Published in final edited form as:

Exp Neurol. 2016 September ; 283(Pt B): 476–488. doi:10.1016/j.expneurol.2016.05.010.

Glial progenitor cell-based treatment of the childhood leukodystrophies

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Abstract

The childhood leukodystrophies comprise a group of hereditary disorders characterized by the absence, malformation or destruction of myelin. These disorders share common clinical, radiological and pathological features, despite their diverse molecular and genetic etiologies. Oligodendrocytes and astrocytes are the major affected cell populations, and are either structurally impaired or metabolically compromised through cell-intrinsic pathology, or are the victims of mis-accumulated toxic byproducts of metabolic derangement. In either case, glial cell replacement using implanted tissue or pluripotent stem cell-derived human neural or glial progenitor cells may comprise a promising strategy for both structural remyelination and metabolic rescue. A broad variety of pediatric white matter disorders, including the primary hypomyelinating disorders, the lysosomal storage disorders, and the broader group of non-lysosomal metabolic leukodystrophies, may all be appropriate candidates for glial progenitor cell-based treatment. Nonetheless, a variety of specific challenges remain before this therapeutic strategy can be applied to children. These include timely diagnosis, before irreparable neuronal injury has ensued; understanding the natural history of the targeted disease; defining the optimal cell phenotype for each disorder; achieving safe and scalable cellular compositions, designing age-appropriate controlled clinical trials; and for autologous therapy of genetic disorders, achieving the safe genetic editing of pluripotent stem cells. Yet these challenges notwithstanding, the promise of glial progenitor cell-based treatment of the childhood myelin disorders offers hope to the many victims of this otherwise largely untreatable class of disease.

Keywords

Stem cells; glial progenitor cells; oligodendrocyte progenitor cells; pluripotent stem cells; cell therapy; myelin; remyelination; white matter; multiple sclerosis; leukodystrophies

Introduction

Glial progenitor cells are present throughout the adult central nervous system (CNS) and are the source of mature oligodendrocytes necessary for myelin maintenance and repair. Direct or indirect injury to oligodendrocytes and/or their progenitors results in myelin loss, and is the basis of a large number of genetic and acquired disorders of the central white matter.

The leukodystrophies in particular are heritable disorders of the CNS white matter, with or without peripheral nervous system (PNS) involvement, that have in common glial cells or myelin sheath abnormalities. As a group, their pathology is characterized by the loss of oligodendrocytes, although their specific cellular mechanisms of disease vary widely; many include significant axonal pathology. As currently defined, the hereditary leukodystrophies exclude the acquired disorders of myelin, such as multiple sclerosis, toxic and vascular disorders affecting myelin, disorders with primary neuronal involvement, and inborn errors of metabolism with predominant systemic symptoms (Vanderver et al., 2015). Instead, newer classification schemes have been proposed to better assign individual disease phenotypes into categories reflecting common pathogenic mechanisms. As such, the major childhood leukodystrophies may be defined as either lysosomal (e.g., Krabbe disease, metachromatic leukodystrophy, Tay-Sachs and the gangliosidoses, Salla disease and other sialic acidurias, and fucosidosis, among others); peroxisomal (adrenoleukodystrophy, peroxisomal biogenesis disorders); and mitochondrial (leukoencephalopathies with lactate elevation), by way of example. Other disorders may be defined by their causally implicated cell type, such as Pelizaeus Merzbacher disease (PMD), which targets myelinating oligodendrocytes (Gow et al., 1998), and Alexander disease, which is caused by astrocytic cytoskeletal pathology (Messing et al., 2012). More traditionally, the hereditary disorders of myelin have been described in three groups according to disease histopathology (Powers, 2004); this parallel nomenclature includes, 1) the hypomyelinating disorders, characterized by decreased or absence formation of myelin, such as PMD and other primary hypomyelinating disorders; 2) the metabolic demyelinating diseases, typically characterized by enzymatic deficiency and the misaccumulation of lipid components of myelin, such as Krabbe disease and MLD; and 3) those disorders resulting from gross tissue loss, such as vanishing white matter disease and Canavan disease. The genetic and biochemical bases as well as the clinical presentations of these and other childhood myelin disorders have been extensively reviewed elsewhere (Helman et al., 2015; Parikh et al., 2015; Pouwels et al., 2014; Powers, 2004).

To address this panoply of disorders, a number of cell-based strategies have been developed to replace lost or deficient oligodendroglia and/or astrocytes, or to serve as vectors for enzyme delivery in metabolic and lysosomal storage disorders (Figure 1). This review will focus on defining those cell types that have been developed to safely achieve effective myelin repair, the diseases for which these cells may prove specifically appropriate, and the pre-clinical studies upon which those predictions are based. We will focus particularly on the hereditary disorders of myelin, for which the development of glial cell-based therapies holds particular promise, given the well-understood molecular and cellular basis of these disorders, and the predominant involvement of glial pathology and dysmyelination in their pathogenesis.

Cellular phenotypes appropriate for treatment of the myelin disorders

Neural stem cells

Neural stem cells (NSCs) are self-renewing and multi-lineage component derivatives of the early neuroepithelium (Gage, 2000; Temple, 2001) that can generate a progeny of the three major neural phenotypes, including neurons, astrocytes and oligodendrocytes. NSCs are most prevalent in the developing brain (Keyoung et al., 2001) but also, although in relatively sparse numbers (Pincus et al., 1998), in the subependymal zone and hippocampus of the adult brain (Eriksson et al., 1998; Goldman, 1998; Kirschenbaum et al., 1994; Pincus et al., 1998). Nonetheless, they can be isolated and purified from adult (Arsenijevic et al., 2001; Pincus et al., 1997; Pincus et al., 1998; Roy et al., 2000), as well as fetal brain (Keyoung et al., 2001; Uchida et al., 2000), and readily expanded *in vitro*, maintaining their phenotypic characteristics. CD133⁺-defined NSCs, and in particular their CD24^{-/lo} fraction, can grow as neurospheres and differentiate largely as neurons and astrocytes *in vitro*. Upon transplantation, NSCs can generate neurons and glia in a context-dependent fashion (Keyoung et al., 2001). As such, they constitute a source of oligodendrocyte progenitors that can be mobilized from endogenous stores to remyelinate CNS lesions (Nait-Oumesmar et al., 1999), as well as to myelinate *in vivo* when grafted in hypomyelinated hosts (Uchida et al., 2000; Yandava et al., 1999). However, their *in vivo* differentiation is difficult to instruct, allowing the potential for both heterotopic neuronal differentiation and astrogliosis; as such, they are inefficient as vectors for focused oligodendrocytic and astrocytic production.

Adult glial progenitor cells

GPCs comprise an already lineage-restricted glial progenitor population, that may be better suited to treat disorders of glia, and more appropriate for myelin disease in particular (Goldman et al., 2012), although they do not carry the sustained mitotic competence and scalability of NSCs. GPCs arise from neural stem cells in the subventricular zone, and migrate during development to populate both the subcortical white matter and cortical gray matter (Roy et al., 1999; Scolding et al., 1998a). They comprise between 3–5% of all cells in the adult human subcortical white matter, and persist in analogous if not higher numbers in the cortex, as has been reported in the adult rodent brain (Dawson et al., 2003).

GPCs are the principal remyelinating cell type of the adult CNS and can give rise to both oligodendrocytes and astrocytes (Tripathi et al., 2010; Zawadzka et al., 2010). While glial progenitors are commonly referred to in the literature as oligodendrocyte progenitor cells (OPCs), human GPCs can give rise to oligodendrocytes or astrocytes until their terminal division, and oligodendrocytes per se are post-mitotic; as a result, the terms GPCs and OPCs refer to the same phenotype in humans; they are identical. For simplicity's sake, we have chosen to refer to both as GPCs throughout this review.

The presence of GPCs in the adult human brain was inferred in several early studies that identified immature oligodendroglia in adult brain tissue (Armstrong et al., 1992; Gogate et al., 1994). It was later that human mitotic GPCs were isolated from adult human white matter dissociates, upon transfection of cells with green fluorescent protein (GFP) placed under the control of the human early promoter (P2) for the oligodendrocyte protein cyclic

nucleotide phosphodiesterase (P/hCNP2), one of the earliest proteins to be synthesized in developing oligodendrocytes (Roy et al., 1999). The GFP⁺ cells initially expressed gangliosides recognized by the monoclonal antibody A2B5 and matured as oligodendrocytes, progressing through a stereotypic sequence of A2B5, O4/sulfatide and O1/galactocerebroside expression (Bansal et al., 1989). This study (Roy et al., 1999) also confirmed that the O4 antibody against sulfatide, commonly used to identify GPCs in rodents, recognized primarily post-mitotic oligodendroglia, and not their mitotic progenitors in humans (Armstrong et al., 1992).

Importantly, when removed to low density, high purity culture, single adult GPCs isolated either by A2B5-targeted immunotagging or transfection with GFP under the control of the CNP2 promoter, revealed their multipotential nature *in vitro* (Nunes et al., 2003). Upon transplantation in the rat brain, cells primarily generated oligodendrocytes and astrocytes in the white matter, although also differentiated as neurons when introduced in neurogenic environments such as the prenatal olfactory stream and hippocampus (Nunes et al., 2003; Windrem et al., 2002). Together, these data established that the local environmental niche plays a strong role in the fate of transplanted GPCs (Nunes et al., 2003; Sim et al., 2009).

Fetal glial progenitors

To obtain a more scalable source of GPCs capable of mediating large-scale myelination, GPCs were subsequently purified from the late second trimester fetal human brain using magnetic sorting to isolate A2B5⁺ cells, followed by FACS depletion of PSA-NCAM⁻ immature neurons included within the A2B5 pool during development (Windrem et al., 2004). These fetal GPCs expressed the NG2-chondroitin sulfate proteoglycan (Scolding et al., 1998b), as well as the PDGF α receptor (Sim et al., 2006). While NG2-reactivity is expressed by pericytes as well as GPCs, the PDGF α receptor is expressed only by GPCs within the brain. On that basis, we isolated cells using an antibody against the PDGF α receptor epitope recognized as CD140a. These cells differentiated and myelinated more rapidly than A2B5⁺/PSA-NCAM⁻ cells, and thus proved an efficient vector for cell-based remyelination (Sim et al., 2011).

