. 2004; Murtie et al. 2005), endothelin 1 (Gadea et al. 2009), and many other factors associated with acute inflammatory lesions, and shown to have OPC mitogenic in tissue culture (Vela et al. 2002). Semaphorins are important regulators of OPC migration following demyelination. Semaphorin 3A impairs OPC recruitment to the demyelinated area, whereas semaphorin 3F overexpression accelerates not only OPC recruitment, but also the remyelination rate (Piaton et al. 2011). The population of areas of demyelination by OPCs is referred to as the "recruitment phase" of remyelination, and involves OPC migration in addition to ongoing proliferation.

For remyelination to proceed, the recruited OPC must next differentiate into remyelinating oligodendrocytes—the differentiation phase. This phase encompasses three distinct steps—establishing contact with the axon to be remyelinated; expression of myelin genes and generation of myelin membrane; and, finally, wrapping and compaction to form the sheath. Despite these being fundamental properties of oligodendrocytes, we still have an incomplete understanding of how axoglial contact is established and how this interaction then regulates, within each individual cell process, the morphological changes that constitute myelination. Nevertheless, some molecules have been shown to contribute to the regulation of differentiation, and it is clear that the differentiation of OPCs into myelinating oligodendrocytes in development and during regeneration share many similarities (Fancy et al. 2011a). FGF plays a key role in inhibiting differentiation as well as in promoting recruitment, and thereby regulates the correct transition from the recruitment to the differentiation phases (Armstrong et al. 2002), and insulin-like growth factor (IGF)-I is another factor that plays major roles in both processes (Mason et al. 2003). Semaphorin 3A, in addition to its role in OPC recruitment (Piaton et al. 2011), is also an inhibitor of OPC differentiation (Syed et al. 2011). LINGO-1, a component of the trimolecular Nogo receptor, has been found to be a negative regulator of oligodendrocyte differentiation in development (Mi et al. 2005), whereas mice deficient in LINGO-1 or treated with an antibody antagonist against LINGO-1 showed increased remyelination and functional recovery from EAE (Mi et al. 2007). The canonical Wnt pathway has recently emerged as a very powerful negative regulator of oligodendrocyte differentiation in both development and remyelination (Fancy et al. 2009; Ye et al. 2009). The nuclear receptor retinoid X receptor-γ (RXR-γ) is a key positive regulator of oligodendrocyte differentiation directly from the analysis of remyelinating tissue (Huang et al. 2011).

However, differences in the regulation of development and regeneration of myelin do occur; the transcription factor Olig1, although essential for developmental myelination (Xin et al. 2005), is less redundant role in remyelination in which it plays a pivotal permissive role in OPC differentiation (Arnett et al. 2004). In contrast, Notch signaling pathway, a negative (Wang et al. 1998) or positive (Hu et al. 2003) regulator of differentiation in development (depending on the ligand), is redundant during remyelination because conditional knockout of the *notch1* gene in OPCs has little or no effect on remyelination (Stidworthy et al. 2004; Zhang et al. 2009). The differentiation-inhibitory function of endothelin-1 has recently been shown to operate via activation of the Notch pathway, supporting a view that, on balance, this pathway impedes terminal differentiation (Hammond et al. 2014).

## Inflammation and Remyelination

The innate immune response to demyelination is important for creating an environment conducive to remyelination. The relationship between inflammation and regeneration is well recognized in many other tissues. However, its

involvement in myelin regeneration has been obscured in a field dominated by the immune-mediated pathology of MS and its various animal models, such as EAE, in which it is unquestionably true that the adaptive immune response mediates tissue damage. Nevertheless, several descriptive studies, using experimental models (Ludwin 1980) and MS tissue (Wolswijk 2002), have pointed to a positive association between inflammation and remyelination. In particular, the role of the innate immune response in remyelination has become apparent, in part, through the use of nonimmune-mediated, toxin-induced models of demyelination. Depletion or pharmacological inhibition of macrophages following toxin-induced demyelination leads to an impairment of remyelination (Kotter et al. 2005; Li et al. 2005b). The proinflammatory cytokines Il-1β and TNF-α, the lymphotoxin-β receptor, and MHCII have all been implicated as mediators of remyelination following cuprizone-induced demyelination (Arnett et al. 2001, 2003; Mason et al. 2001; Plant et al. 2007). A critical role played by phagocytic macrophages is the removal of myelin debris generated during demyelination, because CNS myelin contains proteins inhibitory to OPC differentiation both in vitro and during remyelination (Kotter et al. 2006; Baer et al. 2009). Indeed, old adult mice can be made to remyelinate with the efficiency of young adult mice when provided with young adult macrophages that allow more efficient removal of myelin debris (Ruckh et al. 2012). The observation that macrophage activation enhances myelination by transplanted OPCs in the myelin-free retinal nerve fiber layer points to additional regenerative factors produced by these macrophages (Setzu et al. 2006), one of which has been identified as endothelin 2, a positive regulator of OPC differentiation (Yuen et al. 2013).

A recent study has indicated that the M1 macrophage phenotype is associated with the recruitment phase of remyelination, and that the switch from an inflammatory environment to one dominated by M2 macrophages is causally related to the initiation of differentiation, in part via the production of activin-A (Miron et al. 2013).

## DEMYELINATED CNS AXONS CAN ALSO BE REMYELINATED BY SCHWANN CELLS

CNS remyelination can also be mediated by Schwann cells, the myelin-forming cells of the peripheral nervous system. This occurs in several experimental animal models of demyelination as well as in human demyelinating disease (Snyder et al. 1975; Itoyama et al. 1983, 1985; Dusart et al. 1992; Felts et al. 2005). Schwann cell remyelination occurs preferentially where astrocytes are absent, for example, where they have been killed along with oligodendrocytes by the demyelinating agent (Blakemore 1975; Itoyama et al. 1985). Remyelinating Schwann cells within the CNS were generally thought to migrate into the CNS from PNS sources, such as spinal and cranial roots, meningeal fibers, or autonomic nerves following a breach in the glia limitans (Franklin and Blakemore 1993). In support of this idea, CNS Schwann cell remyelination typically occurs in proximity to spinal and cranial nerves or around blood vessels (Snyder et al. 1975; Duncan and Hoffman 1997; Sim et al. 2002a). However, recent genetic fate-mapping studies have revealed that very few CNS remyelinating Schwann cells are derived from PNS Schwann cells, but instead the majority derive from OPCs (Zawadzka et al. 2010), revealing a remarkable capacity of these cells to differentiate into cells of neural crest lineage as well as other neuroepithelial lineages (astrocytes and oligodendrocytes). Indeed, OPCs have been found to have multilineage competence when removed from autocrine and paracrine influences, serving effectively as multipotential neural stem cells (Belachew et al. 2003; Nunes et al. 2003). Thus, the OPC may be more appropriately considered a broadly multilineage-competent neuroectodermal progenitor, capable of producing not only astrocytes and oligodendroytes, but central neurons as well as peripheral Schwann cells as well, in a context-dependent fashion (Crawford et al. 2014).

The implications of Schwann cell remyelination of CNS axons are unclear. Although both Schwann cell and oligodendrocyte remyelination are associated with a return of saltatory conduction (Smith et al. 1979), their relative

abilities to promote axon survival, a major function of myelin (Nave and Trapp 2008), have yet to be established. Thus, from a clinical perspective, we do not yet know whether OPC differentiation into Schwann cells has a beneficial or deleterious effect compared with oligodendrocyte remyelination.

## CAUSES OF REMYELINATION FAILURE

The efficiency of remyelination is affected by the nondisease-related factors of age and sex (Sim et al. 2002b; Li et al. 2006). These generic factors will have a bearing on the efficiency of remyelination regardless of the disease process involved and will be discussed first.

Like all other regenerative processes, the efficiency of remyelination decreases with age. This manifests as a decrease in the rate at which it occurs, and is likely to have a profound bearing on the outcome of a disease process that in the case of MS can occur over many decades. The age-associated effects on remyelination are owing to a decrease in the efficiency of both OPC recruitment and differentiation (Sim et al. 2002b). Of these two events, the impairment of differentiation is rate-determining, because increasing the provision of OPCs by the overexpression of the OPC mitogen and recruitment factor PDGF following demyelination in old mice does not accelerate remyelination (Woodruff et al. 2004). The impairment of OPC differentiation in aging mirrors the failure of oligodendrocyte differentiation associated with many chronically demyelinated MS plaques (Wolswijk 1998; Kuhlmann et al. 2008).

The basis of the aging effect is likely to lie in age-associated changes in both the extrinsic environmental signals to which OPCs are exposed in remyelinating lesions, and to intrinsic determinants of OPC behavior. An impaired macrophage response in aging, associated with a delay in expression of inflammatory cytokines and chemokines (Zhao et al. 2006), leads to poor clearance of myelin debris and the persistence of myelin-associated differentiation-inhibitory proteins. Changes also occur in the expression of remyelination-associated growth factors following toxin-induced demyelination that are commensurate with delays in OPC activation, recruitment, and differentiation, and are illustrative of age-associated environmental changes in remyelination (Hinks and Franklin 2000). Both in vitro studies, revealing age-associated changes in growth factor responsiveness of adult OPCs of different age (Tang et al. 2000), and in vivo studies demonstrating slower recruitment of transplanted old adult progenitor cells compared with young adult-derived cells into progenitor-depleted white matter (Chari et al. 2003) are indicative of intrinsic changes occurring in OPCs during adult aging. A recent study confirms these observations, revealing a critical age-associated change in the epigenetic regulation of OPC differentiation during remyelination (Shen et al. 2008). Differentiation of OPCs is associated with the recruitment of histone deacetylases (HDACs) to promoter regions of differentiation inhibitors (Marin-Husstege et al. 2002). In old animals, HDAC recruitment is impaired resulting in prolonged expression of these inhibitors, delayed OPC differentiation, and, hence, slower remyelination. This effect can be replicated following induction of demyelination in young animals with the use of the HDAC antagonist valproic acid. A key question relating to the development of remyelination therapies is the extent to which age-associated changes can be reversed (Conboy et al. 2005). This has now been shown using the heterochronic parabiosis model—by parabiotic union of a young adult animal to an old adult animal, the old adult animal can be made to remyelinate with the efficiency of a young adult (Ruckh et al. 2012). This is achieved, in part, by the recruitment of circulating young monocytes to bolster the myelin debris clearance function of the old macrophages.

In addition to these generic factors, remyelination could also be incomplete or fail for disease-specific reasons. The strongest evidence for remyelination failure is provided by MS, and the subsequent discussion will specifically relate to this disease, although the issues discussed will be relevant to other diseases with a demyelinating component. Theoretically, remyelination could fail because of (1) a primary deficiency in progenitor cells; (2) a failure of progenitor

cell recruitment; or (3) a failure of progenitor cell differentiation and maturation.

Early speculation on remyelination failure focused on the first of these mechanisms, in which the process of remyelination itself would deplete an area of CNS of its progenitor cells, so that subsequent episodes of demyelination occurring at or around the same site would fail to remyelinate owing to a lack of OPCs. However, data from experimental studies indicate that OPCs are remarkably efficient at repopulating regions from which they have been depleted (Chari and Blakemore 2002), and that repeat episodes of focal demyelination in the same area neither depletes OPCs nor prevents subsequent remyelination (Penderis et al. 2003a). The situation may be different, however, when the same area of tissue is exposed to a sustained demyelinating insult, where remyelination impairment seems to be a result of, at least in part, to a deficiency in OPC availability (Mason et al. 2004; Armstrong et al. 2006).

In the second mechanism, MS lesions fail to remyelinate not because of a shortage of available progenitor cells, but rather because of a failure of OPC recruitment: proliferation, migration, and repopulation of areas of demyelination. Here, descriptions of demyelinated areas from which oligodendrocyte lineage (OL) cells are absent do indicate that this may account for failure of remyelination, in at least a proportion of lesions. Why lesions should become deficient in OPCs is not clear, but one possibility is that they are direct targets of the disease process within the lesion. The identification of patients with antibodies recognizing OPC-expressed antigens (NG2) supports this possibility (Niehaus et al. 2000). Failure of OPC recruitment into areas of demyelination may arise because of disturbances in the local expression of the OPC migration guidance cues semaphorin 3A and 3F (Williams et al. 2007a). In situations in which OPCs need to be recruited into lesions from surrounding intact tissue, the size of the lesion will clearly have a bearing on the efficiency of remyelination; larger lesions require a greater OPC recruitment impetus than smaller ones, especially in aging where older OPCs appear intrinsically less responsive to recruitment signals.

The best evidence at present supports the third mechanism: a failure of differentiation and maturation, as several sets of observations based on the detection of OL cells within areas of demyelination indicate that this stage of remyelination is most vulnerable to failure in MS. The presence of OPCs apparently unable to differentiate within MS lesions was initially shown with the OL marker O4 (Wolswijk 1998) and subsequently with NG2 (Chang et al. 2000), PLP (to reveal premyelinating oligodendrocytes) (Chang et al. 2002), and Olig2 and Nkx2.2 (Kuhlmann et al. 2008). Even though the density of OPCs within chronic lesions is on average lower than in normal white matter, the density can be as high as that in normal white matter or remyelinated lesions, showing that OPC availability per se is not a limiting factor for remyelination in MS.