Of note, fetal and adult GPCs differed in their migratory and myelinogenic competence. Upon transplantation into the shiverer brain, fetal cells expanded and migrated rapidly, but myelinated more slowly and with less efficiency than did adult GPCs (Windrem et al., 2004). In contrast, adult-derived GPCs were more biased towards oligodendroglial fate, and myelinated both more efficiently and more rapidly (Windrem et al., 2004). This in turn suggests that GPCs isolated at different stages of gestational development – or as the case may be, of *in vitro* aging – may have distinct phenotypic biases and fate regulation

Pluripotential stem cells

Pluripotential cells, including both embryonic stem cells (ESCs) and induced pluripotential cells (iPSCs) offer a readily scalable source of glial progenitor cells that can be expanded and differentiated to lineages of interest. Their expansion potential distinguishes them from fetal tissue-derivatives, which lack both the availability and propagability of ESC-derived cells. When combined with genetic editing strategies for the correction of defined mutations

(Fox et al., 2014; Smith et al., 2015), iPSCs in particular offer the possibility of autologous cell correction of hereditary and metabolic disorders that might otherwise require allogeneic grafts. Such autologous transplantation may obviate the immunosuppression otherwise required of allogeneic transplants (Robinton and Daley, 2012; Vogel and Holden, 2008). Oligodendrocytes were first generated from human ES cells (Hu et al., 2009), and subsequently from iPSCs (Wang et al., 2013). In both cases, the preparation protocols included the serial application of agents intended to mimic the environment experienced by cells during oligodendroglial lineage progression (reviewed in (Fox et al., 2014).

Both hESCs and hiPSCs may be appropriate for allogeneic cell therapy, but iPSCs may be uniquely appropriate for autologous therapy. iPSCs are somatic cells genetically reprogrammed to a pluripotential state. The first successful reprogramming strategies used retroviruses to express four transcription factors (Oct4, Sox2, Klf4 and c-myc) in mouse fibroblasts (Takahashi et al., 2007; Takahashi and Yamanaka, 2006), and later in human cells (Nakagawa et al., 2008; Yu et al., 2007). Given the risks of insertional mutagenesis, aberrations in epigenetic modification, and oncogenesis upon incomplete silencing of reprogramming genes (Gore et al., 2011; Lister et al., 2011; Ramos-Mejia et al., 2010), subsequent studies focused on minimizing residual transgene footprints, so as to create cells more appropriate for replacement therapy. Transgene-free iPSCs were first generated using Cre-mediated recombination of fibroblasts transduced with floxed reprogramming factors (Soldner et al., 2009); upon transplantation to the rat brain, these iPSC-derived neural stem cells exhibited appropriate migration and neural differentiation, with no evidence of tumor formation (Major et al., 2011). Successive generations of “footprint-free” strategies have since been developed using transient transfection with episomal plasmids, infection with Sendai virus, transduction with synthetic mRNA and miRNAs, and piggyback transposons (reviewed in (Rao and Malik, 2012).

Human iPSCs can be selectively differentiated to both neuronal (Karumbayaram et al., 2009; Swistowski et al., 2010; Wernig et al., 2008) and glial fate (Wang et al., 2013). Differentiation to glial progenitor cells (GPCs) was first demonstrated in rodent iPSCs (Czepiel et al., 2011) (Tokumoto et al., 2010) and later in human iPSCs (Pouya et al., 2011; Wang et al., 2013). Transplantation of human iPSC-derived GPCs generated myelin-producing cells *in vivo*, both focally in the lysolecithin-induced demyelinated rat optic chiasm (Pouya et al., 2011) and more broadly throughout the neuraxis in the congenitally demyelinated shiverer mouse (Wang et al., 2013).

As an alternative to the use of somatic cells in generating iPSCs before differentiating to glial fate, several groups have developed strategies for the direct induction of glial phenotype from fibroblasts (Goldman, 2013; Najm et al., 2013; Pang et al., 2011; Yang et al., 2013). These protocols effectively skip the pluripotential stem cell stage, and thus greatly accelerate the induction process. While this strategy has not yet been applied to human somatic cells, one may anticipate that its accomplishment may enable the rapid production of myelinogenic oligodendroglia from autologous sources. Whether such induced GPCs and induced oligodendroglia can be therapeutically useful will depend upon whether they can be expanded after initial induction, and the extent to which they prove functionally homologous to bona fide oligodendroglia.

Mesenchymal cells

Other cell types besides those of the CNS have been proposed as vectors for brain repair, including neural crest derivatives such as olfactory ensheathing cells (Barnett et al., 2000; Coutts et al., 2013) and Schwann cells (Bachelin et al., 2005; Blakemore and Crang, 1985), and non-neural phenotypes such as mesenchymal stromal cells, also known as mesenchymal stem cells (MSCs) (Wei et al., 2013). Among these, MSCs have enjoyed the most interest as therapeutic vectors. MSCs are stromal cells that can be isolated from bone marrow and human cord blood, which are able to differentiate along a variety of mesodermal lineages (Pittenger et al., 1999), and have been proposed as potential therapeutic agents for a wide variety of neural disorders, including neonatal brain injury (Donega et al., 2014; Wei et al., 2015), ischemic stroke (Gutierrez-Fernandez et al., 2013), and the neurodegenerative disorders (Castorina et al., 2015), as well as multiple sclerosis and the inflammatory myelin disorders (Ben-Hur and Goldman, 2008; Einstein and Ben-Hur, 2008). They are not, however, vectors for cell replacement, in that little credible evidence has yet supported the notion that genetically-unmodified MSCs might differentiate along neuroepithelial lineages. Rather, the principal mechanism of action of MSCs for treating disorders of myelin appears to lie in their anti-inflammatory function. Systemically administered MSCs enter the perivascular spaces of the brain parenchyma, and can differentiate therein as tissue macrophages; in that capacity, they may then modulate central immune surveillance (Priller et al., 2001). In the case of lysosomal disorders, MSCs may then act as vehicles for delivery of the defective or deficient enzyme, which may be conveyed to the host cells by mechanisms such as the mannose-6-phosphate pathway (Urayama et al., 2004). By this means, lysosomal enzymes released from wild-type MSCs may be transported to enzyme-deficient neighbors, permitting local correction of disease-specific metabolic defects and substrate deposition. However, MSCs are not vectors for cell replacement per se. As such, although both hematopoietic and mesenchymal stem cell transplants have proved effective in stabilizing some systemic disorders of substrate misaccumulation (Orchard and Tolar, 2010), they have proven of less benefit in patients with primary CNS disorders, in which both significant neuronal and glial loss have often occurred before treatment initiation, and for which parenchymal penetration and dispersal of the donor cells may be problematic.

iPSC-derived glia for modeling neurological disease

Beyond their use as reagents for direct cell replacement, patient-derived iPSCs are potent tools to study the cellular and molecular mechanisms underlying glial dysfunction in myelin disorders, both in vivo and in vitro. As initial proofs-of-concept, patient-specific iPSC-derived glial progenitors and oligodendrocytes have already been generated and modeled from patients with Pelizaeus Merzbacher disease (Numasawa-Kuroiwa et al., 2014) and primary progressive multiple sclerosis (Douvaras et al., 2014) and neural-derived cells from patients with Rett Syndrome (Marchetto et al., 2010; Walsh and Hochedlinger, 2010) and autism spectrum disorders (ASD) (Griesi-Oliveira et al., 2015; Yazawa and Dolmetsch, 2013). In the case of Pelizaeus Merzbacher disease, it has confirmed the mis-accumulation of mutant PLP1 proteins to the endoplasmic reticulum and resultant ER stress with increased apoptotic loss in patient iPSC-derived oligodendrocytes (Numasawa-Kuroiwa et al., 2014). Similarly, Rett syndrome iPSC-derived neurons showed both morphological and

electrophysiological deficits, which allowed the *in vitro* testing of possible drug candidates to rescue the disease-associated synaptic defects (Marchetto et al., 2010). These latter studies in particular highlight the potential use of hiPSC-derived glia not only in disease modeling, but also in the phenotype-specific testing of disease-modifying drug candidates in neural and glial progenitor cells and their derivatives. In addition, the recent development of human glial chimeras derived from patient-specific hiPSCs and hESCs should permit the *in vivo* assessment of these same agents, on disease-specific and patient-derived human cells in live adult animals, a unique capability afforded by this pairing of human iPSC-derived glial production with neonatal CNS chimerization (Goldman et al., 2015).

Myelin restoration in animal models of congenital hypomyelination

The ability of NSCs and GPCs to remyelinate *in vivo* has been tested in a variety of models of congenital hypomyelination. The most widely used of these is the shiverer mouse, a spontaneous loss-of-function mutant deficient in myelin basic protein (MBP), the last five exons of which are deleted in the shiverer allele (Roach et al., 1985). Homozygous mice fail to express immunodetectable MBP, fail to form compact myelin, and thus develop ataxia and seizures, dying by 20–22 weeks of age. Although *shiverer* replicates no known human disease, it provides a platform by which to evaluate the efficacy of transplanted cells to myelinate host axons in hypomyelinated environments.

The first reports of transplantation-mediated remyelination appeared using dissociates of fetal brain tissue, engrafted into the *shiverer* brain (Lachapelle et al., 1983; Lachapelle et al., 1994). Later efforts used progressively more defined donor cell populations, by generating Myc-transduced immortalized (Yandava et al., 1999) and epidermal growth factor (EGF)-responsive (Mitome et al., 2001) neural stem cell lines. Lines of human NSCs derived from CD133-sorted cells have also been reported to produce compact and functional myelin in shiverer (Uchida et al., 2012). Yet the efficiency of oligodendrocyte production from neural stem cells *in vivo* is variable, as cells may also differentiate as neurons and astrocytes, and unpredictably so. To address this issue, we and others have focused on the use of glial progenitor cells, the transit-amplifying later-stage derivatives of neural stem cells.

Mitotic human glial progenitor cells were first isolated using a CNP2 promoter-directed strategy, which permitted the identification of A2B5 as an appropriate antibody for the recognition and isolation of these cells (Roy et al., 1999). Human GPCs were subsequently isolated from both the second trimester fetal forebrain, and from surgically-resected adult subcortical white matter, using A2B5-directed selection in tandem with depletion of PSA-NCAM-defined neuroblasts. These cells achieved significant migration and myelination upon transplantation into neonatal shiverers, resulting in whole neuraxis myelination (Windrem et al., 2004; Windrem et al., 2008), which was associated with both phenotypic and clinical rescue of many of the engrafted mice (Windrem et al., 2008). Subsequent studies focused on isolating a more pure GPC population on the basis of their expression of CD140a, an ectodomain epitope of the human PDGF α receptor, which is expressed by the oligodendrocyte-competent fraction of the A2B5-defined progenitor pool (Sim et al., 2006; Sim et al., 2011). These cells exhibited even more rapid migration and robust myelination

than the A2B5 fraction from which they were largely derived, suggesting their superiority as therapeutic vectors (Sim et al., 2011).

Upon transplantation in the rodent brain, both A2B5 and PDGFR α -defined progenitor pools migrate preferentially throughout the major white matter paths of the forebrain, cerebellum and spinal cord (Figure 2). The cells follow local environmental cues, as they respect the white-grey matter borders and do not transverse the barrier between central and peripheral nervous system. Their differentiation fate is also context dependent, such that when transplanted in the normally myelinated rodent white matter, they primarily persist as glial progenitors and astrocytes, but they differentiate broadly when transplanted in hypomyelinating environments, such as in the shiverer mouse brain or within lyssolecithin-induced demyelinated lesions (Figure 2). Their mitotic competence leads to robust engraftment within the animal models, with an appropriate decrease in the Ki67-defined mitotic pool over time. The differences between fetal and adult-derived GPCs in their lineage competence and migratory, mitotic and differentiation potential (Windrem et al., 2004), recall previously reported differences in cell cycle duration and expansion potential between postnatal and adult rodent GPCs (Noble et al., 1992). While fetal GPCs are capable of generating both oligodendrocytes, astrocytes and their progenitors, in a context-dependent fashion, adult GPCs are committed to the oligodendroglial lineage fate. Upon transplantation, fetal cells expand and migrate more readily, with slower but progressive differentiation and myelination, as opposed to adult cells, which disperse and divide less but myelinate faster and more efficiently (Windrem et al., 2004).

Given the inherent heterogeneity, variable provenance, unpredictable availability and ethical concerns regarding the use of fetal tissue-derived cells, methods have been developed for producing GPCs from human pluripotent cells. Similar efficiency in generating glial progenitors, myelinating oligodendrocytes and astrocytes was shown upon transplantation in the shiverer mouse brain (Wang et al., 2013) and later using iPSCs-derived from patients with multiple sclerosis (Douvaras et al., 2014). Pluripotent ES-derived oligodendrocyte progenitor cells showed efficient repair in the radiation-induced demyelinated rat brain (Piao et al., 2015). A later stage in oligodendroglial lineage was targeted (O4 stage), which resulted in high differentiation percentage and rapid myelination, with no co-astrocytic generation and no mitotic competence. Tumor formation was not observed in any of these experiments.

Engrafted hGPCs can remyelinate and rescue a murine model of hypomyelination

To address the potential use of GPCs as cellular vectors for the treatment of congenital disorders of myelin, Windrem et al. (2008) generated a multisite neonatal injection protocol, that included injections in the corpus callosum, internal capsule, cerebellar and brainstem white matter, to ensure that implanted GPCs had contiguous access to all major white matter tracts of the neuraxis, without having to traverse intervening grey matter. This protocol resulted in significant cell dispersal and, ultimately, effective myelination throughout the entire brain, brainstem, cerebellum, spinal cord and roots of recipient mice, both using fetal-derived (Windrem et al., 2008) and iPSC-derived GPCs (Wang et al., 2013) (Figure 3).

Donor-derived myelin effectively unsheathed and enwrapped host axons, exhibited normal compaction and ultrastructure, and restored both nodes of Ranvier and transcallosum conduction velocities in transplanted mice (Figure 3). Most importantly, these animals enjoyed markedly prolonged survival related to the untreated controls, with many frankly rescued. The rescued animals exhibited a nearly normal phenotype and neurologic function and a significant proportion achieved normal lifespans. Transplantation of both tissue-derived and iPSC-derived GPCs resulted in rescue of the shiverer mouse. Interestingly, more than half of the mice transplanted with iPSC-derived grafts lived beyond 6 months, a higher percentage compared to the recipients of fetal-derived cells, suggesting the superiority of iPSC-derived GPCs as therapeutic vectors. These experiments further underline the potential of human GPCs obtained from different sources in the treatment of congenital disorders of myelin.

Disease targets for neural and glial progenitor cell therapy

The disorders most suited for cell therapy are those with well-understood etiology and pathophysiology, in which only one or a few discrete cell types are affected. Among those, disorders primarily involving the glia, which include the congenital disorders of myelin, are particularly attractive targets, as these typically result from primary pathology limited to oligodendrocytes and/or astrocytes, and the etiology is reasonably well understood for many of them. In contrast, disorders that affect both glia and/or neurons comprise more difficult targets, given the multiplicity of cell types involved by the disease process, and the difficulty of restoring neurons and their networks to their premorbid state.

The childhood disorders of white matter can be parsed into three principal groups, defined as such by their underlying pathophysiology: 1) the hypomyelinating disorders, characterized by decreased or aberrant myelin formation; 2) the metabolic demyelinations, characterized by myelin loss after initially normal formation, and often associated with the appearance of toxic metabolites and misaccumulation of metabolic substrates; and, 3) the non-metabolic disorders of myelin, such as ischemic injury, often resulting in gross tissue destruction and loss. In all of these, cell-based treatment strategies aim to replace the abnormal glial cells with oligodendrocytes capable of forming functional myelin, as well as to serve as vehicles for the delivery of wild-type enzymes whose replacement might be sufficient to restore or maintain normal myelin by the deficient host cells.

Hypomyelinating disorders

Pelizaeus-Merzbacher disease (PMD) is an X-linked hereditary disorder of myelin formation caused by mutations in the proteolipid protein 1 (PLP1) gene, which encodes the proteolipid protein of myelinating oligodendroglia (Garbern et al., 1999). Patients suffer from prominent hypotonia, nystagmus and developmental delay. The phenotype is a result of decreased or abnormally formed PLP protein and leads to significant mortality and morbidity. PMD is a very attractive target for cell therapy because the primarily affected cell type, the myelinating oligodendrocyte, is identified and their replacement by progenitors capable of differentiation and functional myelin formation can restore myelination. The ability of these cells to generate compact and functional myelin upon transplantation in the symptomatic

and asymptomatic shiverer-immunodeficient mice brain, (Uchida et al., 2012), along with the capability of human GPCs to rescue a murine model of congenital hypomyelination (Goldman et al., 2012; Wang et al., 2013; Windrem et al., 2008), were the basis for the first phase 1 clinical trial assessing intracerebral transplantation of human neural stem cells in four patients with congenital PMD (NCT01005004) (Gupta et al., 2012). The trial investigators reported a favorable safety profile at 1-year after transplantation, by both clinical and radiological evaluation, but the efficacy of these neural stem cell grafts requires further evaluation with time. Long-term follow-up safety and preliminary efficacy outcomes, including clinical neurodevelopmental assessment, radiological evaluation and neurophysiological evaluation (electroencephalogram and somatosensory evoked potentials) will be assessed 4 years after transplantation (NCT01391637).

Similarly, other hypomyelinating disorders, such as the PMD variant hereditary spastic paraparesis type 2 (Steenweg et al., 2010), the PMD-like disorder associated with mutations in the gap junction protein connexin 47 (Hobson and Garbern, 2012), the broadly hypomyelinated 18q- syndrome (Steenweg et al., 2010) the thyroid hormone transporter (MCT8) deficiency of Allen-Herdon-Dudley syndrome (Vaurs-Barriere et al., 2009) and others (Pouwels et al., 2014), may also be considered viable candidates for oligodendrocyte replacement therapy. That said, several of these disorders are not well characterized, and may exhibit either peripheral nervous system or visceral organ involvement, neither of which is treated via centrally-delivered GPCs. As such, careful assessment of each disease and each patient will need to be performed before attempting intracerebral progenitor cell-based therapy.

Metabolic demyelinating disorders

These disorders are characterized by an enzymatic deficit that leads to accumulation of abnormal metabolites, which become toxic to glial cells and/or neurons. The aims of cell-based therapy for such disorders are to replace the enzymatic deficit, either by allogeneic transplantation of glial cells or by autologous transplantation of genetically modified cells expressing normal or supplemented levels of the defective enzyme, and possibly by concurrent cell replacement. Both neural and glial progenitor cells can act as vehicles for enzyme delivery, which can integrate into the deficient host cells and target the lysosome through the mannose-6-phosphate receptor pathway (Jeyakumar et al., 2005; Urayama et al., 2004).

NSCs transplantation in models of mucopolysaccharidoses (MPS) first revealed the therapeutic potential of cell therapy in metabolic disorders involving the central nervous system. Intraventricular transplantation of transduced murine NSCs, and later fetal-derived human NSCs (Buchet et al., 2002; Meng et al., 2003), restored beta-glucuronidase levels in an animal model of mucopolysaccharidoses VII (Sly disease) (Snyder et al., 1995). In GM2 gangliosidosis (Tay-Sachs and Sandhoff diseases), NSCs transplantation also improved survival with significant enzymatic delivery, both via wild-type murine NSCs (Lacorazza et al., 1996), and via human NSCs transduced to overexpress β -hexosaminidase (Lee et al., 2007). Recipients of human NSCs showed significant survival benefit, even when transplanted after the onset of symptomatic disease (Jeyakumar et al., 2009), suggesting

favorable effects in subjects already manifesting disease, a situation more often encountered clinically.

In neuronal ceroid lipofuscinosis (NCL), neurodegenerative disorders caused by a various lysosomal enzymatic defects which result in excessive accumulation of lipofuscin, transplantation of NCSs also showed favorable results with decreased accumulation of this toxic pigment (Tamaki et al., 2009). Following the encouraging pre-clinical studies, a phase I clinical trial was initiated in 2006 for infantile and late infantile NCL, caused by deficiency of the lysosomal enzyme palmitoyl-protein thioesterase 1 (PPT1) or tripeptidyl peptidase 1 (TTP1), respectively. The trial consisted of allograft transplantation of a banked human NSC line in the brain parenchyma of 6 patients with advanced disease (NCT00337636). This phase 1 study was the first conducting intracerebral stem cell transplantation in the pediatric brain; while it reported an acceptable safety profile, it claimed no evidence of functional efficacy (Selden et al., 2013).