One possible explanation for this failure of differentiation is that chronically demyelinated lesions contain factors that inhibit progenitor differentiation. First implicated was the Notch-jagged pathway, a negative regulator of OPC differentiation. Notch and its downstream activator Hes5 were detected in OPCs and jagged in astrocytes within chronic demyelinated MS lesions (John et al. 2002). However, the expression of Notch by OPCs and jagged by other cells within lesions undergoing remyelination and, more informatively, the limited remyelination phenotype in experimental models following conditional deletion of Notch in OL cells, suggest that Notch-jagged signaling is not a critical negative regulator of remyelination (Stidworthy et al. 2004; Zhang et al. 2009). The ability of inhibitors of γ-secretase, an enzyme involved in the Notch pathway, to enhance recovery following EAE, might be indicative of an inhibitory role for Notch signaling in remyelination, but is difficult to interpret given the many cellular targets of γ-secretase, as well as the concurrent expression of Notch by inflammatory effector cells.

Other potential inhibitory factors have been identified in other experimental and pathological studies. The accumulation of hyaluronan, a glycosaminoglycan inhibitor of OPC differentiation within MS lesions, may contribute to an environment that is not conducive to remyeli-

nation by inhibiting OPC function via TLR2 signaling (Back et al. 2005; Sloane et al. 2010). The demyelinated axon itself has been implicated in remyelination failure, because demyelinated axons have been shown to express the adhesion molecule PSA-NCAM (Charles et al. 2002), which inhibits myelination in cell culture (Charles et al. 2000). The possibility that OPCs within areas of demyelination might be regulated by electrical activity (or lack of) in (Gibson et al. 2014), or synaptic input from (Etxeberria et al. 2010), demyelinated axons represents an exciting new development in the understanding of the complexity of regulatory factors that govern remyelination, and by extension remyelination failure.

Although many studies in the last few years have concentrated on putative inhibitory signals to account for the failure of OPCs to undergo complete differentiation within demyelinated MS plaques, an alternative explanation is that these lesions fail to remyelinate because of a deficiency of signals that induce differentiation. This hypothesis, based on the absence of differentiation factors, is difficult to prove but is consistent with a model of remyelination in which the acute inflammatory events play a key role in progenitor activation and in creating an environment conducive to remyelination (see above). Whereas MS lesions are rarely devoid of any inflammatory activity, chronic lesions are relatively noninflammatory compared with acute lesions, and constitute a less active environment in which OPC differentiation might become quiescent. Acute inflammatory lesions are characterized by reactive astrocytes that are the source of many remyelination-signaling factors (Williams et al. 2007b; Moore et al. 2011). In contrast, chronic quiescent lesions are characterized by scarring astrocytes that are transcriptionally quiet compared with reactive astrocytes. The scarring astrocyte is better viewed as a consequence of remyelination failure and not its cause. Thus, neither the reactive nor the scarring astrocytes—both contributing to astrogliosis—are likely drivers of remyelination failure.

The two possibilities that remyelination failure reflects the presence of negative factors or the absence of positive factors are not of course mutually exclusive. Moreover, it has become apparent from many studies in recent years that there are a multitude of interacting factors, both environmental and intrinsic, that guide the behavior of OL cells through the various stages of remyelination. Efficient remyelination may depend as much on the precise timing of action as on the presence or absence of these factors. This is called the "dysregulation hypothesis" in which remyelination failure reflects an inappropriate sequence of events (Franklin 2002; Franklin and Ffrench-Constant 2008; Fancy et al. 2011a). Although the causes of remyelination failure in a disease as complex and variable as MS are likely to be many, we still regard this hypothesis as useful for understanding remyelination failure in the majority of cases.

## ENHANCING ENDOGENOUS REMYELINATION

Because remyelination can occur completely and, because the cells responsible are abundant throughout the adult CNS, even within demyelinated lesions, a conceptually attractive approach to enhancing remyelination is to target the endogenous regenerative process (Franklin and Gallo 2014). This approach is predicated on the view that if the mechanisms of remyelination can be understood and nonredundant pathways described, then the causes of remyelination failure and, hence, plausible therapeutic targets will be identified. From the preceding sections it will be clear that remyelination failure is associated with either insufficient OPC recruitment or, more commonly, failed OPC and oligodendrocyte differentiation. However, the underlying biology of these two phases of remyelination is different, and sometimes mutually exclusive. Therefore, the implication is that prorecruitment therapies may not promote remyelination in which the primary problem is OPC differentiation, and vice versa.

A further consideration in the development of therapies intended to enhance remyelination therapies is the use of appropriate animal models. In the chronic demyelinated plaques of MS, remyelination is assumed to have failed; hence, the requirement is for an intervention that will

reactivate a dormant process. In contrast, in many of the demyelination models used to test enhancement of remyelination, such as the toxin-based models, remyelination does not fail. In those models, one can only achieve the acceleration of an already effective ongoing process. That said, assessing the temporal dynamics of remyelination in MS tissue, and whether it has stopped or merely slowed, is difficult to assess from the "snapshots" provided by biopsy or postmortem tissue. Nevertheless, this problem can, in part, be overcome in two ways. First, using aged animals, in which the slow rate of remyelination is suboptimal, presents an opportunity for assessing its enhancement. Second, by modifying standard lesion models in which the endogenous repair process is compromised, such as in the chronic cuprizone model (Armstrong et al. 2006). The Theiler's virus-induced demyelination model has also proven useful for demonstrating enhanced remyelination (Njenga et al. 1999). However, assessment of remyelination has proven especially complex in EAE, in which the processes of demyelination and remyelination can occur concurrently. This can make it very difficult to distinguish an effect that renders the environment less hostile to remyelination, allowing it to proceed at its natural rate, from one in which the rate of remyelination is actually accelerated. For example, systemic delivery of putative remyelination-enhancing factors can affect the balance of myelin damage and regeneration via effects on cells other than oligodendroglial cells, such as those of the immune system. This may account for the discrepancy in the studies of IGF-I and glial growth factor (GGF)-2 administered systemically in EAE, compared with those in which these agents are delivered locally in nonimmune-mediated models of demyelination (Yao et al. 1995; Cannella et al. 1998; O'Leary et al. 2002; Penderis et al. 2003b).

Despite caveats regarding models and methods of analysis, several recent studies have provided proofs-of-principle for the therapeutic enhancement of remyelination. An especially intriguing line of investigation has been the identification of polyreactive IgM autoantibodies that react with oligodendrocyte surface antigens and promote remyelination (Warrington et al. 2000), although the mechanisms of this effect remain unclear.

Over the last few years, a number of pathways have emerged amenable to pharmacological manipulation that hold considerable promise for the development of drug-based remyelination enhancing medicines (Fancy et al. 2010). First, humanized monoclonal antibodies against LINGO-1 have been developed and are already in phase I clinical trials (Mi et al. 2007). Second, the development of pharmacological inhibitors against the Wnt pathway in cancer therapy, suggests that it might be possible, in the near future, to assess the use of Wnt inhibitors to stimulate OPC differentiation. Particularly relevant here is the recent report that small molecule inhibitors of tankyrase, a ADP-polyribosylating enzyme that stabilizes Axin and thereby releases OPCs from Wnt-pathway mediated differentiation block, can induce precocious OPC differentiation and thus accelerate remyelination (Fancy et al. 2011b). Third, chemical agonists and antagonists of RXR signaling, or rexinoids, are widely available and are showing promise in the treatment of certain types of cancers and metabolic disorders (Altucci et al. 2007). When cultured OPCs are exposed to the RXR selective antagonists, HX531 and PA452, oligodendrocyte differentiation is severely impaired (Huang et al. 2011). In contrast, when OPCs are exposed to the RXR agonists, 9-*cis*-retinoic acid (9cRA), HX630, or PA024, oligodendrocytes are stimulated to differentiate and form myelin membrane-like sheets in culture. Further experiments testing the effect of 9cRA on aged rats, which received focal demyelination, resulted in the significant acceleration of remyelination (Fig. 2). Fourth, rolipram, a PDE inhibitor that is both safe and widely used clinically, been shown to enhance remyelination and may therefore provide a readily "translate-able" regenerative medicine for demyelinating disease (Syed et al. 2013). Fifth, several lines of evidence point toward the clinical potential of hormone-based remyelination therapies (Hussain et al. 2013). Sixth, modulators of the receptor tyrosine phosphatase β/ζ (PTPRZ1), itself a modulator of β-catenin signaling and, hence, of Wnt pathway tone, have

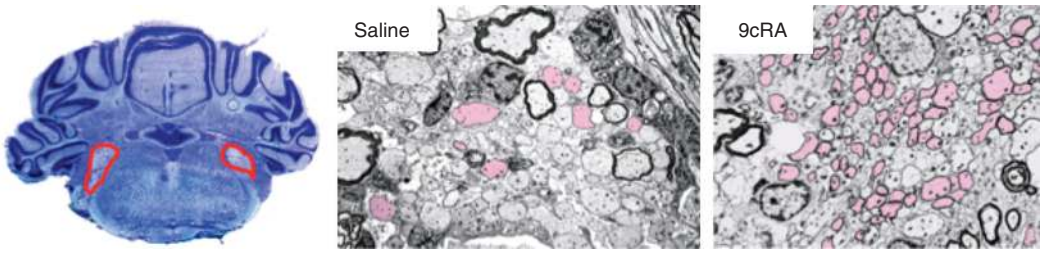


**Figure 2.** Systemic delivery of RXR agonists enhance endogenous remyelination in aged animals. Age is a major cause of declining efficiency of endogenous remyelination, largely because of an age-associate decline in the ability of OPCs to differentiate into remyelinating oigodendocytes. The nucleoreceptor RXR-γ is a positive regulator of OPC differentiation. When the RXR agonist 9-*cis*-retinoic acid is given to aged mice, in which focal demyelination has been generated in the caudal cerebellar peduncles (red outline in brain cross section on *left*) by injection of ethidium bromide, there is a marked acceleration in the generation of new remyelinating oligodendrocytes compared with saline injected controls. This is evident in the two electron micrographs in which demyelinated axons lack an electron dense myelin sheath, whereas newly remyelinated axons (pseudo-colored in pink) have thin myelin sheaths typical of remyelination that contrast with the thick myelin sheaths of normally myelinated axons evident at the lesion edges (see Huang et al. 2011).



been shown to modulate oligodendrocyte differentiation by human OPCs in vitro (Sim et al. 2006; McClain et al. 2012), and the availability of small molecule modulators of PTPRZ1 suggests the potential for targeting this pathway in vivo as well. Finally, the cholinergic agonist benztropine has been proposed as capable of inducing terminal oligodendrocytic differentiation from OPCs, an effect that was evident in experimental models of demyelination as well as in vitro (Deshmukh et al. 2013).

## ENHANCING REMYELINATION BY GLIAL PROGENITOR CELL TRANSPLANTATION

The mobilization of endogenous oligodendrocyte progenitor cells may be appropriate for a variety of disorders of progressive dys- or demyelination. However, a broad swath of neurological disease involves the structural loss of white matter, reflecting frank loss of both oligodendrocytes and their progenitors, and often of their associated fibrous astrocytes. These disorders include ischemic and traumatic demyelination, as well as demyelination associated with sustained autoimmune inflammation and insults as diverse as chemotherapy and radiotherapy. In addition, the hereditary and metabolic disorders of white matter, the pediatric leukodystrophies and lysosomal storage disorders in particular, are intrinsically refractory to any approach aimed at activating resident progenitors—which themselves carry the culpable mutation. Together, these diverse disorders of myelin require extensive tissue repair, and in the case of the pediatric leukodystrophies, even whole neuraxis myelination. Thus, any practical cell therapeutic for the myelin disorders must provide large numbers of progenitors biased to oligodendrocyte differentiation and myelinogenesis. This requirement has prompted the development of human OPCs from a variety of potential sources, which include fetal and adult human tissue (Roy et al. 1999; Dietrich et al. 2002; Windrem et al. 2002, 2004), embryonic stem cells (Izrael et al. 2007; Hu et al. 2009), and induced pluripotential stem cells (Wang et al. 2013). Each of these sources has now been shown able to generate potentially myelinogenic oligodendrocytes (Fig. 3), and each has its own strengths and weaknesses as a cellular therapeutic (Goldman et al. 2012). Of note, OPCs have also been recently generated directly from mouse fibroblasts as well (Najm et al. 2013; Yang et al. 2013); although this feat has yet to be reproduced using human fibroblasts, it nonetheless heralds the likely imminent development of yet another source of clinically appropriate OPCs (Goldman 2013).