Another lysosomal disorder manifesting significant CNS pathology is Niemann-Pick type A disease, which is caused by a deficiency of acid sphingomyelinase, resulting in sphingomyelin mis-accumulation and consequent glial pathology. In a mouse model of Niemann-Pick A, transplantation of murine NSCs engineered to overexpress human acid sphingomyelinase yielded the correction of enzyme levels, with reversal of lysosomal pathology and clearance of the toxic metabolites (Shihabuddin et al., 2004). Krabbe disease, or globoid cell leukodystrophy, another lysosomal disease primarily affecting the white matter, might also be a beneficiary of GPC-based treatment, similarly with the dual intention of enzyme correction and cell replacement. Krabbe disease is caused by a loss-of-function mutation in galactocerebrosidase (GalC), the loss of which results in oligodendrocytic death and demyelination. Kondo and colleagues (2005) elegantly demonstrated that GalC can be transferred from wild-type cells to GalC deficient oligodendrocytes, by transplanting GalC deficient oligodendrocytes taken from *twitcher* mice into the hypomyelinated *shiverer* mouse brain. The transplanted GalC-null *twitcher* cells achieved normal levels of GalC, presumably transferred from GalC-expressing though MBP-null host glia; the *twitcher* cells then generated normal myelin (Kondo et al., 2005). In a related study, intracerebral transplant of murine NSCs restored GalC to detectable levels in the *twitcher* brain (Pellegatta et al., 2006). Subsequent studies using NSCs genetically engineered to overexpress GalC yielded improvements in cell survival and distribution, as well as intracerebral enzyme levels and lifespan (Neri et al., 2011), and highlighted the need to provide adequate levels of the deficient enzyme throughout the whole CNS. More recently, the control of enzyme expression level was achieved in HSCs, by adding a microRNA which down-regulates GalC translation, thus avoiding the toxicity associated with excessive enzymatic production (Gentner et al., 2010; Orchard and Wagner, 2011). One can anticipate the adoption of such methods for use in NSCs and GPCs, so as to optimize enzyme delivery to the brain while minimizing toxicity to the donor cell pool.

Metachromatic leukodystrophy (MLD) is a lysosomal leukodystrophy caused by deficiency of arylsulfatase A (ARSA), which is necessary for breakdown of cerebroside 3-sulfatase. ARSA deficiency results in the accumulation of sulfatide in myelinating cells of both the CNS and PNS. Intracerebral cell grafts of murine neural precursors overexpressing

arylsulfatase A resulted in sulfatide reduction and modest improvement of a murine ARSA-deficient model (Givogri et al., 2006; Kawabata et al., 2006). Similar results were subsequently obtained using neural progenitors derived from ESCs and iPSCs (Doerr et al., 2015; Klein et al., 2006). Although the donor cells were capable of *in vivo* oligodendroglial and astroglial differentiation, they did so at low frequency, possibly accounting for the relatively modest treatment-associated suppression of demyelination with disease progression. Glial progenitor cells may prove better vectors than earlier neural stem or precursor cells for treating these leukodystrophies, by virtue of their more efficient *in vivo* expansion, dispersal and mature glial differentiation.

In both Krabbe disease and MLD, as well as in the mucopolysaccharidoses, therapies solely directed towards the CNS have shown only modest improvements in survival, likely due to progression of concurrent visceral and PNS disease. Indeed, many of the hereditary leukodystrophies have concurrent PNS disease and systemic involvement, beyond the neurologic phenotype. Combining different therapies may then be necessary for functional rescue, a strategy that has proven effective in animal models (Hawkins-Salsbury et al., 2015; Ricca et al., 2015). As a case in point, synergistic improvements in lifespan were noted upon combination of hematopoietic stem cell transplant with intracerebral cell and gene therapy in arylsulfatase-deficient MLD mice (Ricca et al., 2015).

Disease targets of mesenchymal and hematopoietic stem cell-based therapy

Although primarily designed as a means of delivering enzyme activity to deficient visceral sites, transplantation of hematopoietic stem cells, umbilical cord stem cells, and mesenchymal cells have also been attempted for enzyme replacement in the CNS. This strategy depends upon the ability of donor cell-derived macrophages to populate the CNS, whether as perivascular macrophages or as microglia-like cells, where they might provide wild-type enzyme to otherwise deficient host brain. Accordingly, allogeneic umbilical cord stem cell transplants have been found to slow disease progression in both Krabbe and MLD disease, but only when performed early, either pre-symptomatically or in the earliest symptomatic stage (Escolar et al., 2005; Martin et al., 2013; Solders et al., 2014). The lack of adequate response seen in patients with already-symptomatic disease may in part be due to the slow rate of macrophage colonization of the brain in these patients. To address this possibility, hematopoietic stem cells were transduced to overexpress the defective enzyme was assessed in MLD, in which it yielded improved outcomes in late infantile onset disease (Biffi et al., 2013). On that basis, a phase I/II clinical trial is currently the use of arylsulfatase-overexpressing donor cells in pre-symptomatic or early symptomatic MLD (NCT01560182). While the long-term safety of this approach remains unclear, given the possibility of insertional mutagenesis associated with integrating viral vectors, newer methods of genetic editing – once their own reliability and safety are optimized – might be expected to improve the safety profile of such studies going forward.

Non-lysosomal leukodystrophies

Childhood ataxia with central nervous system hypomyelination (CACH) or vanishing white matter disease (VWM) is an autosomal recessive disease caused by mutations affecting any of the 5 subunits of the eukaryotic translation initiation factor (EIF2B2), resulting in rapid neurological deterioration triggered by head trauma, fever or other stress-inducing event (van der Knaap et al., 2002). The disease predominantly affects the white matter, leading to cystic degeneration and frank cavitation. At a microscopic level, there is both oligodendrocytic (van der Knaap et al., 2006) and astrocytic pathology (Bugiani et al., 2011). As such, the implantation of GPCs may prove a viable approach towards replacing the genetically defective cells and restoring both normal glia and functional myelin to affected patients. A variety of other non-lysosomal hereditary disorders exist, which may be variably amenable to GPC-based cell therapy, depending upon the extent to which pathology is limited to glia, and whether the affected glial cells are replaceable by immigrating donor cells. Among those is adrenoleukodystrophy (ALD), a peroxisomal leukodystrophy characterized by fatty acid metabolism abnormalities and consequent myelin pathology, Alexander disease, characterized by mutations in the astrocytic glial fibrillary acidic protein (GFAP) which result in secondary oligodendroglial involvement, Canavan disease, caused by mutations in the aspartoacylase (ASPA) gene which encodes for aspartoacylase and results in neuronal dysfunction and myelin degeneration by virtue of accumulation of *n*-acetyl L-aspartate (NAA) in neurons and oligodendroglial precursors, among others.

Whether GPC grafts will be effective vectors for therapy in these and other leukodystrophies will depend upon their ability to integrate within the host, compete successfully with the endogenous progenitor pool, and do so in a time frame rapid enough to provide meaningful benefit before disease progression supervenes. The concurrence of peripheral nervous system disease in many disorders of CNS myelin, adrenoleukodystrophy being a prototypic example, also comprises a significant limitation, as GPCs do not cross the CNS-PNS barrier (Windrem et al., 2008). Further studies, conducted on a disease-by-disease basis in pathologically-appropriate animal models, will be needed to identify and validate those disease targets most appropriate for CNS cell therapy using human GPCs.

White matter disorders of the perinatal brain

Cerebral palsy encompasses a group of disorders that result from perinatal injury. Although not without overlap, those can be divided into disorders of prematurity, which include both periventricular leukoencephalomalacia (PVL) and germinal matrix hemorrhage leading to intraventricular hemorrhage (IVH), and disorders affecting the term brain, which include neonatal hypoxic ischemic injury due to asphyxia (HIE). Macroscopically, PVL is characterized by diffuse astrogliosis, associated with microscopic necrosis and axonopathy (Buser et al., 2012); cystic necrosis is another traditional feature of the pathology, which is now rarely seen with recent advances in perinatal care. GPCs and pre-myelinating oligodendrocytes are especially abundant precisely when the risk window period for PVL is the highest. Upon insult, they appear to undergo maturation arrest, by virtue of their particular vulnerability to oxidative stress and excitotoxicity, resulting in consequent hypomyelination (Fancy et al., 2009; Goldman and Osorio, 2014; Volpe, 2009). Replacement of these abnormal cells by healthy, myelinogenic GPCs is an attractive

therapeutic strategy. However, the environmental changes triggered by the initial insult, which include significant changes in the extracellular matrix (Buser et al., 2012), as well as in local astroglial and microglial activation, may not be permissive for oligodendroglial differentiation. In addition, the co-existent injuries to subplate and GABAergic neurons noted in these children may be irreversible (Back and Miller, 2014; Haynes et al., 2008; Volpe, 2009). Pre-clinical animal studies evaluating intracerebral transplantation of neural cells are lacking, because of the scarcity of animal models capable of reliably modeling human prematurity and neonatal brain injury (Kinney and Volpe, 2012). Large animals such as the sheep and the baboon best simulate this complex pathology (Inder et al., 2005; McClure et al., 2008); however those models are expensive and difficult to maintain (Silbereis et al., 2010; Yawno et al., 2013). HIE, which typically affects term or near-term neonates, has an injury pattern that differs from that seen in either perinatal IVH and PVL, in that HIE affects both cortical and subcortical grey matter to a larger extent, although diffuse white matter involvement may also be evident, depending upon the underlying etiology to the ischemic insult (Gunn and Bennet, 2009). Accordingly, intracerebral transplantation of NSCs has been associated with only modest functional improvement, which appeared more related to enhanced endogenous brain repair, rather to cell replacement per se (Bennet et al., 2012; Daadi et al., 2010; Titomanlio et al., 2011). Given the extensive neuronal involvement and loss in HIE, it is not as promising a potential target for repair as PVL.

Choice of donor phenotype for treating the congenital myelin disorders

The cell type most suited for white matter repair – whether oligodendrocytes, astrocytes or their common progenitor - will necessarily vary as a function of the underlying disease process and basis for demyelination. For the hereditary disorders of myelin, glial progenitor cells would seem especially appropriate as candidates for cell replacement. GPCs sorted on the basis of A2B5 or CD140a retain mitotic competence, are highly migratory, and disperse broadly throughout both the central white matter tracts, where they can become either oligodendrocytes or astroglia. Interestingly, GPCs show age-related distinctions in their lineage potential, which may influence the stage of donor cell differentiation at which transplantation is most effective for given disease targets. Fetal-derived and CD140a-sorted GPCs, which are highly migratory but differentiate slowly, might best be used to target disorders requiring significant and widespread cell repopulation and replacement, as in the case of the enzymatic deficiencies, lysosomal storage disorders and metabolic disorders of myelin. In contrast, adult GPCs or those aged to an adult phenotype *in vitro* before transplant, may prove superior vectors for diseases of acute oligodendrocytic loss, such as may occur after inflammatory or ischemic demyelinating events, because of their more rapid remyelination *in vivo* (Windrem et al., 2004). In that regard, cells selected at later stages of oligodendroglial differentiation, based on the presence of the O4 sulfatide antigen, exhibit rapid differentiation and myelination *in vivo*; however, they are no longer mitotically competent, exhibit less astrocytic potential, and are less migratory, likely limiting their use to regionally-restricted myelin disorders solely affecting oligodendrocytes.