Regardless of their derivation, to be practical, safe, and effective as therapeutic vectors,

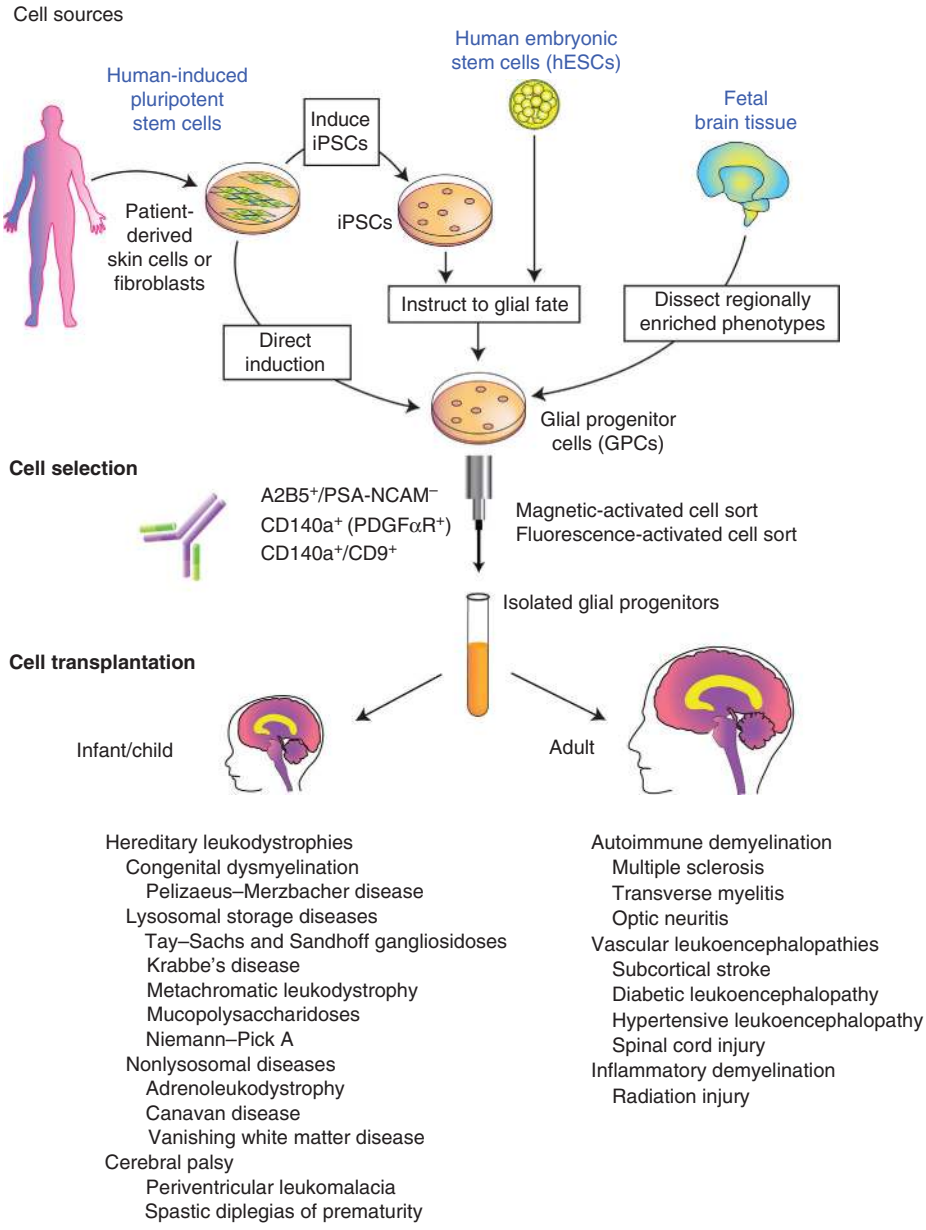


**Figure 3.** Multiple sources of human OPCs can target a broad spectrum of myelin disorders. This schematic shows the major potential sources of myelinogenic central oligodendrocytes and their progenitor cells (OPCs). These include human tissue, embryonic stem cells, induced pluripotential cells, and OPCs directly induced from somatic cells. OPCs may be isolated directly from all of these, on the basis of surface antigens that define several serially generated phenotypes within the oligodendroglial lineage. On transplantation to the brains of afflicted children or adults, the cells may be capable of widespread migration and myelinogenesis. The *bottom* of the figure provides a list of the major myelin disorders that might be approached through this general strategy of cell-based remyelination from introduced hOPCs. (From Goldman et al. 2012; adapted, with permission, from the author.)

OPCs must be deliverable with a uniform myelinogenic phenotype, in both reliable purity and significant quantity. To address this need, several methods for isolating human OPCs from mixed cell populations have been developed (Roy et al. 1999; Nunes et al. 2003). In particular, the surface antigen–based purification of human OPCs, using both fluorescence, activated cell sorting (FACS) and magnetic cell sorting (MACS), has allowed the assessment of these cells in a variety of animal models of congenital dysmyelination (Windrem et al. 2002, 2004). In shiverer mice, fetal- and adult-derived human OPCs behave differently after neonatal xenograft in that isolates of OPCs derived from adult white matter are able to myelinate recipient brain much more rapidly than fetal OPCs; adult-derived progenitors achieved widespread myelination by just 4 wk after graft, whereas cells derived from second trimester fetuses took over 3 mo to do so (Fig. 4) (Windrem et al. 2004). In contrast, fetal OPCs emigrated more widely and engrafted more efficiently, differentiating as astrocytes in gray matter and oligodendrocytes in white matter.

The broad dispersal and context-dependent differentiation of fetal human OPCs suggests their utility in a broad array of both congenital and acquired myelin disorders. However, these cells must be sourced from fetal tissues, whether from abortuses or pathological samples. Despite the significant mitotic competence of these cells, they remain finite in both initial number and in expansion competence, necessitating the periodic reacquisition, isolation, and expansion of OPCs from new donors. Recent efforts have thus focused on the generation of myelinogenic OPCs from pluripotential human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells as well.

## PLURIPOTENTIAL STEM CELLS AS A SOURCE OF MYELINOGENIC PROGENITORS

Following the first report of myelination in the injured spinal cord by implanted murine ES cells (Brustle et al. 1999), oligodendrocytes derived from human ES cells (ESCs) were reported to similarly myelinate demyelinated foci in spinal cord contusions (Nistor et al. 2005). Human ES cells were later shown to generate myelin in the rodent brain (Zhang et al. 2001), and subsequent studies with improved differentiation protocols reported more efficient glial and oligodendrocytic differentiation (Izrael et al. 2007; Hu et al. 2009). Nonetheless, none of these studies isolated glial progenitors or oligodendrocytes before transplantation, nor did any follow animals for the long periods of time required to ensure the phenotypic stability and safety of the engrafted cells. This is problematic because any persistent undifferentiated ES cells in the donor pool retain the potential to generate teratomas or neuroepithelial tumors after implantation (Roy et al. 2006; Pruszak et al. 2009). As a result of these considerations, stringent purification of committed OPCs will likely be important to the safe use of any human embryonic stem (hES) cell–derived therapeutic. In addition, long-term survival studies over broad dose ranges will need to be performed in experimental animals to ensure the lack of tumorigenicity of hES and human-induced pluripotent stem cells (hiPSC) derivatives, recognizing that the long in vitro differentiation protocols that may mitigate the risk of tumorigenesis do not come without a price; prolonged differentiation may limit the expansion and dispersal capability, and, ultimately, the utility of the engrafted donor cells.

ESC-based cell therapy suffers not only from the risk of tumorigenesis, and the trade-offs involved in mitigating that risk, but also from the possibility of rejection of incompatible immunophenotypes, and hence a need for long-term immunosuppression in graft recipients. Enthusiasm has thus developed for the potential use of autologous grafts of OPCs derived from induced pluripotential cells (iPSCs), as a source of new oligodendrocytes for myelin repair. iPSCs are pluripotential cells that have been generated by the reprogramming of somatic cells to a less phenotypically committed stem cell ground state, by the forced expression of a small set of transcription factors that permit the self-renewing stem cell phenotype (Stadtfeld and Hochedlinger 2010). Most typically, iPSCs have been generated from somatic cells cotransduced with a number

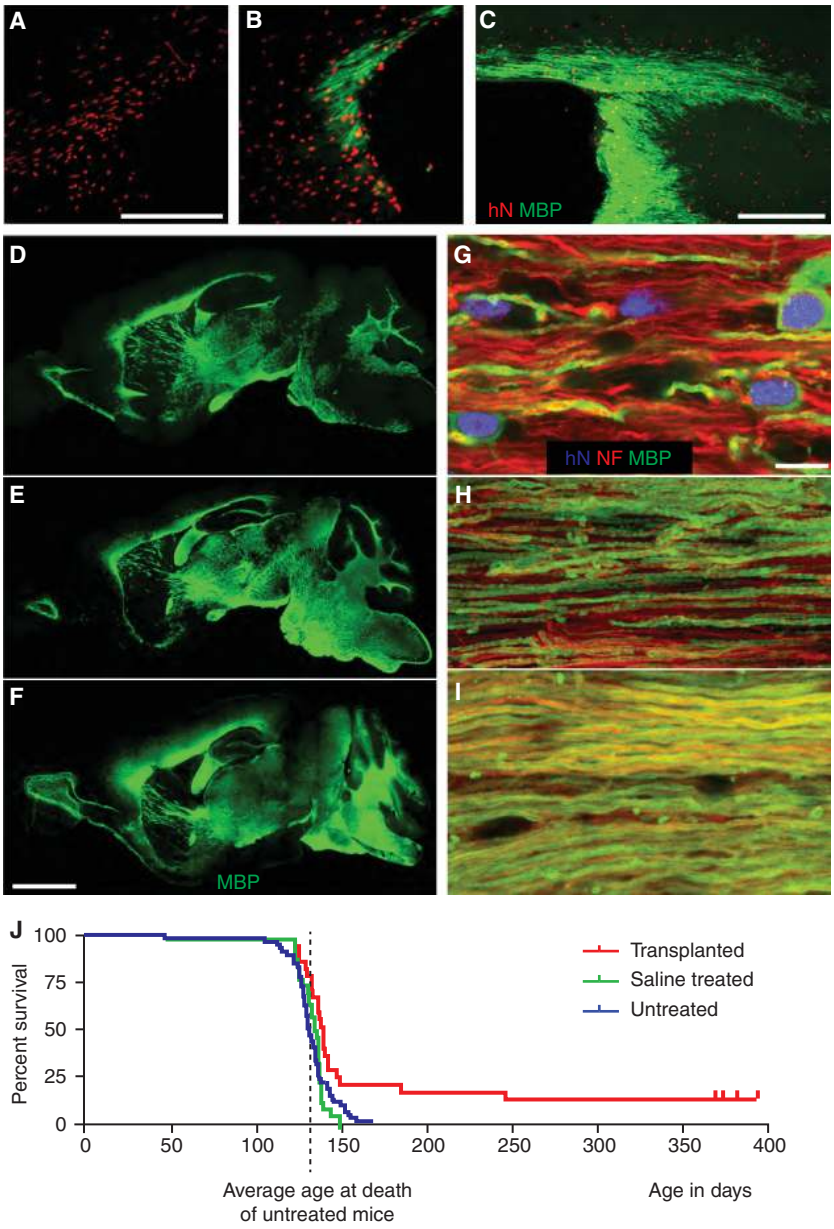


**Figure 4.** Fetal- and adult-derived human OPCs are distinct in their remyelination competence. (*A*) Fetal-derived human $hNA^{+}$ OPCs (red) expressed no detectable MBP at 4 wk after neonatal graft into myelin-deficient and immunodeficient shiverer ($MBP^{shi/shi}$) × $rag2^{-/-}$ mice; no myelin development was noted in these mice until 12 wk. (*B*) In contrast, adult-derived OPCs (red) achieved dense MBP expression (green) by 4 wk after neonatal graft. (*C*) Low-power coronal images of callosal–fimbrial junction of shiverer recipient, showing dense myelination 12 wk after perinatal graft of adult human OPCs. (*D–F*) Sagittal sections of fetal hOPC-implanted mice immunolabeled for MBP (green) at (*D*) 20 wk, (*E*) 35 wk, and (*F*) 52 wk. Fetal hOPCs dispersed more effectively and expanded more than adult OPCs. By 1 yr, fetal hOPC-derived myelination appeared complete throughout the forebrain and hindbrain, and indeed the entire central neuraxis. (*G–I*) Corresponding confocal optical sections of transplanted shiverer mouse corpus callosum taken at (*G*) 20 wk, (*H*) 35 wk, and (*I*) 52 wk after fetal hOPC graft immunolabeled for neurofilament (red) and MBP (green), reveal the progressive increase in axonal ensheathment with time. (*J*) Kaplan–Meier survival plot of immunodeficient shiverer mice, either engrafted with human OPCs at birth (red), injected with saline (green), or untreated (blue). Most died between 18 and 21 wk. However, a fraction of engrafted mice (23% to >1 yr in this series) lived substantially longer than any control mouse; these rescued mice have lived normal life spans (typically >2 yr), with substantial recovery of neurological phenotypic. Scale bars, 100 μm (*A*); 1 mm (*C*); 2.5 mm (*F*); 10 μm (*G*). (Images *A–C* from Windrem et al. 2004 and *D–I* from Windrem et al. 2008; reprinted, with permission, from the authors.)

of stem cell–associated transcription factors, including POU5F1/OCT4, SOX2, MYC, KLF4, and NANOG (Yamanaka 2007, 2008), or alternatively, with either miRNAs that modulate the expression of these proteins, or small molecules that mimic their actions (Bu et al. 2004; Lin et al. 2009). iPSCs have the decided advantage over hES cells of being sources of lineage-restricted phenotypes that can be autologously transplanted back to the subjects from which they were generated, obviating the need for posttransplant immune suppression. The methods previously described for generating OPCs from ES cells have proven adaptable to iPSCs as well, and yield both glial progenitors and terminally differentiated oligodendrocytes that are highly myelinogenic in vivo (Czepiel et al. 2011). More recent advances in optimizing the methods for generating OPCs from human iPS and ES cells have led to the production of highly enriched populations of human OPCs that appear highly efficient at myelinogenesis in vivo, while manifesting no evidence of tumorigenic potential (Fig. 5) (Wang et al. 2013).