Challenges for the use of GPC grafts in the pediatric leukodystrophies

Despite the strong animal data now supporting the potential of transplanted human GPCs for treating the childhood white matter disorders, a number of milestones still need to be achieved before pluripotent cell-derived GPCs can be introduced into clinical trial. Both hESC and hiPSCs will likely need to be maintained under feeder-free and xenogen-free culture conditions, under standardized conditions compliant with good manufacturing practice (GMP). Definitive efficacy studies will need to be done, to the extent possible, over patient-analogous dose ranges in disease-appropriate models, while definitive safety and biodistribution studies will also need to be done, with GMP-compliant GPCs. Furthermore, the routes and means of administration will need to be carefully designed, especially since GPCs intended for structural repair will need to be delivered intracerebrally. As such, human delivery conditions will need to be mimicked in animal models to the extent possible, to ensure both reliable delivery and dosing (Chiu and Rao, 2011).

The additional safety considerations that arise with the use of pluripotential cell-derived GPCs, in particular when derived from reprogrammed and genetically-edited founders, also need to be specifically addressed. Indeed, these considerations alone may delay initial clinical trials of hESC and hiPSC GPCs in the leukodystrophies for several years (Li et al., 2014; Rao and Malik, 2012); pluripotential cells have potential toxicities – undifferentiated growth and tumorigenesis in particular - that must be proactively considered and effectively abrogated before their clinical use can be advanced. We and others have developed prolonged in vitro differentiation strategies coupled with immunoisolation and cell sorting to greatly minimize the risks attendant in pluripotential cell-derived GPCs (Wang et al., 2013), but more definitive long-term safety studies using GMP-compliant hESC-derived GPCs are still needed before patient transplantation. Even the choice of pluripotential cell must be considered. Reprogramming somatic cells to the iPSC stage, even without subsequent genetic correction, may be attended by mutations and chromosomal alterations, changes that may both hinder the use and complicate the regulatory oversight of iPSC derivatives. As such, hESCs may be the preferred source of transplantable GPCs, at least over the initial formative years of cell therapy of the pediatric brain. Yet hESCs do not have the potential for autologous delivery that iPSCs have, so that hESC transplantation is fundamentally allogeneic, and will necessitate some period of immunosuppression in transplant recipients. We have recently reviewed the limitations of both iPSC and hESC-derived GPC therapeutics elsewhere (Goldman, 2016), and refer the reader to that paper for a more in depth discussion of the operational and clinical issues associated with the choice of cell source.

The timing of transplantation will be critical to the success of any clinical trial of the leukodystrophies, and yet this will be a highly disease-specific in nature. It would seem axiomatic that the sooner a therapeutic transplant is delivered, the better, not only because of disease progression, but also because local changes in the tissue environment secondary to the disease may be non-permissive to graft survival, dispersal and appropriate differentiation. Yet few of the leukodystrophies potentially amenable to glial progenitor transplantation are routinely screened in newborns (Duffner et al., 2009; Theda et al., 2014), such that children with sporadic or autosomal recessive disorders typically only present to medical attention after symptom onset (Helman et al., 2015; Parikh et al., 2015). This

presents a paradox, in that whereas in practice most treatment efforts will be directed at already-symptomatic children with relatively advanced disease, much of the preclinical disease modeling thus far has been based on early postnatal transplants into presymptomatic mice. As a result, further testing is needed to assess the efficacy of GPC delivery in children already manifesting disease. That additional body of desired evidence, attesting to the ability of hGPCs to invade, disperse and differentiate in the postnatal brain, will add to the preclinical studies needed to advance this approach to clinical trials, but will at the same time help to ensure the success of this approach.

A related issue in which progress may be delayed by the mismatch between mouse models and human disease is the extent to which PNS involvement can complicate the human leukodystrophies, but often tends to be less apparent in mice, whose short lifespans can inaccurately minimize the role of less-rapidly evolving PNS disease. For disorders that include significant PNS pathology, such as many of the lysosomal storage diseases, effective treatment strategies will need to include systemic delivery of either replacement enzyme, or of enzymatically wild-type cells able to integrate systemic nerves, such as MSCs. As such, the combination of intracerebral hGPC grafts with systemic enzyme replacement therapy or MSCs may be necessary to treat these maladies (Hawkins-Salsbury et al., 2015; Ricca et al., 2015).

Overview

In childhood disorders affecting myelin, oligodendrocytes and/or their progenitors are either diseased, in the case of the hereditary and metabolic leukodystrophies, or lost, as in perinatal brain injury. In this broad set of disorders, glial progenitor cell transplants offer the promise of oligodendrocytic and astrocytic replacement, and remyelination, throughout the diseased white matter. We may now isolate and expand myelinogenic neural and glial progenitor cells from both fetal and adult human brain, and can produce analogous populations of human GPCs from both iPSCs and hESCs. Each of these cellular reagents can rescue pre-clinical models of congenital dysmyelination, and all are potential sources of allogeneic cell-based remyelination. In addition, with the advent of genetic editing technologies, and their pairing with autologously-derived hiPSCs, we may now consider the patient-specific repair, replacement of autologously-derived cells, conceivably permitting the structural as well as functional recovery from inherited disorders of myelin formation and metabolism. That said, each disease target as a group, and every patient individually, will need to be separately evaluated in regards to the specific disease environment into which cells will be introduced and in which they will expand and mature, and how that environment might influence the efficacy as well as the safety of the intended cellular therapeutic. Judiciously applied, such hGPC-based cell therapeutics may provide benefit not only to the hereditary disorders of myelin, but also to disease phenotypes as varied as the inflammatory demyelinating disorders, the ischemic demyelination of cerebral palsy, and no doubt others as the glial contributions to neurological disease become more clear.

Acknowledgments

Dr. Goldman is supported by the Novo Nordisk Foundation, NINDS, NIMH, the National Multiple Sclerosis Society, CHDI, New York Stem Cell Science (NYSTEM), the Mathers Charitable Foundation, Lundbeck

Foundation, Adelson Medical Research Foundation, the ALS Association and PML Consortium. Dr. Osorio is supported by a grant from the American Child Neurology Society. We thank Drs. Su Wang, Martha Windrem and Abdellatif Benraiss for their contribution of images for this review.

References

- Armstrong RC, Dorn HH, Kufta CV, Friedman E, Dubois-Dalcq ME. Pre-oligodendrocytes from adult human CNS. *J Neurosci*. 1992; 12:1538–1547. [PubMed: 1556607]
- Arsenijevic Y, Villemure JG, Brunet JF, Bloch JJ, Deglon N, Kostic C, Zurn A, Aebischer P. Isolation of multipotent neural precursors residing in the cortex of the adult human brain. *Exp Neurol*. 2001; 170:48–62. [PubMed: 11421583]
- Bachelin C, Lachapelle F, Girard C, Moissonnier P, Serguera-Lagache C, Mallet J, Fontaine D, Chojnowski A, Le Guern E, Nait-Oumesmar B, et al. Efficient myelin repair in the macaque spinal cord by autologous grafts of Schwann cells. *Brain : a journal of neurology*. 2005; 128:540–549. [PubMed: 15689363]
- Back SA, Miller SP. Brain injury in premature neonates: A primary cerebral dysmaturation disorder? *Annals of neurology*. 2014; 75:469–486. [PubMed: 24615937]
- Bansal R, Warrington AE, Gard AL, Ranscht B, Pfeiffer SE. Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development. *Journal of neuroscience research*. 1989; 24:548–557. [PubMed: 2600978]
- Barnett SC, Alexander CL, Iwashita Y, Gilson JM, Crowther J, Clark L, Dunn LT, Papanastassiou V, Kennedy PG, Franklin RJ. Identification of a human olfactory ensheathing cell that can effect transplant-mediated remyelination of demyelinated CNS axons. *Brain : a journal of neurology*. 2000; 123(Pt 8):1581–1588. [PubMed: 10908188]
- Ben-Hur T, Goldman SA. Prospects of cell therapy for disorders of myelin. *Ann N Y Acad Sci*. 2008; 1142:218–249. [PubMed: 18990129]
- Bennet L, Tan S, Van den Heuvel L, Derrick M, Groenendaal F, van Bel F, Juul S, Back SA, Northington F, Robertson NJ, et al. Cell therapy for neonatal hypoxia-ischemia and cerebral palsy. *Ann Neurol*. 2012; 71:589–600. [PubMed: 22522476]
- Biffi A, Montini E, Loriglioli L, Cesani M, Fumagalli F, Plati T, Baldoli C, Martino S, Calabria A, Canale S, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science*. 2013; 341:1233158. [PubMed: 23845948]
- Blakemore WF, Crang AJ. The use of cultured autologous Schwann cells to remyelinate areas of persistent demyelination in the central nervous system. *J Neurol Sci*. 1985; 70:207–223. [PubMed: 4056820]
- Buchet D, Serguera C, Zennou V, Charneau P, Mallet J. Long-term expression of beta-glucuronidase by genetically modified human neural progenitor cells grafted into the mouse central nervous system. *Molecular and cellular neurosciences*. 2002; 19:389–401. [PubMed: 11906211]
- 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. Defective glial maturation in vanishing white matter disease. *J Neuropathol Exp Neurol*. 2011; 70:69–82. [PubMed: 21157376]
- Buser JR, Maire J, Riddle A, Gong X, Nguyen T, Nelson K, Luo NL, Ren J, Struve J, Sherman LS, et al. Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. *Ann Neurol*. 2012; 71:93–109. [PubMed: 22275256]
- Castorina A, Szychlinska MA, Marzagalli R, Musumeci G. Mesenchymal stem cells-based therapy as a potential treatment in neurodegenerative disorders: is the escape from senescence an answer? *Neural Regen Res*. 2015; 10:850–858. [PubMed: 26199588]
- Chiu AY, Rao MS. Cell-based therapy for neural disorders--anticipating challenges. *Neurotherapeutics*. 2011; 8:744–752. [PubMed: 21901585]
- Coutts DJ, Humphries CE, Zhao C, Plant GW, Franklin RJ. Embryonic-derived olfactory ensheathing cells remyelinate focal areas of spinal cord demyelination more efficiently than neonatal or adult-derived cells. *Cell Transplant*. 2013; 22:1249–1261. [PubMed: 23031825]
- Czepiel M, Balasubramanian V, Schaafsma W, Stancic M, Mikkers H, Huisman C, Boddeke E, Copray S. Differentiation of induced pluripotent stem cells into functional oligodendrocytes. *Glia*. 2011; 59:882–892. [PubMed: 21438010]

- Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R, Jiang K, Sun G, Wu JC, Steinberg GK. Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. *Stroke; a journal of cerebral circulation*. 2010; 41:516–523.
- Dawson MR, Polito A, Levine JM, Reynolds R. NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Molecular and cellular neurosciences*. 2003; 24:476–488. [PubMed: 14572468]
- Doerr J, Bockenhoff A, Ewald B, Ladewig J, Eckhardt M, Gieselmann V, Matzner U, Brustle O, Koch P. Arylsulfatase A Overexpressing Human iPSC-derived Neural Cells Reduce CNS Sulfatide Storage in a Mouse Model of Metachromatic Leukodystrophy. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2015
- Donega V, Nijboer CH, van Tilborg G, Dijkhuizen RM, Kavelaars A, Heijnen CJ. Intranasally administered mesenchymal stem cells promote a regenerative niche for repair of neonatal ischemic brain injury. *Experimental neurology*. 2014; 261:53–64. [PubMed: 24945601]
- Douvaras P, Wang J, Zimmer M, Hanchuk S, O'Bara MA, Sadiq S, Sim FJ, Goldman J, Fossati V. Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells. *Stem cell reports*. 2014; 3:250–259. [PubMed: 25254339]
- Duffner PK, Caggana M, Orsini JJ, Wenger DA, Patterson MC, Crosley CJ, Kurtzberg J, Arnold GL, Escolar ML, Adams DJ, et al. Newborn screening for Krabbe disease: the New York State model. *Pediatr Neurol*. 2009; 40:245–252. discussion 253–245. [PubMed: 19302934]
- Einstein O, Ben-Hur T. The changing face of neural stem cell therapy in neurologic diseases. *Archives of neurology*. 2008; 65:452–456. [PubMed: 18413466]
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. *Nature medicine*. 1998; 4:1313–1317.
- Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S, Wenger DA, Pietryga D, Wall D, Champagne M, et al. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *N Engl J Med*. 2005; 352:2069–2081. [PubMed: 15901860]
- Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJ, Rowitch DH. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes Dev*. 2009; 23:1571–1585. [PubMed: 19515974]
- Fox JJ, Daley GQ, Goldman SA, Huard J, Kamp TJ, Trucco M. Stem cell therapy. Use of differentiated pluripotent stem cells as replacement therapy for treating disease. *Science*. 2014; 345:1247391. [PubMed: 25146295]
- Gage FH. Mammalian neural stem cells. *Science*. 2000; 287:1433–1438. [PubMed: 10688783]
- Garbern J, Cambi F, Shy M, Kamholz J. The molecular pathogenesis of Pelizaeus-Merzbacher disease. *Arch Neurol*. 1999; 56:1210–1214. [PubMed: 10520936]
- Gentner B, Visigalli I, Hiramatsu H, Lechman E, Ungari S, Giustacchini A, Schira G, Amendola M, Quattrini A, Martino S, et al. Identification of Hematopoietic Stem Cell-Specific miRNAs Enables Gene Therapy of Globoid Cell Leukodystrophy. *Science translational medicine*. 2010; 2:58ra84.
- Givogri MI, Galbiati F, Fasano S, Amadio S, Perani L, Superchi D, Morana P, Del Carro U, Marchesini S, Brambilla R, et al. Oligodendroglial progenitor cell therapy limits central neurological deficits in mice with metachromatic leukodystrophy. *J Neurosci*. 2006; 26:3109–3119. [PubMed: 16554462]
- Gogate N, Verma L, Zhou JM, Milward E, Rusten R, O'Connor M, Kufra C, Kim J, Hudson L, Dubois-Dalcq M. Plasticity in the adult human oligodendrocyte lineage. *J Neurosci*. 1994; 14:4571–4587. [PubMed: 7519254]
- Goldman SA. Adult neurogenesis: from canaries to the clinic. *J Neurobiol*. 1998; 36:267–286. [PubMed: 9712309]
- Goldman SA. White matter from fibroblasts. *Nat Biotechnol*. 2013; 31:412–413. [PubMed: 23657393]
- Goldman SA. Stem and progenitor cell-based therapy of the central nervous system: Hopes, hype, and wishful thinking. *Cell Stem Cell*. 2016; 18:174–188. [PubMed: 26849304]
- Goldman SA, Nedergaard M, Windrem MS. Glial progenitor cell-based treatment and modeling of neurological disease. *Science*. 2012; 338:491–495. [PubMed: 23112326]

- Goldman SA, Nedergaard M, Windrem MS. Modeling cognition and disease using human glial chimeric mice. *Glia*. 2015; 63:1483–1493. [PubMed: 26010831]
- Goldman SA, Osorio J. So many progenitors, so little myelin. *Nature neuroscience*. 2014; 17:483–485. [PubMed: 24671061]
- Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, Canto I, Giorgetti A, Israel MA, Kiskinis E, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature*. 2011; 471:63–67. [PubMed: 21368825]
- Gow A, Southwood CM, ALR. Disrupted proteolipid protein trafficking results in oligodendrocyte apoptosis in an animal model of Pelizaeus-Merzbacher disease. *The Journal of Cell Biology*. 1998; 140:925–934. [PubMed: 9472043]
- Griesi-Oliveira K, Acab A, Gupta AR, Sunaga DY, Chailangkarn T, Nicol X, Nunez Y, Walker MF, Murdoch JD, Sanders SJ, et al. Modeling non-syndromic autism and the impact of TRPC6 disruption in human neurons. *Mol Psychiatry*. 2015; 20:1350–1365. [PubMed: 25385366]
- Gunn AJ, Bennet L. Fetal hypoxia insults and patterns of brain injury: insights from animal models. *Clinics in perinatology*. 2009; 36:579–593. [PubMed: 19732615]
- Gupta N, Henry RG, Strober J, Kang SM, Lim DA, Bucci M, Caverzasi E, Gaetano L, Mandelli ML, Ryan T, et al. Neural Stem Cell Engraftment and Myelination in the Human Brain. *Science Translational Medicine*. 2012; 4:155ra137–155ra137.
- Gutierrez-Fernandez M, Rodriguez-Frutos B, Ramos-Cejudo J, Teresa Vallejo-Cremades M, Fuentes B, Cerdan S, Diez-Tejedor E. Effects of intravenous administration of allogenic bone marrow- and adipose tissue-derived mesenchymal stem cells on functional recovery and brain repair markers in experimental ischemic stroke. *Stem Cell Res Ther*. 2013; 4:11. [PubMed: 23356495]
- Hawkins-Salsbury JA, Shea L, Jiang X, Hunter DA, Guzman AM, Reddy AS, Qin EY, Li Y, Gray SJ, Ory DS, et al. Mechanism-based combination treatment dramatically increases therapeutic efficacy in murine globoid cell leukodystrophy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2015; 35:6495–6505. [PubMed: 25904800]
- Haynes RL, Billiards SS, Borenstein NS, Volpe JJ, Kinney HC. Diffuse axonal injury in periventricular leukomalacia as determined by apoptotic marker fractin. *Pediatr Res*. 2008; 63:656–661. [PubMed: 18520330]
- Helman G, Van Haren K, Bonkowski JL, Bernard G, Pizzino A, Braverman N, Suhr D, Patterson MC, Ali Fatemi S, Leonard J, et al. Disease specific therapies in leukodystrophies and leukoencephalopathies. *Molecular genetics and metabolism*. 2015; 114:527–536. [PubMed: 25684057]
- Hobson GM, Garbern JY. Pelizaeus-Merzbacher disease, Pelizaeus-Merzbacher-like disease 1, and related hypomyelinating disorders. *Semin Neurol*. 2012; 32:62–67. [PubMed: 22422208]
- Hu BY, Du ZW, Li XJ, Ayala M, Zhang SC. Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects. *Development*. 2009; 136:1443–1452. [PubMed: 19363151]
- Inder T, Neil J, Kroenke C, Dieni S, Yoder B, Rees S. Investigation of cerebral development and injury in the prematurely born primate by magnetic resonance imaging and histopathology. *Dev Neurosci*. 2005; 27:100–111. [PubMed: 16046843]
- Jeyakumar M, Dwek RA, Butters TD, Platt FM. Storage solutions: treating lysosomal disorders of the brain. *Nat Rev Neurosci*. 2005; 6:713–725. [PubMed: 16049428]
- Jeyakumar M, Lee JP, Sibson NR, Lowe JP, Stuckey DJ, Tester K, Fu G, Newlin R, Smith DA, Snyder EY, et al. Neural stem cell transplantation benefits a monogenic neurometabolic disorder during the symptomatic phase of disease. *Stem cells*. 2009; 27:2362–2370. [PubMed: 19591217]
- Karumbayaram S, Novitch BG, Patterson M, Umbach JA, Richter L, Lindgren A, Conway AE, Clark AT, Goldman SA, Plath K, et al. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem cells*. 2009; 27:806–811. [PubMed: 19350680]
- Kawabata K, Migita M, Mochizuki H, Miyake K, Igarashi T, Fukunaga Y, Shimada T. Ex vivo cell-mediated gene therapy for metachromatic leukodystrophy using neurospheres. *Brain research*. 2006; 1094:13–23. [PubMed: 16729983]
- Keyoung HM, Roy NS, Benraiss A, Louissaint A Jr, Suzuki A, Hashimoto M, Rashbaum WK, Okano H, Goldman SA. High-yield selection and extraction of two promoter-defined phenotypes of