The capability to now produce scalable quantities of highly myelinogenic iPSC-derived OPCs allows us to now realistically consider their use as clinical cell therapeutics, in particular, the use of iPSC-derived oligodendrocytes for autologous treatment of myelin disorders. Such an iPSC-based strategy is likely appropriate for vascular, traumatic, and inflammatory demyelination, in which no underlying genetic lesion exists, and in which sufficient axonal numbers remain for remyelination to be beneficial. In addition, with the advent of genetic editing approaches, such as TALEN and Crispr/Cas-mediated technologies, by which genetic mutations may be corrected in parental iPSC lines (Gaj et al. 2013), genetically modified and corrected OPCs may be generated from patients harboring pathological mutations, and transplanted back into those same patients. Such genetically corrected autologous grafts may permit the treatment of genetic disorders of myelin with curative intent, yet without the need for immune suppression.

Yet, notwithstanding the great promise of iPSC technology, OPCs derived from iPSCs may share the same risks as those derived from hES cells, in terms of both unintended differentiation of lineage-unrestricted contaminants, as well as frank tumorigenesis. Just as with the use of OPCs derived from hES cells, those generated from iPSCs will likely need to be purified before clinical use, so as to minimize the risk of tumorigenesis from undifferentiated contaminants accompanying the transplanted cell populations. These risks may soon be overcome in light of the newly developed sorting techniques for enriching OPCs to purity (Windrem et al. 2004; Sim et al. 2011). Nonetheless, additional concerns may yet slow the introduction of iPSC derivatives to the clinic (Mattis and Svendsen 2011). iPSCs may retain some of the epigenetic marks—the DNA methylation and histone acetylation patterns of chromosomal architecture—of the donor cells from which they are derived (Stadtfeld et al. 2010) so that the cell type of origin may influence the differentiation competence of iPS cells derived from different tissue sources (Polo et al. 2010). This is of potential concern, in that if iPS cells do not undergo complete reprogramming, then their derived OPCs might be expected to differ from their tissue or hES-derived homologs (Kim et al. 2010; Polo et al. 2010). That said, whether any such differences will prove meaningful in clinical practice remains to be seen.

OPCs may be generated not only from pluripotential cells, but also directly from somatic cells, using phenotype-defining transcription factors. As noted, several recent studies have reported the direct induction of both OPCs and oligodendrocytes from fibroblasts using targeted overexpression of defined proglial transcripts (Najm et al. 2013; Yang et al. 2013). Such avoidance of pluripotential intermediates in the generation of glial progenitors may mitigate the risk of tumorigenesis, yet this too will need to be established in practice. Yet, whether OPCs are directly induced from a somatic phenotype or derived from pluripotential cells, it is reasonable to expect that future studies will need to accomplish the stringent isolation of lineage-restricted OPCs before transplant, as a necessary safety step in the execution of OPC-based remyelination.

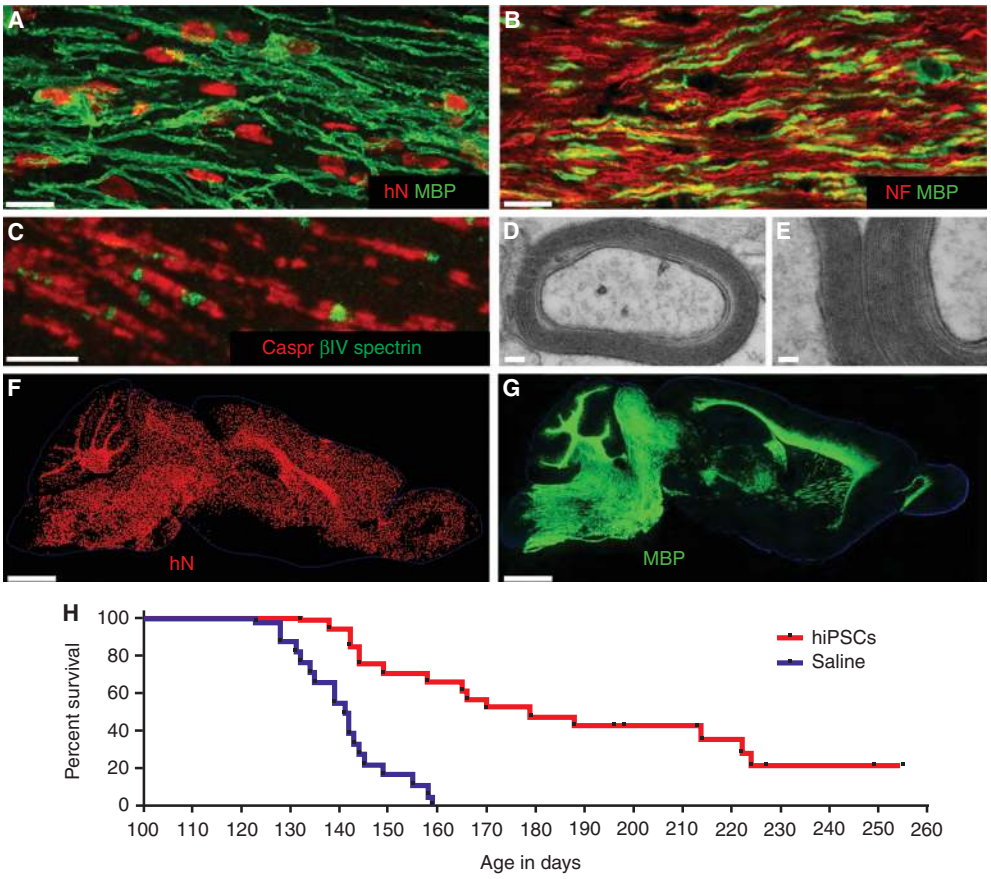


**Figure 5.** hiPSC OPCs are efficient agents for therapeutic myelination. High-power confocal images of the callosal white matter of mice engrafted with hiPSC OPCs. (*A*) Dense donor-derived myelination of single axons is evident, with myelin basic protein (MBP, green) production by donor-derived cells (human nuclear antigen, red), and (*B*) ensheathment of host axons (neurofilament, red) by donor-derived myelin (green). (*C*) iPSC OPC-derived oligodendrocytic differentiation and myelination permitted the composition of architecturally appropriate nodes of Ranvier in transplanted shiverers. Oligodendrocytic paranodal Caspr protein (red) is seen here flanking βIV spectrin (green)-defined nodes. (*D*,*E*) Electron micrographs of iPSC oligodendrocyte-derived myelin in the corpus callosum at 40 wk. Thick myelin wraps with alternating major dense and intraperiod lines, characteristic of mature myelin, are evident. (*F*,*G*) hiPSC OPC dispersal and donor-derived myelination of shiverer forebrain. (*F*) Dot map indicating the distribution of hiPSC OPC-derived cells at 7 mo, following neonatal engraftment. Widespread dispersal and chimerization by hiPSC OPCs is evident (hNA, red). (*G*) Extensive donor-derived (MBP, green) myelination is evident; sampled 1 mm lateral to *F*. MBP immunoreactivity (green) is all donor derived. (*H*) Kaplan–Meier plot of the survival of iPSC-OPC-implanted versus saline-injected mice. Remaining engrafted mice were killed for electron microscopy at ≥270 d. Scale bars, *A* and *B*, 5 μm; *C*, 5 μm; *D*, 200 nm; *E*, 100 nm; *F* and *G*, 2 mm.

## PEDIATRIC TARGETS OF NEURAL STEM CELL- AND GLIAL PROGENITOR CELL-BASED THERAPIES

Tens of thousands of children in the United States suffer from diseases of myelin failure or loss. These include the metabolic demyelinations, such as adrenoleukodystrophy; the lysosomal storage disorders, such as metachromatic leukodystrophy; the neuronal ceroid lipofuscinoses, mucopolysaccharidoses, and gangliosidoses; Niemann–Pick and Krabbe's diseases;

the hypomyelinating diseases, such as Pelizaeus–Merzbacher disease and hereditary spastic paraplegia; the myelinoclastic disorders, vanishing white matter disease, and Canavan's disease; dysmyelinating disorders, such as Alexander disease (Brenner et al. 2001; Dietrich et al. 2005; Li et al. 2005a; Bugiani et al. 2011); and, most commonly of all, periventricular leukomalacia, the most common form of cerebral palsy, which may largely be a result of a perinatal loss of oligodendrocytes and their precursors (Back and Rivkees 2004; Follett et al. 2004; Robinson et al. 2005). Their mechanistic heterogeneity notwithstanding, all of these conditions include the prominent loss of oligodendrocytes and central myelin, highlighting their potential attractiveness as targets for cell replacement.

## Neonatal Delivery of OPCs for Enzymatic Reconstitution and Myelin Preservation

Because OPC engraftment is both widespread and associated with astrocytic as well as oligodendrocytic production, glial progenitors would seem an especially promising vehicle for dispersing functionally competent glia throughout otherwise diseased and/or enzyme-deficient brain parenchyma. The lysosomal storage disorders present especially attractive targets in this regard (Snyder et al. 1995), 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 (Urayama et al. 2004). As a result, a relatively small number of donor glia may provide sufficient enzymatic activity to correct the underlying catalytic deficit and storage disorder of a much larger number of host cells (Lacorazza et al. 1996; Jeyakumar et al. 2005). The intracerebral delivery of OPCs would thus seem an especially attractive approach for treating those demyelinating diseases associated with enzyme deficiencies specific to brain. By way of example, metachromatic leukodystrophy (MLD) is characterized by deficient expression of arylsulfatase A (ARSA), which results in sulfatide misaccumulation and oligodendrocyte loss. Mesenchymal and hematopoietic stem cell grafts have proven unable to correct the CNS manifestations of this disorder (Koc et al. 2002). In contrast, experimental models of MLD have responded well to OPC grafts (Givogri et al. 2006), suggesting that the broader dispersal competence and greater histiotypic appropriateness of orthotopically engrafted OPCs might provide significant therapeutic advantages relative to nonneural phenotypes. Similarly, although asymptomatic Krabbe patients transplanted with umbilical cord stem cells manifested slower disease progression than untreated controls, the benefits of transplantation to children engrafted after symptom onset seemed minimal (Escolar et al. 2005). Yet, the intracerebral parenchymal infiltration of stromal derivatives is also minimal, suggesting that treatment of these children with native, brain-penetrant neural stem cells (NSCs) or glial progenitors might comprise a more promising treatment strategy (Pellegatta et al. 2006).

In the same vein, the neuronal ceroid lipofuscinoses (NCLs), including Batten disease, will likely require neural cell grafts for their cell-based treatment, as the enzymes deficient in this class of disorders are largely neural in expression. Experimentally, when human NSCs were engrafted into PPT1 mutant mice, which model infantile NCL, the cells dispersed broadly and corrected some of the lipofuscin misaccumulation of these animals (Tamaki et al. 2009). However, no survival data were reported in this study, so it remains unclear whether NSC engraftment is sufficient to slow disease progression in this model. Nonetheless, on the basis of the available data, a clinical trial to assess the use of human neural stem cell allografts in treating both infantile and late infantile forms of NCL was undertaken (clinicaltrials.gov, identifier NCT00337636). This limited phase I safety trial did not address any functional or therapeutic endpoints, but its initiation speaks to the efforts that may be anticipated in developing the use of engrafted NSCs and OPCs as vehicles for intracerebral enzyme replacement, in the lysosomal storage disorders as well as in the broader category of disorders of brain metabolism characterized by aberrant catabolism.

## Neonatal Delivery of OPCs for Structural Myelin Replacement

For myelin replacement, OPCs might reasonably be considered more effective vectors than neural stem cells, given their glial-restricted phenotype in vivo, their oligodendrocytic bias in hypomyelinated CNS, and their efficient remyelination of structurally demyelinated tissue. To assess the potential of OPC-based treatment for congenital dysmyelination, Windrem and colleagues transplanted sorted human OPCs of both fetal and adult origin into newborn shiverer mice, a dysmyelinated mouse deficient in myelin basic protein (Windrem et al. 2002, 2004). This work followed similar studies in which both native and immortalized murine NSCs had been transplanted into shiverers; each had yielded some degree of context-dependent differentiation and myelination (Yandava et al. 1999; Mitome et al. 2001). On that basis, Windrem et al. extracted fetal OPCs from late second-trimester forebrain, as well as adult OPCs from surgically resected subcortical white matter; both were isolated by either fluorescence-activated or immunomagnetic sorting, based on the phenotype A2B5$^{+}$/PSA-NCAM$^{-}$. When the cells were transplanted into recipient shiverers as highly enriched isolates, both fetal and adult OPCs spread widely throughout the brain, developing as astrocytes and oligodendrocytes (Fig. 4). Notably, the cells did so in a highly context-dependent fashion, such that those donor cells that engrafted presumptive white matter developed as myelinogenic oligodendrocytes and fibrous astrocytes, while those invading cortical and subcortical gray differentiated largely as protoplasmic astrocytes (Windrem et al. 2004).