- neural stem cells from the fetal human brain. *Nat Biotechnol.* 2001; 19:843–850. [PubMed: 11533643]
- Kinney HC, Volpe JJ. Modeling the encephalopathy of prematurity in animals: the important role of translational research. *Neurol Res Int.* 2012; 2012:295389. [PubMed: 22685653]
- Kirschenbaum B, Nedergaard M, Preuss A, Barami K, Fraser RA, Goldman SA. In vitro neuronal production and differentiation by precursor cells derived from the adult human forebrain. *Cereb Cortex.* 1994; 4:576–589. [PubMed: 7703685]
- Klein D, Schmandt T, Muth-Kohne E, Perez-Bouza A, Segschneider M, Gieselmann V, Brustle O. Embryonic stem cell-based reduction of central nervous system sulfatide storage in an animal model of metachromatic leukodystrophy. *Gene Ther.* 2006; 13:1686–1695. [PubMed: 16871228]
- Kondo Y, Wenger DA, Gallo V, Duncan ID. Galactocerebrosidase-deficient oligodendrocytes maintain stable central myelin by exogenous replacement of the missing enzyme in mice. *Proceedings of the National Academy of Sciences of the United States of America.* 2005; 102:18670–18675. [PubMed: 16352725]
- Lachapelle F, Gumpel M, Baulac M, Jacque C, Duc P, Baumann N. Transplantation of CNS fragments into the brain of shiverer mutant mice: extensive myelination by implanted oligodendrocytes. I. Immunohistochemical studies. *Dev Neurosci.* 1983; 6:325–334. [PubMed: 6085571]
- Lachapelle F, Gumpel M, Baumann N. Contribution of transplantations to the understanding of the role of the PLP gene. *Neurochem Res.* 1994; 19:1083–1090. [PubMed: 7528353]
- Lacorazza HD, Flax JD, Snyder EY, Jendoubi M. Expression of human beta-hexosaminidase alpha-subunit gene (the gene defect of Tay-Sachs disease) in mouse brains upon engraftment of transduced progenitor cells. *Nature medicine.* 1996; 2:424–429.
- Lee JP, Jeyakumar M, Gonzalez R, Takahashi H, Lee PJ, Baek RC, Clark D, Rose H, Fu G, Clarke J, et al. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nature medicine.* 2007; 13:439–447.
- Li M, Suzuki K, Kim NY, Liu GH, Izpisua Belmonte JC. A cut above the rest: targeted genome editing technologies in human pluripotent stem cells. *The Journal of biological chemistry.* 2014; 289:4594–4599. [PubMed: 24362028]
- Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O'Malley R, Castanon R, Klugman S, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature.* 2011; 471:68–73. [PubMed: 21289626]
- Major T, Menon J, Auyeung G, Soldner F, Hockemeyer D, Jaenisch R, Tabar V. Transgene excision has no impact on in vivo integration of human iPS derived neural precursors. *PloS one.* 2011; 6:e24687. [PubMed: 21961042]
- Marchetto MC, Carroumeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell.* 2010; 143:527–539. [PubMed: 21074045]
- Martin HR, Poe MD, Provenzale JM, Kurtzberg J, Mendizabal A, Escolar ML. Neurodevelopmental outcomes of umbilical cord blood transplantation in metachromatic leukodystrophy. *Biol Blood Marrow Transplant.* 2013; 19:616–624. [PubMed: 23348427]
- McClure MM, Riddle A, Manese M, Luo NL, Rorvik DA, Kelly KA, Barlow CH, Kelly JJ, Vincore K, Roberts CT, et al. Cerebral blood flow heterogeneity in preterm sheep: lack of physiologic support for vascular boundary zones in fetal cerebral white matter. *J Cereb Blood Flow Metab.* 2008; 28:995–1008. [PubMed: 18091757]
- Meng XL, Shen JS, Ohashi T, Maeda H, Kim SU, Eto Y. Brain transplantation of genetically engineered human neural stem cells globally corrects brain lesions in the mucopolysaccharidosis type VII mouse. *J Neurosci Res.* 2003; 74:266–277. [PubMed: 14515356]
- Messing A, Brenner M, Feany MB, Nedergaard M, Goldman JE. Alexander disease. *J Neurosci.* 2012; 32:5017–5023. [PubMed: 22496548]
- Mitome M, Low HP, van den Pol A, Nunnari JJ, Wolf MK, Billings-Gagliardi S, Schwartz WJ. Towards the reconstruction of central nervous system white matter using neural precursor cells. *Brain.* 2001; 124:2147–2161. [PubMed: 11673317]

- Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Baron-Van Evercooren A. Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci.* 1999; 11:4357–4366. [PubMed: 10594662]
- Najm FJ, Lager AM, Zaremba A, Wyatt K, Capriarello AV, Factor DC, Karl RT, Maeda T, Miller RH, Tesar PJ. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. *Nat Biotechnol.* 2013; 31:426–433. [PubMed: 23584611]
- Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol.* 2008; 26:101–106. [PubMed: 18059259]
- Neri M, Ricca A, di Girolamo I, Alcalá-Franco B, Cavazzin C, Orlacchio A, Martino S, Naldini L, Gritti A. Neural stem cell gene therapy ameliorates pathology and function in a mouse model of globoid cell leukodystrophy. *Stem cells.* 2011; 29:1559–1571. [PubMed: 21809420]
- Noble M, Wren D, Wolswijk G. The O-2A(adult) progenitor cell: a glial stem cell of the adult central nervous system. *Semin Cell Biol.* 1992; 3:413–422. [PubMed: 1489973]
- Numasawa-Kuroiwa Y, Okada Y, Shibata S, Kishi N, Akamatsu W, Shoji M, Nakanishi A, Oyama M, Osaka H, Inoue K, et al. Involvement of ER Stress in Dysmyelination of Pelizaeus-Merzbacher Disease with PLP1 Missense Mutations Shown by iPSC-Derived Oligodendrocytes. *Stem cell reports.* 2014; 2:648–661. [PubMed: 24936452]
- Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G 2nd, Jiang L, Kang J, Nedergaard M, Goldman SA. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nature medicine.* 2003; 9:439–447.
- Orchard PJ, Tolar J. Transplant outcomes in leukodystrophies. *Seminars in hematology.* 2010; 47:70–78. [PubMed: 20109614]
- Orchard PJ, Wagner JE. Leukodystrophy and gene therapy with a dimmer switch. *The New England journal of medicine.* 2011; 364:572–573. [PubMed: 21306246]
- Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Sudhof TC, et al. Induction of human neuronal cells by defined transcription factors. *Nature.* 2011; 476:220–223. [PubMed: 21617644]
- Parikh S, Bernard G, Leventer RJ, van der Knaap MS, van Hove J, Pizzino A, McNeill NH, Helman G, Simons C, Schmidt JL, et al. A clinical approach to the diagnosis of patients with leukodystrophies and genetic leukoencephalopathies. *Molecular genetics and metabolism.* 2015; 114:501–515. [PubMed: 25655951]
- Pellegatta S, Tunici P, Poliani PL, Dolcetta D, Cajola L, Colombelli C, Ciusani E, Di Donato S, Finocchiaro G. The therapeutic potential of neural stem/progenitor cells in murine globoid cell leukodystrophy is conditioned by macrophage/microglia activation. *Neurobiol Dis.* 2006; 21:314–323. [PubMed: 16199167]
- Piao J, Major T, Auyeung G, Policarpio E, Menon J, Droms L, Gutin P, Uryu K, Tchieu J, Soulet D, et al. Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation. *Cell stem cell.* 2015; 16:198–210. [PubMed: 25658373]
- Pincus DW, Harrison-Restelli C, Barry J, Goodman RR, Fraser RA, Nedergaard M, Goldman SA. In vitro neurogenesis by adult human epileptic temporal neocortex. *Clin Neurosurg.* 1997; 44:17–25. [PubMed: 10079997]
- Pincus DW, Keyoung HM, Harrison-Restelli C, Goodman RR, Fraser RA, Edgar M, Sakakibara S, Okano H, Nedergaard M, Goldman SA. Fibroblast growth factor-2/brain-derived neurotrophic factor-associated maturation of new neurons generated from adult human subependymal cells. *Ann Neurol.* 1998; 43:576–585. [PubMed: 9585351]
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science (New York, NY).* 1999; 284:143–147.
- Pouwels PJ, Vanderver A, Bernard G, Wolf NI, Dreha-Kulczewski SF, Deoni SC, Bertini E, Kohlschütter A, Richardson W, Ffrench-Constant C, et al. Hypomyelinating leukodystrophies: translational research progress and prospects. *Annals of neurology.* 2014; 76:5–19. [PubMed: 24916848]

- Pouya A, Satarian L, Kiani S, Javan M, Baharvand H. Human induced pluripotent stem cells differentiation into oligodendrocyte progenitors and transplantation in a rat model of optic chiasm demyelination. *PloS one*. 2011; 6:e27925. [PubMed: 22125639]
- Powers, J. *Myelin Biology and Disorders*. San Diego: Elsevier Academic Press; 2004.
- Priller J, Flugel A, Wehner T, Boentert M, Haas CA, Prinz M, Fernandez-Klett F, Prass K, Bechmann I, de Boer BA, et al. Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nature medicine*. 2001; 7:1356–1361.
- Ramos-Mejia V, Munoz-Lopez M, Garcia-Perez JL, Menendez P. iPSC lines that do not silence the expression of the ectopic reprogramming factors may display enhanced propensity to genomic instability. *Cell Res*. 2010; 20:1092–1095. [PubMed: 20820191]
- Rao MS, Malik N. Assessing iPSC reprogramming methods for their suitability in translational medicine. *J Cell Biochem*. 2012; 113:3061–3068. [PubMed: 22573568]
- Ricca A, Rufo N, Ungari S, Morena F, Martino S, Kulik W, Alberizzi V, Bolino A, Bianchi F, Del Carro U, et al. Combined gene/cell therapies provide long-term and pervasive rescue of multiple pathological symptoms in a murine model of globoid cell leukodystrophy. *Human molecular genetics*. 2015; 24:3372–3389. [PubMed: 25749991]
- Roach A, Takahashi N, Pravtcheva D, Ruddell F, Hood L. Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. *Cell*. 1985; 42:149–155. [PubMed: 2410137]
- Robinton DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. *Nature*. 2012; 481:295–305. [PubMed: 22258608]
- Roy NS, Benraiss A, Wang S, Fraser RA, Goodman R, Couldwell WT, Nedergaard M, Kawaguchi A, Okano H, Goldman SA. Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone. *J Neurosci Res*. 2000; 59:321–331. [PubMed: 10679767]
- Roy NS, Wang S, Harrison-Restelli C, Benraiss A, Fraser RA, Gravel M, Braun PE, Goldman SA. Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter. *J Neurosci*. 1999; 19:9986–9995. [PubMed: 10559406]
- Scolding N, Franklin R, Stevens S, Heldin CH, Compston A, Newcombe J. Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of multiple sclerosis. *Brain*. 1998a; 121(Pt 12):2221–2228. [PubMed: 9874475]
- Scolding NJ, Morgan BP, Compston DA. The expression of complement regulatory proteins by adult human oligodendrocytes. *J Neuroimmunol*. 1998b; 84:69–75. [PubMed: 9600710]
- Selden NR, Al-Uzri A, Huhn SL, Koch TK, Sikora DM, Nguyen-Driver MD, Guillaume DJ, Koh JL, Gultekin SH, Anderson JC, et al. Central nervous system stem cell transplantation for children with neuronal ceroid lipofuscinosis. *J Neurosurg Pediatr*. 2013; 11:643–652. [PubMed: 23581634]
- Shihabuddin LS, Numan S, Huff MR, Dodge JC, Clarke J, Macauley SL, Yang W, Taksir TV, Parsons G, Passini MA, et al. Intracerebral transplantation of adult mouse neural progenitor cells into the Niemann-Pick-A mouse leads to a marked decrease in lysosomal storage pathology. *J Neurosci*. 2004; 24:10642–10651. [PubMed: 15564580]
- Silbereis JC, Huang EJ, Back SA, Rowitch DH. Towards improved animal models of neonatal white matter injury associated with cerebral palsy. *Dis Model Mech*. 2010; 3:678–688. [PubMed: 21030421]
- Sim FJ, Lang JK, Waldau B, Roy NS, Schwartz TE, Pilcher WH, Chandross KJ, Natesan S, Merrill JE, Goldman SA. Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation. *Ann Neurol*. 2006; 59:763–779. [PubMed: 16634042]
- Sim FJ, McClain CR, Schanz SJ, Protack TL, Windrem MS, Goldman SA. CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. *Nature biotechnology*. 2011; 29:934–941.
- Sim FJ, Windrem MS, Goldman SA. Fate determination of adult human glial progenitor cells. *Neuron glia biology*. 2009; 5:45–55. [PubMed: 19807941]