Following a single neonatal intracallosal injection, the majority of donor cells engrafted the presumptive white matter, such that the corpus callosum and capsules densely expressed myelin basic protein throughout the forebrain white matter tracts (Windrem et al. 2004). Donor-derived myelin effectively ensheathed host shiverer axons, as validated by both confocal imaging, and by the ultrastructural observation of donor-derived myelin with major dense lines, indicating effective myelin compaction, of which native shiverer oligodendrocytes are incapable. Confocal analysis also revealed nodes of Ranvier between donor-derived myelinated segments, and transcallosal conduction velocities were normalized in the OPC-transplanted mice. On that basis, Windrem and colleagues developed a five-site injection protocol for achieving broader dispersal of OPCs in the recipient CNS. Using this approach, cell dispersal was achieved throughout the entire neuraxis, and was associated with effective whole-neuraxis myelination, which pervaded the spinal cord and roots as well as the entire brain, including the brainstem, cerebellum, and cranial nerve roots. This was associated with significantly and substantially prolonged survivals in transplanted shiverer mice, with frank rescue and phenotypic recovery of a large minority (Windrem et al. 2008); whereas untreated shiverers invariably die by 20 wk of age, some engrafted animals achieved normal murine life spans (Fig. 4). These data strongly suggested the feasibility of neonatal OPC implantation as a strategy for treating the congenital disorders of myelin formation and maintenance. Later studies refined the criteria for selecting myelinogenic progenitors, by identifying the PDGFα receptor epitope CD140a as recognizing the entire population of potentially oligodendrocytic cells (Sim et al. 2011). OPCs selected on the basis of CD140a expression proved superior to those selected on the basis of A2B5 in their efficiency, rapidity of differentiation, and ultimate extent of myelination, while nonetheless being highly migratory; as such, they served to supplant the latter as a preferred cellular vector for therapeutic remyelination (Fig. 6).

## Challenges in the Use of Glial Progenitor Grafts for the Childhood Myelin Disorders

Cell-based therapies comprise a broad platform for the potential amelioration of enzymatic and storage disorders, in that a common strategy of intracerebral delivery of OPCs may prove broadly applicable across a variety of specific enzymatic disorders. In practice though, given treatment regimens will need to be tightly calibrated to

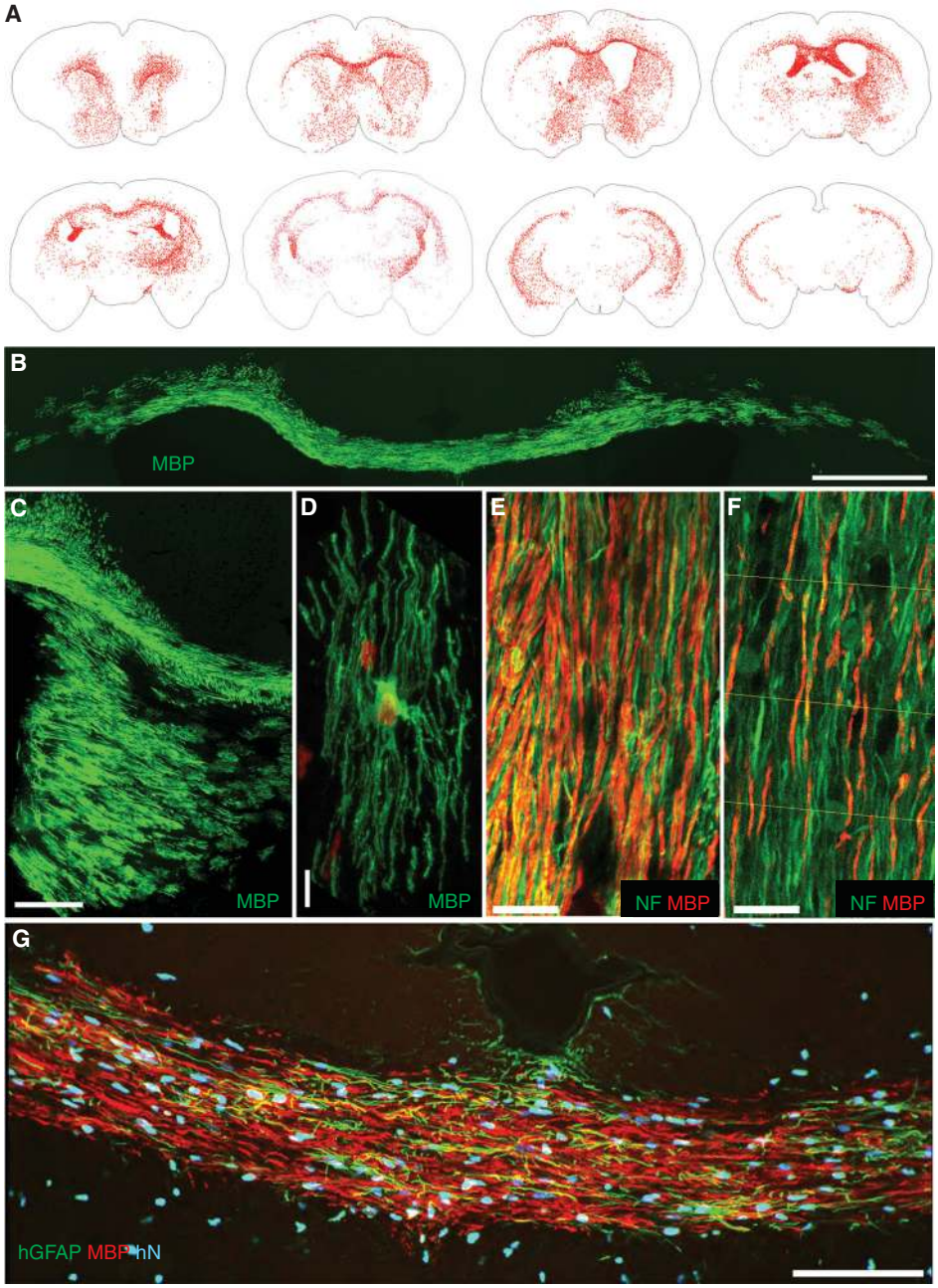


**Figure 6.** CD140-isolated OPCs are able to broadly migrate and efficiently myelinate. (*A*) Computer-assisted drawings of 14-μm sections of a 12-wk-old shiverer × rag2-null mouse, transplanted bilaterally in the corpus callosum with 100,000 CD140a$^+$ cells. Red dots represent individual cells labeled with antihuman nuclear antigen. (*B*) The corpus callosum of an engrafted shiverer mouse at 12 wk, stained for MBP, showing substantial donor-derived myelin. (*C*) A photomicrograph of the corpus callosum and fimbria in another engrafted mouse. (*D*) An individual oligodendrocyte, stained for antihuman nuclear antigen (red). (*E*,*F*) Ensheathment of host mouse axons (neurofilament, green) at 12 wk by CD140a$^+$ (*E*) or A2B5$^+$ (*F*) human fetal cells, showing the more rapid and robust axonal myelination by CD140a$^+$ cells. (*G*) A CD140a$^+$ cell-engrafted shiverer callosum at 12 wk, immunostained for MBP, human GFAP, and human nuclear antigen, showing robust production of hGFAP$^+$ astrocytes as well as MBP$^+$ oligodendrocytes. Scale bars, *B*, 500 μm; *C*, 200 μm; *D*, 10 μm; *E* and *F*, 20 μm. (Images from Sim et al. 2011; reprinted, with permission, from the authors.)

specific disease phenotypes and stages. Little data are available as to the number or proportion of wild-type cells required to achieve local correction of enzymatic activity and substrate clearance in any storage disorder, and these values will likely need to be empirically derived for different disease targets. Similarly, the efficiency and extent of myelination required to achieve significant benefit in the hypomyelinating disorders remains unclear, and will depend on disease extent and duration as much as donor cell dispersal and myelinogenic competence in the disease environment. These caveats notwithstanding, there is considerable reason for optimism that cell-based therapy of the pediatric myelin disorders, including not only the storage disorders, but also the primary dysmyelinations, such as Pelizaeus–Merzbacher, vanishing white matter disease, and selected forms of cerebral palsy, may soon prove both feasible and effective (Goldman 2011). Indeed, a recent phase I trial of implanted neural stem cells reported the safety of NSC implants into children with Pelizaeus–Merzbacher disease, and tentatively claimed evidence of new myelin formation at the sites of implantation (Gupta et al. 2012). One may expect that with the development of improved and scalable preparations of migratory and lineage-restricted human OPCs, whether of tissue or pluripotential cell origin, that both long-term safety and tangible benefit may soon be demonstrated in these especially needy pediatric patient populations.

## ADULT DISEASE TARGETS OF GLIAL PROGENITOR CELL-BASED TREATMENT

In adults, oligodendrocytic loss is causal in diseases as diverse as traumatic brain and spinal cord injury, MS and its variants, and hypertensive and diabetic white matter loss. In addition, the prominent role of oligodendrocytic loss in both vascular dementia and age-related white matter loss is becoming increasingly recognized. All of these are potential targets of glial progenitor cell replacement therapy, although the disease environment may limit the feasibility of this approach in ways not encountered in pediatric disease targets (Franklin and Ffrench-Constant 2008). For instance, the chronically ischemic brain tissue of diabetic and hypertensive patients with small vessel disease may present an inhospitable environment for graft acceptance, and may require aggressive treatment of the underlying vascular disorders before any cell replacement strategy may be considered. Similarly, the inflammatory disease environment of patients with autoimmune demyelination presents its own challenges, which need to be overcome before cell-based remyelination can succeed. Nonetheless, current disease-modifying strategies for treating both vascular and autoimmune diseases have advanced to the point where transplant-based remyelination of adult targets may now be feasible (Goldman et al. 2012).

### Multiple Sclerosis and Autoimmune Demyelination

Most experimental models of cell-based remyelination have focused on MS, which, as noted, is characterized by both inflammatory myelinolysis and degenerative axonal loss. The attraction of MS as a therapeutic target derives from its high incidence and extraordinary prevalence, given its typical onset in youth and long disease course. Over 200 young adults are diagnosed weekly, with more than 300,000 affected in the United States alone. Within 10 years, roughly one-half of MS patients develop the progressive neurodegeneration of secondary progressive MS. In the past, MS had not been an actively researched target for cell therapy, because there was limited enthusiasm for introducing new cells into an active disease environment, one essentially primed for allograft rejection. Yet, the recent development of new approaches toward CNS immune modulation have so substantially diminished disease recurrence, as to make the use of cell replacement strategies for restoring lost myelin and repairing already-demyelinated lesions more tenable.

In that regard, both NSCs and OPCs have been assessed as potential cell therapeutics for myelin repair in a variety of models of acquired demyelination (Pluchino et al. 2004; Franklin and Kotter 2008). As cellular vectors for remye-

lination, NSCs have the advantage of sustained expansion competence, but their efficiency of oligodendrocytic differentiation and myelination appears low. Their systemic administration into mice subjected to experimental allergic encephalomyelitis resulted in improved remyelination (Pluchino et al. 2003), although not by contributing new oligodendrocytes, but rather through central immunosuppression by inducing the programmed cell death of blood-borne, CNS-infiltrating $T_H1$ cells (Pluchino et al. 2004; Einstein and Ben-Hur 2008). In contrast, the intracerebral delivery of OPCs into demyelinated brain may offer a feasible strategy for direct remyelination. Using this approach, when we transplanted adult human OPCs directly into lysolecithin-induced demyelinating lesions within the adult rat brain, the cells quickly matured as oligodendrocytes and myelinated residual denuded host axons, although with lower efficiency than that noted using similar donor cells in congenitally hypomyelinated brain (Windrem et al. 2002). In this regard, in a recent study focusing on a new model of axon-sparing noninflammatory demyelination, Duncan and colleagues found that remyelination of denuded adult axons occurs readily and efficiently from endogenous progenitors (Duncan et al. 2009). Thus, donor as well as endogenous OPCs would seem likely to be effective cellular vectors for remyelination, provided there is a permissive axonal substrate.

### Challenges in the Use of OPC Grafts for Adult Demyelinating Disorders

Regardless of the cell-autonomous capabilities of donor OPCs, the complexity of the adult disease environment, which may include latent inflammatory activity and underlying axonal loss, vascular insufficiency, local gliosis, and chronic inflammatory cells, may all conspire to make adult targets less approachable than their pediatric counterparts (Franklin 2002). Indeed, as noted previously, in some disease settings, endogenous progenitors may be intrinsically competent to remyelinate adult demyelinated brain, but are impeded from doing so in aged animals by a deficient innate immune system, which can be rescued by immune reconstitution from a younger parabiotic partner (Ruckh et al. 2012).

Clearly then, any cell-based therapeutic strategies for adult demyelination, especially those intended to remyelinate both acute and chronic lesions of multiple sclerosis, will require aggressive disease modification as well as rigorous stratification to define those patients with sufficient axonal integrity to potentially benefit from this approach. That said, the noted improvement in immune modulators and disease-modifying therapies offers hope that OPC-based cell therapy may soon evolve as a viable treatment option for the restoration of both lost myelin and compromised function to afflicted patients.

## CONCLUDING REMARKS

Our understanding of the biology of oligodendrocytes and their progenitors, as well as of myelin and its disorders, has increased tremendously over the past several years, aided by new technologies in genomics and stem cell biology, transgenic and chimeric animal models of disease, and imaging and cell signaling analysis. These new insights provide real hope that triggering remyelination from pharmacologically mobilized endogenous progenitors and using tissue- and stem cell–derived oligodendrocyte progenitor cells as transplantable agents for myelin replacement are both viable strategies for clinical myelin repair, which may each soon be ready for the leap from bench-to-bedside. We must remain cautious though, even as our enthusiasm builds, as the risks of these new approaches are also only now becoming understood and surmounted. That said, given the rapidly accelerating growth in remyelination biology over the past decade, progress over the next decade seems assured to be as scientifically exciting as it promises to be therapeutically meaningful.

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## REFERENCES

Albert M, Antel J, Bruck W, Stadelmann C. 2007. Extensive cortical remyelination in patients with chronic multiple sclerosis. *Brain Pathol* **17:** 129–138.

Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR, Gronemeyer H. 2007. RAR and RXR modulation in cancer and metabolic disease. *Nat Rev Drug Discov* **6:** 793–810.

Armstrong RC, Le TQ, Frost EE, Borke RC, Vana AC. 2002. Absence of fibroblast growth factor 2 promotes oligodendroglial repopulation of demyelinated white matter. *J Neurosci* **22:** 8574–8585.

Armstrong RC, Le TQ, Flint NC, Vana AC, Zhou YX. 2006. Endogenous cell repair of chronic demyelination. *J Neuropathol Exp Neurol* **65:** 245–256.

Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JPY. 2001. TNFα promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci* **4:** 1116–1122.

Arnett HA, Wang Y, Matsushima GK, Suzuki K, Ting JP. 2003. Functional genomic analysis of remyelination reveals importance of inflammation in oligodendrocyte regeneration. *J Neurosci* **23:** 9824–9832.

Arnett HA, Fancy SPJ, Alberta JA, Zhao C, Plant SR, Raine CS, Rowitch DH, Franklin RJM, Stiles CD. 2004. The bHLH transcription factor Olig1 is required for repair of demyelinated lesions in the CNS. *Science* **306:** 2111–2115.

Back SA, Rivkees SA. 2004. Emerging concepts in periventricular white matter injury. *Semin Perinatol* **28:** 405–414.

Back SA, Tuohy TM, Chen H, Wallingford N, Craig A, Struve J, Luo NL, Banine F, Liu Y, Chang A, et al. 2005. Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. *Nat Med* **11:** 966–972.

Baer AS, Syed YA, Kang SU, Mitteregger D, Vig R, Ffrench-Constant C, Franklin RJM, Altmann F, Lubec G, Kotter MR. 2009. Myelin-mediated inhibition of oligodendrocyte precursor differentiation can be overcome by pharmacological modulation of Fyn-RhoA and protein kinase C signalling. *Brain* **132:** 465–481.

Belachew S, Chittajallu R, Aguirre AA, Yuan X, Kirby M, Anderson S, Gallo V. 2003. Postnatal NG2 proteoglycan–expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J Cell Biol* **161:** 169–186.

Blakemore WF. 1975. Remyelination by Schwann cells of axons demyelinated by intraspinal injection of 6-aminonicotinamide in the rat. *J Neurocytol* **4:** 745–757.

Blakemore WF, Franklin RJM. 2008. Remyelination in experimental models of toxin-induced demyelination. *Curr Top Microbiol Immunol* **318:** 193–212.

Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, Messing A. 2001. Mutations in *GFAP*, encoding glial fibrillary acidic protein, are associated with Alexander disease. *Nat Genet* **27:** 117–120.

Brinkmann BG, Agarwal A, Serada MW, Garratt AN, Mueller T, Wende H, Stassart RM, Nawaz S, Humml C, Velanac V, et al. 2008. Neuregulin-1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system. *Neuron* **59:** 581–594.

Brustle O, Jones KN, Learish RD, Karram K, Choudhary K, Wiestler OD, Duncan ID, McKay RDG. 1999. Embryonic stem cell–derived glial precursors: A source of myelinating transplants. *Science* **285:** 754–756.

Bu J, Banki A, Wu Q, Nishiyama A. 2004. Increased $NG2^+$ glial cell proliferation and oligodendrocyte generation in the hypomyelinating mutant shiverer. *Glia* **48:** 51–63.

Bugiani M, Boor I, van Kollenburg B, Postma N, Polder E, van Berkel C, van Kesteren RE, Windrem MS, Hol EM, Scheper GC, et al. 2011. Defective glial maturation in vanishing white matter disease. *J Neuropathol Exp Neurol* **70:** 69–82.

Caillava C, Vandenbosch R, Jablonska B, Deboux C, Spigoni G, Gallo V, Malgrange B, Baron-Van Evercooren A. 2011. Cdk2 loss accelerates precursor differentiation and remyelination in the adult central nervous system. *J Cell Biol* **193:** 397–407.

Cannella B, Hoban CJ, Gao YL, Garcia-Arenas R, Lawson D, Marchionni M, Gwynne D, Raine CS. 1998. The neuregulin, glial growth factor 2, diminishes autoimmune demyelination and enhances remyelination in a chronic relapsing model for multiple sclerosis. *Proc Natl Acad Sci* **95:** 10100–10105.

Chang A, Nishiyama A, Peterson J, Prineas J, Trapp BD. 2000. NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. *J Neurosci* **20:** 6404–6412.

Chang A, Tourtellotte WW, Rudick R, Trapp BD. 2002. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. *N Engl J Med* **346:** 165–173.

Chari DM, Blakemore WF. 2002. Efficient recolonisation of progenitor-depleted areas of the CNS by adult oligodendrocyte progenitor cells. *Glia* **37:** 307–313.

Chari DM, Crang AJ, Blakemore WF. 2003. Decline in rate of colonization of oligodendrocyte progenitor cell (OPC)-depleted tissue by adult OPCs with age. *J Neuropathol Exp Neurol* **62:** 908–916.

Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, Zalc B, Lubetzki C. 2000. Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. *Proc Natl Acad Sci* **97:** 7585–7590.

Charles P, Reynolds R, Seilhean D, Rougon G, Aigrot MS, Niezgoda A, Zalc B, Lubetzki C. 2002. Re-expression of PSA-NCAM by demyelinated axons: An inhibitor or remyelination in multiple sclerosis? *Brain* **125:** 1972–1979.

Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. 2005. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433:** 760–764.

Crawford AH, Stockley JH, Tripathi RB, Richardson WD, Franklin RJM. 2014. Oligodendrocyte progenitors: Adult stem cells of the central nervous system? *Exp Neurol* **260:** 50–55.

Crockett DP, Burshteyn M, Garcia C, Muggironi M, Casaccia-Bonnefil P. 2005. Number of oligodendrocyte progenitors recruited to the lesioned spinal cord is modulated by the levels of the cell cycle regulatory protein p27Kip-1. *Glia* **49:** 301–308.

Czepiel M, Balasubramaniyan V, Schaafsma W, Stancic M, Mikkers H, Huisman C, Boddeke E, Copray S. 2011. Differentiation of induced pluripotent stem cells into functional oligodendrocytes. *Glia* **59:** 882–892.

Dawson MRL, Polito A, Levine JM, Reynolds R. 2003. NG2-expressing glial progenitor cells: An abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci* **24:** 476–488.

Deshmukh VA, Tardif V, Lyssiotis CA, Green CC, Kerman B, Kim HJ, Padmanabhan K, Swoboda JG, Ahmad I, Kondo T, et al. 2013. A regenerative approach to the treatment of multiple sclerosis. *Nature* **502:** 327–332.

Dietrich J, Noble M, Mayer-Proschel M. 2002. Characterization of A2B5$^+$ glial precursor cells from cryopreserved human fetal brain progenitor cells. *Glia* **40:** 65–77.

Dietrich J, Lacagnina M, Gass D, Richfield E, Mayer-Proschel M, Noble M, Torres C, Proschel C. 2005. *EIF2B5* mutations compromise GFAP$^+$ astrocyte generation in vanishing white matter leukodystrophy. *Nat Med* **11:** 277–283.

Duncan ID, Hoffman RL. 1997. Schwann cell invasion of the central nervous system of the myelin mutants. *J Anatomy* **190:** 35–49.

Duncan ID, Brower A, Kondo Y, Curlee JF Jr, Schultz RD. 2009. Extensive remyelination of the CNS leads to functional recovery. *Proc Natl Acad Sci* **106:** 6832–6836.

Dusart I, Marty S, Peschanski M. 1992. Demyelination and remyelination by Schwann cells and oligodendrocytes after kainate-induced neuronal depletion in the central nervous system. *Neuroscience* **5:** 137–148.

Edgar JM, McLaughlin M, Yool D, Zhang SC, Fowler JH, Montague P, Barrie JA, McCulloch MC, Duncan ID, Garbern J, et al. 2004. Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic paraplegia. *J Cell Biol* **166:** 121–131.

Einstein O, Ben-Hur T. 2008. The changing face of neural stem cell therapy in neurologic diseases. *Arch Neurol* **65:** 452–456.

Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S, Wenger DA, Pietryga D, Wall D, Champagne M, et al. 2005. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *N Engl J Med* **352:** 2069–2081.

Etxeberria A, Mangin JM, Aguirre A, Gallo V. 2010. Adult-born SVZ progenitors receive transient synapses during remyelination in corpus callosum. *Nat Neurosci* **13:** 287–289.

Fancy SPJ, Zhao C, Franklin RJM. 2004. Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. *Mol Cell Neurosci* **27:** 247–254.

Fancy SPJ, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJM, Rowitch DH. 2009. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes Dev* **23:** 1571–1585.

Fancy SPJ, Kotter MR, Harrington EP, Huang JK, Zhao C, Rowitch DH, Franklin RJM. 2010. Overcoming remyelination failure in multiple sclerosis and other myelin disorders. *Exp Neurol* **225:** 18–23.

Fancy SPJ, Chan JR, Baranzini SE, Franklin RJM, Rowitch DH. 2011a. Myelin regeneration: A recapitulation of development? *Annu Rev Neurosci* **34:** 19–41.

Fancy SPJ, Harrington EP, Yuen TJ, Silbereis JC, Zhao C, Baranzini SE, Bruce CC, Otero JJ, Huang EJ, Nusse R, et al. 2011b. Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. *Nat Neurosci* **14:** 1009–1016.

Felts PA, Woolston AM, Fernando HB, Asquith S, Gregson NA, Mizzi OJ, Smith KJ. 2005. Inflammation and primary demyelination induced by the intraspinal injection of lipopolysaccharide. *Brain* **128:** 1649–1666.

Flores AI, Narayanan SP, Morse EN, Shick HE, Yin X, Kidd G, Avila RL, Kirschner DA, Macklin WB. 2008. Constitutively active Akt induces enhanced myelination in the CNS. *J Neurosci* **28:** 7174–7183.

Follett PL, Deng W, Dai W, Talos DM, Massillon LJ, Rosenberg PA, Volpe JJ, Jensen FE. 2004. Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: A protective role for topiramate. *J Neurosci* **24:** 4412–4420.

Franklin RJM. 2002. Why does remyelination fail in multiple sclerosis? *Nat Rev Neurosci* **3:** 705–714.

Franklin RJM, Blakemore WF. 1993. Requirements for Schwann cell migration within CNS environments: A viewpoint. *Int J Dev Neurosci* **11:** 641–649.

Franklin RJM, Ffrench-Constant C. 2008. Remyelination in the CNS: From biology to therapy. *Nat Rev Neurosci* **9:** 839–855.

Franklin RJM, Gallo V. 2014. The translational biology of remyelination: Past, present, and future. *Glia* doi: 10.1002/glia.22622.

Franklin RJM, Hinks GL. 1999. Understanding CNS remyelination: Clues from developmental and regeneration biology. *J Neurosci Res* **58:** 207–213.

Franklin RJM, Kotter MR. 2008. The biology of CNS remyelination: The key to therapeutic advances. *J Neurol* **255:** 19–25.

Gadea A, Aguirre A, Haydar TF, Gallo V. 2009. Endothelin-1 regulates oligodendrocyte development. *J Neurosci* **29:** 10047–10062.

Gaj T, Gersbach CA, Barbas CF III. 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* **31:** 397–405.

Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, Klugmann M, Nave KA, Sistermans EA, van der Knaap MS, Bird TD, et al. 2002. Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-

dependent axonal degeneration in the absence of demyelination and inflammation. *Brain* **125:** 551–561.

Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, Inema I, Miller SE, Bieri G, Zuchero JB, et al. 2014. Neuronal activity promotes oligodendrogenesis and adpative myelination in the mammalian brain. *Science* **344:** 1252304.

Givogri MI, Galbiati F, Fasano S, Amadio S, Perani L, Superchi D, Morana P, Del Carro U, Marchesini S, Brambilla R, et al. 2006. Oligodendroglial progenitor cell therapy limits central neurological deficits in mice with metachromatic leukodystrophy. *J Neurosci* **26:** 3109–3119.

Glezer I, Lapointe A, Rivest S. 2006. Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. *FASEB J* **20:** 750–752.

Goldman SA. 2011. Progenitor cell-based treatment of the pediatric myelin disorders. *Arch Neurol* **68:** 848–856.

Goldman SA. 2013. White matter from fibroblasts. *Nat Biotechnol* **31:** 412–413.

Goldman SA, Nedergaard M, Windrem MS. 2012. Glial progenitor cell-based treatment and modeling of neurological disease. *Science* **338:** 491–495.

Goldschmidt T, Antel J, Konig FB, Bruck W, Kuhlmann T. 2009. Remyelination capacity of the MS brain decreases with disease chronicity. *Neurology* **72:** 1914–1921.

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, et al. 1998. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* **280:** 1610–1613.

Gupta N, Henry RG, Strober J, Kang SM, Lim DA, Bucci M, Caverzasi E, Gaetano L, Mandelli ML, Ryan T, et al. 2012. Neural stem cell engraftment and myelination in the human brain. *Sci Transl Med* **4:** 155ra137.

Hammond TR, Gadea A, Dupree J, Kerninon C, Nait-Oumesmar B, Aguirre A, Gallo V. 2014. Astrocyte-derived endothelin-1 inhibits remyelination through Notch activation. *Neuron* **81:** 588–602.

Hampton DW, Anderson J, Pryce G, Irvine KA, Giovannoni G, Fawcett JW, Compston A, Franklin RJM, Baker D, Chandran S. 2008. An experimental model of secondary progressive multiple sclerosis that shows regional variation in gliosis, remyelination, axonal and neuronal loss. *J Neuro Immunol* **201–202:** 200–211.

Harrington EP, Zhao C, Fancy SPJ, Kaing S, Franklin RJM, Rowitch DH. 2010. Oligodendrocyte *PTEN* required for myelin and axonal integrity not remyelination. *Ann Neurol* **68:** 703–726.

Hinks GL, Franklin RJM. 1999. Distinctive patterns of PDGF-A, FGF-2, IGF-I and TGF-β1 gene expression during remyelination of experimentally induced spinal cord demyelination. *Mol Cell Neurosci* **14:** 153–168.

Hinks GL, Franklin RJM. 2000. Delayed changes in growth factor gene expression during slow remyelination in the CNS of aged rats. *Mol Cell Neurosci* **16:** 542–556.

Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J Neurosci* **20:** 2218–2228.

Hu QD, Ang BT, Karsak M, Hu WP, Cui XY, Duka T, Takeda Y, Chia W, Sankar N, Ng YK, et al. 2003. F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. *Cell* **115:** 163–175.

Hu BY, Du ZW, Zhang SC. 2009. Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat Protoc* **4:** 1614–1622.

Huang JK, Jarjour AA, Nait Oumesmar B, Kerninon C, Williams A, Krezel W, Kagechika H, Bauer J, Zhao C, Baron van Evercooren A, et al. 2011. Retinoid X receptor γ signaling accelerates CNS remyelination. *Nat Neurosci* **14:** 45–53.

Hussain R, Ghoumari AM, Bielecki B, Steibel J, Boehm N, Liere P, Macklin WB, Kumar N, Habert R, Mhaouty-Kodja S, et al. 2013. The neural androgen receptor: A therapeutic target for myelin repair in chronic demyelination. *Brain* **136:** 132–146.

Irvine KA, Blakemore WF. 2008. Remyelination protects axons from demyelination-associated axon degeneration. *Brain* **131:** 1464–1477.

Itoyama Y, Webster HD, Richardson EP Jr, Trapp BD. 1983. Schwann cell remyelination of demyelinated axons in spinal cord multiple sclerosis lesions. *Ann Neurol* **14:** 339–346.

Itoyama Y, Ohnishi A, Tateishi J, Kuroiwa Y, Webster HD. 1985. Spinal cord multiple sclerosis lesions in Japanese patients: Schwann cell remyelination occurs in areas that lack glial fibrillary acidic protein (GFAP). *Acta Neuropathol (Berl)* **65:** 217–223.

Izrael M, Zhang P, Kaufman R, Shinder V, Ella R, Amit M, Itskovitz-Eldor J, Chebath J, Revel M. 2007. Human oligodendrocytes derived from embryonic stem cells: Effect of noggin on phenotypic differentiation in vitro and on myelination in vivo. *Mol Cell Neurosci* **34:** 310–323.

Jeffery ND, Crang AJ, O'Leary MT, Hodge SJ, Blakemore WF. 1999. Behavioural consequences of oligodendrocyte progenitor cell transplantation into demyelinating lesions in rat spinal cord. *Eur J Neurosci* **11:** 1508–1514.

Jeyakumar M, Dwek RA, Butters TD, Platt FM. 2005. Storage solutions: Treating lysosomal disorders of the brain. *Nat Rev Neurosci* **6:** 713–725.

John GR, Shankar SL, Shafit-Zagardo B, Massimi A, Lee SC, Raine CS, Brosnan CF. 2002. Multiple sclerosis: Re-expression of a developmental pathway that restricts oligodendrocyte maturation. *Nat Med* **8:** 1115–1121.

Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, et al. 2010. Epigenetic memory in induced pluripotent stem cells. *Nature* **467:** 285–290.

Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W. 2002. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant* **30:** 215–222.

Kornek B, Storch MK, Weissert R, Wallstroem E, Stefferl A, Olsson T, Linington C, Schmidbauer M, Lassmann H. 2000. Multiple sclerosis and chronic autoimmune encephalomyelitis: A comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. *Am J Pathol* **157:** 267–276.

Kotter MR, Zhao C, van Rooijen N, Franklin RJM. 2005. Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. *Neurobiol Dis* **18:** 166–175.

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Kotter MR, Li WW, Zhao C, Franklin RJM. 2006. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *J Neurosci* **26:** 328–332.

Kuhlmann T, Miron V, Cui Q, Wegner C, Antel J, Bruck W. 2008. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. *Brain* **131:** 1749–1758.

Lacorazza HD, Flax JD, Snyder EY, Jendoubi M. 1996. Expression of human β-hexosaminidase α-subunit gene (the gene defect of Tay–Sachs disease) in mouse brains upon engraftment of transduced progenitor cells. *Nat Med* **2:** 424–429.

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA. 2003. Disruption of *Cnp1* uncouples oligodendroglial functions in axonal support and myelination. *Nat Genet* **33:** 366–374.

Lasiene J, Shupe L, Perlmutter S, Horner P. 2008. No evidence for chronic demyelination in spared axons after spinal cord injury in a mouse. *J Neurosci* **28:** 3887–3896.

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang P-W, et al. 2012. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* **487:** 44–448.

Li R, Johnson AB, Salomons G, Goldman JE, Naidu S, Quinlan R, Cree B, Ruyle SZ, Banwell B, D'Hooghe M, et al. 2005a. Glial fibrillary acidic protein mutations in infantile, juvenile, and adult forms of Alexander disease. *Ann Neurol* **57:** 310–326.

Li WW, Setzu A, Zhao C, Franklin RJM. 2005b. Minocycline-mediated inhibition of microglia activation impairs oligodendrocyte progenitor cell responses and remyelination in a non-immune model of demyelination. *J Neuroimmunol* **158:** 58–66.

Li WW, Penderis J, Zhao C, Schumacher M, Franklin RJM. 2006. Females remyelinate more efficiently than males following demyelination in the aged but not young adult CNS. *Exp Neurol* **202:** 250–254.

Liebetanz D, Merkler D. 2006. Effects of commissural de- and remyelination on motor skill behaviour in the cuprizone mouse model of multiple sclerosis. *Exp Neurol* **202:** 217–224.

Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R, Lin X, Hahm HS, Hao E, Hayek A, et al. 2009. A chemical platform for improved induction of human iPSCs. *Nat Method* **6:** 805–808.

Linington C, Engelhardt B, Kapocs G, Lassman H. 1992. Induction of persistently demyelinated lesions in the rat following the repeated adoptive transfer of encephalitogenic T cells and demyelinating antibody. *J Neuroimmunol* **40:** 219–224.

Ludwin SK. 1980. Chronic demyelination inhibits remyelination in the central nervous system. *Lab Invest* **43:** 382–387.

Marin-Husstege M, Muggironi M, Liu A, Casaccia-Bonnefil P. 2002. Histone deacetylase activity is necessary for oligodendrocyte lineage progression. *J Neurosci* **22:** 10333–10345.

Mason JL, Suzuki K, Chaplin DD, Matsushima GK. 2001. Interleukin-1β promotes repair of the CNS. *J Neurosci* **21:** 7046–7052.

Mason JL, Xuan S, Dragatsis I, Efstratiadis A, Goldman JE. 2003. Insulin-like growth factor (IGF) signaling through type 1 IGF receptor plays an important role in remyelination. *J Neurosci* **23:** 7710–7718.

Mason JL, Toews A, Hostettler JD, Morell P, Suzuki K, Goldman JE, Matsushima GK. 2004. Oligodendrocytes and progenitors become progressively depleted within chronically demyelinated lesions. *Am J Pathol* **164:** 1673–1682.

Mattis VB, Svendsen CN. 2011. Induced pluripotent stem cells: A new revolution for clinical neurology? *Lancet Neurol* **10:** 383–394.

McClain CR, Sim FJ, Goldman SA. 2012. Pleiotrophin suppression of receptor protein tyrosine phosphatase-β/ζ maintains the self-renewal competence of fetal human oligodendrocyte progenitor cells. *J Neurosci* **32:** 15066–15075.

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. 2006. Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* **26:** 7907–7918.

Merkler D, Ernsting T, Kerschensteiner M, Bruck W, Stadelmann C. 2006. A new focal EAE model of cortical demyelination: Multiple sclerosis-like lesions with rapid resolution of inflammation and extensive remyelination. *Brain* **129:** 1972–1983.

Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, Shao Z, Chang J, Thill G, Levesque M, Zhang M, et al. 2005. LINGO-1 negatively regulates myelination by oligodendrocytes. *Nat Neurosci* **8:** 745–751.

Mi S, Hu B, Hahm K, Luo Y, Kam Hui ES, Yuan Q, Wong WM, Wang L, Su H, Chu TH, et al. 2007. LINGO-1 antagonist promotes spinal cord remyelination and axonal integrity in MOG-induced experimental autoimmune encephalomyelitis. *Nat Med* **13:** 1228–1233.

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA. 2004. Axonal neuregulin-1 regulates myelin sheath thickness. *Science* **304:** 700–703.

Miron VE, Boyd A, Zhao JW, Yuen TJ, Ruckh JM, Shadrach JL, vang Wijngaarden P, Wagers AJ, Williams A, Franklin RJM, et al. 2013. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci* **16:** 1211–1218.

Mitome M, Low HP, van deb Pol A, Nunnari JJ, Wolf MK, Billings-Gagliardi S, Schwartz WJ. 2001. Towards the reconstruction of central nervous system white matter using neural precursor cells. *Brain* **124:** 2147–2161.

Moore CS, Milner R, Nishiyama A, Frausto RF, Serwanski DR, Pagarigan RR, Whitton JL, Miller RH, Crocker SJ. 2011. Astrocytic tissue inhibitor of metalloproteinase-1 (TIMP-1) promotes oligodendrocyte differentiation and enhances CNS myelination. *J Neurosci* **31:** 6247–6254.

Morrison BM, Lee Y, Rothstein JD. 2013. Oligodendroglia: Metabolic supporters of axons. *Trends Cell Biol* **23:** 644–651.

Murtie JC, Zhou YX, Le TQ, Vana AC, Armstrong RC. 2005. PDGF and FGF2 pathways regulate distinct oligodendrocyte lineage responses in experimental demyelination with spontaneous remyelination. *Neurobiol Dis* **19:** 171–182.

Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Van Evercooren AB. 1999. Progenitor cells

of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci* **11:** 4357–4366.

Najm FJ, Lager AM, Zaremba A, Wyatt K, Caprariello AV, Factor DC, Karl RT, Maeda T, Miller RH, Tesar PJ. 2013. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. *Nat Biotechnol* **31:** 426–433.

Nave KA, Trapp BD. 2008. Axon-glial signaling and the glial support of axon function. *Annu Rev Neurosci* **31:** 535–561.

Niehaus A, Shi J, Grzenkowski M, Diers-Fenger M, Archelos J, Hartung HP, Toyka K, Bruck W, Trotter J. 2000. Patients with active relapsing-remitting multiple sclerosis synthesize antibodies recognizing oligodendrocyte progenitor cell surface protein: Implications for remyelination. *Ann Neurol* **48:** 362–371.

Nistor GI, Totoiu MO, Haque N, Carpenter MK, Keirstead HS. 2005. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia* **49:** 385–396.

Njenga MK, Murray PD, McGavern D, Lin X, Drescher KM, Rodriguez M. 1999. Absence of spontaneous central nervous system remyelination in class II–deficient mice infected with Theiler's virus. *J Neuropathol Exp Neurol* **58:** 78–91.

Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G, Jiang L, Kang J, Nedergaard M, Goldman SA. 2003. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nat Med* **9:** 439–447.

O'Leary MT, Hinks GL, Charlton HM, Franklin RJM. 2002. Increasing local levels of IGF-I mRNA expression using adenoviral vectors does not alter oligodendrocyte remyelination in the CNS of aged rats. *Mol Cell Neurosci* **19:** 32–42.

Patani R, Balaratnam M, Vora A, Reynolds R. 2007. Remyelination can be extensive in multiple sclerosis despite a long disease course. *Neuropathol Appl Neurobiol* **33:** 277–287.

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Bruck W, Lucchinetti C, Lassmann H. 2006. Remyelination is extensive in a subset of multiple sclerosis patients. *Brain* **129:** 3165–3172.

Pellegatta S, Tunici P, Poliani PL, Dolcetta D, Cajola L, Colombelli C, Ciusani E, Di Donato S, Finocchiaro G. 2006. The therapeutic potential of neural stem/progenitor cells in murine globoid cell leukodystrophy is conditioned by macrophage/microglia activation. *Neurobiol Dis* **21:** 314–323.

Penderis J, Shields SA, Franklin RJM. 2003a. Impaired remyelination and depletion of oligodendrocyte progenitors does not occur following repeated episodes of focal demyelination in the rat CNS. *Brain* **126:** 1382–1391.

Penderis J, Woodruff RH, Lakatos A, Li WW, Dunning MD, Zhao C, Marchionni M, Franklin RJM. 2003b. Increasing local levels of neuregulin (glial growth factor-2) by direct infusion into areas of demyelination does not alter remyelination in the rat CNS. *Eur J Neurosci* **18:** 2253–2264.

Piaton G, Williams A, Seilhean D, Lubetzki C. 2009. Remyelination in multiple sclerosis. *Prog Brain Res* **175:** 453–464.

Piaton G, Aigrot MS, Williams A, Moyon S, Tepavcevic V, Moutkine I, Gras J, Matho KS, Schmitt A, Soellner H, et al. 2011. Class 3 semaphorins influence oligodendrocyte precursor recruitment and remyelination in adult central nervous system. *Brain* **134:** 1156–1167.

Plant SR, Iocca HA, Wang Y, Thrash JC, O'Connor BP, Arnett HA, Fu YX, Carson MJ, Ting JP. 2007. Lymphotoxin β receptor (LtβR): Dual roles in demyelination and remyelination and successful therapeutic intervention using LtβR-Ig protein. *J Neurosci* **27:** 7429–7437.

Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U, Amadio S, Bergami A, et al. 2003. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* **422:** 688–694.

Pluchino S, Furlan R, Martino G. 2004. Cell-based remyelinating therapies in multiple sclerosis: Evidence from experimental studies. *Curr Opin Neurol* **17:** 247–255.

Pohl HB, Porcheri C, Mueggler T, Bachmann LC, Martino G, Riethmacher D, Franklin RJM, Rudin M, Suter U. 2011. Genetically induced adult oligodendrocyte cell death is associated with poor myelin clearance, reduced remyelination, and axonal damage. *J Neurosci* **31:** 1069–1080.

Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, et al. 2010. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol* **28:** 848–855.

Powers BE, Sellers DL, Lovelett EA, Cheung W, Aalami SP, Zapertov N, Maris DO, Horner PJ. 2013. Remyelination reporter reveals prolonged refinement of spontaneously regenerated myelin. *Proc Natl Acad Sci* **110:** 4075–4080.

Prayoonwiwat N, Rodriguez M. 1993. The potential for oligodendrocyte proliferation during demyelinating disease. *J Neuropathol Exp Neurol* **52:** 55–63.

Pruszak J, Ludwig W, Blak A, Alavian K, Isacson O. 2009. CD15, CD24, and CD29 define a surface biomarker code for neural lineage differentiation of stem cells. *Stem Cells* **27:** 2928–2940.

Rhodes KE, Raivich G, Fawcett JW. 2006. The injury response of oligodendrocyte precursor cells is induced by platelets, macrophages and inflammation-associated cytokines. *Neuroscience* **140:** 87–100.

Richardson WD, Young KM, Tripathi RB, McKenzie I. 2011. NG2-glia as multipotent neural stem cells: Fact or fantasy? *Neuron* **70:** 661–673.

Robinson S, Petelenz K, Li Q, Cohen ML, Dechant A, Tabrizi N, Bucek M, Lust D, Miller RH. 2005. Developmental changes induced by graded prenatal systemic hypoxic-ischemic insults in rats. *Neurobiol Dis* **18:** 568–581.

Roy NS, Wang S, Harrison-Restelli C, Benraiss A, Fraser RA, Gravel M, Braun PE, Goldman SA. 1999. Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter. *J Neurosci* **19:** 9986–9995.

Roy NS, Cleren C, Singh S, Yang L, Beal MF, Goldman SA. 2006. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by co-culture with telo-

merase-immortalized midbrain astrocytes. *Nat Med* **12:** 1259–1268.

Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJM. 2012. Rejuvenation of regeneration in the aging central nervous system. *Cell Stem Cell* **10:** 96–103.

Setzu A, Lathia JD, Zhao C, Wells KA, Rao M, Ffrench-Constant C, Franklin RJM. 2006. Inflammation stimulates myelination by transplanted oligodendrocyte precursor cells. *Glia* **54:** 297–303.

Shen S, Sandoval J, Swiss V, Li J, Dupree J, Franklin RJM, Casaccia-Bonnefil P. 2008. Age-dependent epigenetic control of differentiation inhibitors: A critical determinant of remyelination efficiency. *Nat Neurosci* **11:** 1024–1034.

Sim FJ, Zhao C, Li WW, Lakatos A, Franklin RJM. 2002a. Expression of the POU domain transcription factors SCIP/Oct-6 and Brn-2 is associated with Schwann cell but not oligodendrocyte remyelination of the CNS. *Mol Cell Neurosci* **20:** 669–682.

Sim FJ, Zhao C, Penderis J, Franklin RJM. 2002b. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J Neurosci* **22:** 2451–2459.

Sim FJ, Lang JK, Waldau B, Roy NS, Schwartz TE, Pilcher WH, Chandross KJ, Natesan S, Merrill JE, Goldman SA. 2006. Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation. *Ann Neurol* **59:** 763–779.

Sim FJ, McClain CR, Schanz SJ, Protack TL, Windrem MS, Goldman SA. 2011. CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. *Nat Biotechnol* **29:** 934–941.

Sloane JA, Batt C, Ma Y, Harris ZM, Trapp B, Vartanian T. 2010. Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. *Proc Natl Acad Sci* **107:** 11555–11560.

Smith KJ, Blakemore WF, McDonald WI. 1979. Central remyelination restores secure conduction. *Nature* **280:** 395–396.

Snyder DH, Valsamis MP, Stone SH, Raine CS. 1975. Progressive demyelination and reparative phenomena in chronic experimental allergic encephalomyelitis. *J Neuropathol Exp Neurol* **34:** 209–221.

Snyder EY, Taylor RM, Wolfe JH. 1995. Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. *Nature* **374:** 367–370.

Stadtfeld M, Hochedlinger K. 2010. Induced pluripotency: History, mechanisms, and applications. *Genes Dev* **24:** 2239–2263.

Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, Kono T, Shioda T, Hochedlinger K. 2010. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* **465:** 175–181.

Stidworthy MF, Genoud S, Suter U, Mantei N, Franklin RJM. 2003. Quantifying the early stages of remyelination following cuprizone-induced demyelination. *Brain Pathol* **13:** 329–339.

Stidworthy MF, Genoud S, Li WW, Leone DP, Mantei N, Suter U, Franklin RJM. 2004. Notch1 and Jagged1 are expressed after CNS demyelination but are not a major rate-determining factor during remyelination. *Brain* **127:** 1928–1941.

Syed YA, Hand E, Mobius W, Zhao C, Hofer M, Nave KA, Kotter MR. 2011. Inhibition of CNS remyelination by the presence of semaphorin 3A. *J Neurosci* **31:** 3719–3728.

Syed YA, Baer A, Hofer MP, Gonzalez GA, Rundle J, Myrta S, Huang JK, Zhao C, Rossner MJ, Trotter MW, et al. 2013. Inhibition of phosphodiesterase-4 promotes oligodendrocyte precursor cell differentiation and enhances CNS remyelination. *EMBO Mol Med* **5:** 1918–1934.

Tamaki SJ, Jacobs Y, Dohse M, Capela A, Cooper JD, Reitsma M, He D, Tushinski R, Belichenko PV, Salehi A, et al. 2009. Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. *Cell Stem Cell* **5:** 310–319.

Tang DG, Tokumoto YM, Raff MC. 2000. Long-term culture of purified postnatal oligodendrocyte precursor cells. Evidence for an intrinsic maturation program that plays out over months. *J Cell Biol* **148:** 971–984.

Trapp BD, Nave KA. 2008. Multiple sclerosis: An immune or neurodegenerative disorder? *Annu Rev Neurosci* **31:** 247–269.

Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD. 2010. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. *J Neurosci* **30:** 16383–16390.

Urayama A, Grubb JH, Sly WS, Banks WA. 2004. Developmentally regulated mannose 6-phosphate receptor-mediated transport of a lysosomal enzyme across the blood–brain barrier. *Proc Natl Acad Sci* **101:** 12658–12663.

Vela JM, Molina-Holgado E, Arevalo-Martin A, Almazan G, Guaza C. 2002. Interleukin-1 regulates proliferation and differentiation of oligodendrocyte progenitor cells. *Mol Cell Neurosci* **20:** 489–502.

Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, Weinmaster G, Barres BA. 1998. Notch receptor activation inhibits oligodendrocyte differentiation. *Neuron* **21:** 63–75.

Wang S, Bates J, Li X, Schanz S, Chandler-Militello D, Levine C, Maherali N, Studer L, Hochedlinger K, Windrem M, et al. 2013. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell* **12:** 252–264.

Warrington AE, Asakura K, Bieber AJ, Ciric B, Van KV, Kaveri SV, Kyle RA, Pease LR, Rodriguez M. 2000. Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis. *Proc Natl Acad Sci* **97:** 6820–6825.

Watanabe M, Hadzic T, Nishiyama A. 2004. Transient upregulation of Nkx2.2 expression in oligodendrocyte lineage cells during remyelination. *Glia* **46:** 311–322.

Werner HB, Kuhlmann K, Shen S, Uecker M, Schardt A, Dimova K, Orfaniotou F, Dhaunchak A, Brinkmann BG, Mobius W, et al. 2007. Proteolipid protein is required for transport of sirtuin 2 into CNS myelin. *J Neurosci* **27:** 7717–7730.

Williams A, Piaton G, Aigrot MS, Belhadi A, Theaudin M, Petermann F, Thomas JL, Zalc B, Lubetzki C. 2007a. Sem-

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aphorin 3A and 3F: Key players in myelin repair in multiple sclerosis? *Brain* **130:** 2554–2565.

Williams A, Piaton G, Lubetzki C. 2007b. Astrocytes—Friends or foes in multiple sclerosis? *Glia* **55:** 1300–1312.

Windrem MS, Roy NS, Wang J, Nunes M, Benraiss A, Goodman R, McKhann GM, Goldman SA. 2002. Progenitor cells derived from the adult human subcortical white matter disperse and differentiate as oligodendrocytes within demyelinated lesions of the rat brain. *J Neurosci Res* **69:** 966–975.

Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G, Roy NS, Goldman SA. 2004. Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nat Med* **10:** 93–97.

Windrem MS, Schanz SJ, Guo M, Tian GF, Washco V, Stanwood N, Rasband M, Roy NS, Nedergaard M, Havton LA, et al. 2008. Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. *Cell Stem Cell* **2:** 553–565.

Wolswijk G. 1998. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. *J Neurosci* **18:** 601–609.

Wolswijk G. 2002. Oligodendrocyte precursor cells in the demyelinated multiple sclerosis spinal cord. *Brain* **125:** 338–349.

Wolswijk G, Noble M. 1989. Identification of an adult-specific glial progenitor cell. *Development* **105:** 387–400.

Wolswijk G, Noble M. 1992. Cooperation between PDGF and FGF converts slowly dividing $O\text{–}2A^{adult}$ progenitors to rapidly dividing cells with characteristics of $O\text{-}2A^{perinatal}$ progenitor cells. *J Cell Biol* **118:** 889–900.

Woodruff RH, Fruttiger M, Richardson WD, Franklin RJM. 2004. Platelet-derived growth factor regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination. *Mol Cell Neurosci* **25:** 252–262.

Xin M, Yue T, Ma Z, Wu FF, Gow A, Lu QR. 2005. Myelinogenesis and axonal recognition by oligodendrocytes in brain are uncoupled in *Olig1*-null mice. *J Neurosci* **25:** 1354–1365.

Yamanaka S. 2007. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* **1:** 39–49.

Yamanaka S. 2008. Pluripotency and nuclear reprogramming. *Philos Trans R Soc B* **363:** 2079–2087.

Yandava BD, Billinghurst LL, Snyder EY. 1999. "Global" cell replacement is feasible via neural stem cell transplantation: Evidence from the dysmyelinated shiverer mouse brain. *Proc Natl Acad Sci* **96:** 7029–7034.

Yang N, Zuchero JB, Ahlenius H, Marro S, Ng YH, Vierbuchen T, Hawkins JS, Geissler R, Barres BA, Wernig M. 2013. Generation of oligodendroglial cells by direct lineage conversion. *Nat Biotechnol* **31:** 434–439.

Yao DL, Liu X, Hudson LD, Webster Hd. 1995. Insulin-like growth factor I treatment reduces demyelination and up-regulates gene expression of myelin-related proteins in experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci* **92:** 6190–6194.

Ye F, Chen Y, Hoang T, Montgomery RL, Zhao XH, Bu H, Hu T, Taketo MM, van Es JH, Clevers H, et al. 2009. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the β-catenin–TCF interaction. *Nat Neurosci* **12:** 829–838.

Yuen TJ, Johnson KR, Miron VE, Zhao C, Quandt J, Harrisingh MC, McFarland HF, Franklin RJM, Ffrench-Constant C. 2013. Identification of endothelin-2 as an inflammatory factor that promotes CNS remyelination. *Brain* **136:** 1035–1047.

Zawadzka M, Rivers LE, Fancy SPJ, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, et al. 2010. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell Stem Cell* **6:** 578–590.

Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. 2001. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol* **19:** 1129–1133.

Zhang Y, Argaw AT, Gurfein BT, Zameer A, Snyder BJ, Ge C, Lu QR, Rowitch DH, Raine CS, Brosnan CF, et al. 2009. Notch1 signaling plays a role in regulating precursor differentiation during CNS remyelination. *Proc Natl Acad Sci* **106:** 19162–19167.

Zhao C, Li WW, Franklin RJM. 2006. Differences in the early inflammatory responses to toxin-induced demyelination are associated with the age-related decline in CNS remyelination. *Neurobiol Aging* **27:** 1298–1307.

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