- Smith C, Abalde-Atristain L, He C, Brodsky BR, Braunstein EM, Chaudhari P, Jang YY, Cheng L, Ye Z. Efficient and allele-specific genome editing of disease loci in human iPSCs. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2015; 23:570–577. [PubMed: 25418680]
- Snyder EY, Taylor RM, Wolfe JH. Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. *Nature*. 1995; 374:367–370. [PubMed: 7885477]
- Solders M, Martin DA, Andersson C, Remberger M, Andersson T, Ringden O, Solders G. Hematopoietic SCT: a useful treatment for late metachromatic leukodystrophy. *Bone Marrow Transplant*. 2014; 49:1046–1051. [PubMed: 24797185]
- Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell*. 2009; 136:964–977. [PubMed: 19269371]
- Steenweg ME, Vanderver A, Blaser S, Bizzi A, de Koning TJ, Mancini GM, van Wieringen WN, Barkhof F, Wolf NI, van der Knaap MS. Magnetic resonance imaging pattern recognition in hypomyelinating disorders. *Brain : a journal of neurology*. 2010; 133:2971–2982. [PubMed: 20881161]
- Swistowski A, Peng J, Liu Q, Mali P, Rao MS, Cheng L, Zeng X. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. *Stem cells*. 2010; 28:1893–1904. [PubMed: 20715183]
- Takahashi K, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc*. 2007; 2:3081–3089. [PubMed: 18079707]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
- Tamaki SJ, Jacobs Y, Dohse M, Capela A, Cooper JD, Reitsma M, He D, Tushinski R, Belichenko PV, Salehi A, et al. Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. *Cell Stem Cell*. 2009; 5:310–319. [PubMed: 19733542]
- Temple S. The development of neural stem cells. *Nature*. 2001; 414:112–117. [PubMed: 11689956]
- Theda C, Gibbons K, Defor TE, Donohue PK, Golden WC, Kline AD, Gulamali-Majid F, Panny SR, Hubbard WC, Jones RO, et al. Newborn screening for X-linked adrenoleukodystrophy: further evidence high throughput screening is feasible. *Molecular genetics and metabolism*. 2014; 111:55–57. [PubMed: 24268529]
- Titomanlio L, Kavelaars A, Dalous J, Mani S, El Ghouzzi V, Heijnen C, Baud O, Gressens P. Stem cell therapy for neonatal brain injury: perspectives and challenges. *Ann Neurol*. 2011; 70:698–712. [PubMed: 22162055]
- Tokumoto Y, Ogawa S, Nagamune T, Miyake J. Comparison of efficiency of terminal differentiation of oligodendrocytes from induced pluripotent stem cells versus embryonic stem cells in vitro. *J Biosci Bioeng*. 2010; 109:622–628. [PubMed: 20471604]
- Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. *J Neurosci*. 2010; 30:16383–16390. [PubMed: 21123584]
- Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH, Weissman IL. Direct isolation of human central nervous system stem cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97:14720–14725. [PubMed: 11121071]
- Uchida N, Chen K, Dohse M, Hansen KD, Dean J, Buser JR, Riddle A, Beardsley DJ, Wan Y, Gong X, et al. Human neural stem cells induce functional myelination in mice with severe dysmyelination. *Sci Transl Med*. 2012; 4:155ra136.
- Urayama A, Grubb JH, Sly WS, Banks WA. Developmentally regulated mannose 6-phosphate receptor-mediated transport of a lysosomal enzyme across the blood-brain barrier. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:12658–12663. [PubMed: 15314220]
- van der Knaap MS, Leegwater PA, Konst AA, Visser A, Naidu S, Oudejans CB, Schutgens RB, Pronk JC. Mutations in each of the five subunits of translation initiation factor eIF2B can cause

- leukoencephalopathy with vanishing white matter. *Ann Neurol*. 2002; 51:264–270. [PubMed: 11835386]
- van der Knaap MS, Pronk JC, Scheper GC. Vanishing white matter disease. *Lancet Neurol*. 2006; 5:413–423. [PubMed: 16632312]
- Vanderver A, Prust M, Tonduti D, Mochel F, Hussey HM, Helman G, Garbern J, Eichler F, Labauge P, Aubourg P, et al. Case definition and classification of leukodystrophies and leukoencephalopathies. *Molecular genetics and metabolism*. 2015; 114:494–500. [PubMed: 25649058]
- Vaurs-Barriere C, Deville M, Sarret C, Giraud G, Des Portes V, Prats-Vinas JM, De Michele G, Dan B, Brady AF, Boespflug-Tanguy O, et al. Pelizaeus-Merzbacher-Like disease presentation of MCT8 mutated male subjects. *Ann Neurol*. 2009; 65:114–118. [PubMed: 19194886]
- Vogel G, Holden C. Stem cells. Ethics questions add to concerns about NIH lines. *Science*. 2008; 321:756–757.
- Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol*. 2009; 8:110–124. [PubMed: 19081519]
- Walsh RM, Hochedlinger K. Modeling Rett syndrome with stem cells. *Cell*. 2010; 143:499–500. [PubMed: 21074040]
- Wang S, Bates J, Li X, Schanz S, Chandler-Militello D, Levine C, Maherali N, Studer L, Hochedlinger K, Windrem M, et al. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell*. 2013; 12:252–264. [PubMed: 23395447]
- Wei X, Yang X, Han ZP, Qu FF, Shao L, Shi YF. Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol Sin*. 2013; 34:747–754. [PubMed: 23736003]
- Wei ZZ, Gu X, Ferdinand A, Lee JH, Ji X, Ji XM, Yu SP, Wei L. Intranasal delivery of bone marrow mesenchymal stem cells improved neurovascular regeneration and rescued neuropsychiatric deficits after neonatal stroke in rats. *Cell Transplant*. 2015; 24:391–402. [PubMed: 25647744]
- Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, Broccoli V, Constantine-Paton M, Isacson O, Jaenisch R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:5856–5861. [PubMed: 18391196]
- Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G 2nd, Roy NS, Goldman SA. Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nature medicine*. 2004; 10:93–97.
- Windrem MS, Roy NS, Wang J, Nunes M, Benraiss A, Goodman R, McKhann GM 2nd, Goldman SA. 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*. 2002; 69:966–975. [PubMed: 12205690]
- Windrem MS, Schanz SJ, Guo M, Tian GF, Washco V, Stanwood N, Rasband M, Roy NS, Nedergaard M, Havton LA, et al. Neonatal Chimerization with Human Glial Progenitor Cells Can Both Remyelinate and Rescue the Otherwise Lethally Hypomyelinated Shiverer Mouse. *Cell Stem Cell*. 2008; 2:553–565. [PubMed: 18522848]
- Yandava BD, Billingham LL, Snyder EY. “Global” cell replacement is feasible via neural stem cell transplantation: evidence from the dysmyelinated shiverer mouse brain. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96:7029–7034. [PubMed: 10359833]
- Yang N, Zuchero JB, Ahlenius H, Marro S, Ng YH, Vierbuchen T, Hawkins JS, Geissler R, Barres BA, Wernig M. Generation of oligodendroglial cells by direct lineage conversion. *Nat Biotechnol*. 2013; 31:434–439. [PubMed: 23584610]
- Yawno T, Schuilwerwe J, Moss TJ, Vosdoganes P, Westover AJ, Afandi E, Jenkin G, Wallace EM, Miller SL. Human amnion epithelial cells reduce fetal brain injury in response to intrauterine inflammation. *Developmental neuroscience*. 2013; 35:272–282. [PubMed: 23571644]
- Yazawa M, Dolmetsch RE. Modeling Timothy syndrome with iPSC cells. *J Cardiovasc Transl Res*. 2013; 6:1–9. [PubMed: 23299782]

- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–1920. [PubMed: 18029452]
- Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, et al. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell Stem Cell*. 2010; 6:578–590. [PubMed: 20569695]

Figure 1. Sources and targets of human glial progenitor cells

This schematic illustrates the principal sources of clinically-scalable and engraftable human glial progenitor cells, and highlights some of the principal opportunities for their use in treating the childhood disorders of central myelin.

Figure 2. CD140a-defined glial progenitor cells migrate broadly when introduced to the adult brain

Human fetal-derived GPCs broadly migrate within the major white matter tracts upon transplantation in the adult rat brain. Adult wild type male rats (8–12 wks old) were transplanted with 100,000 fetal-derived human CD140a+ GPCs and sacrificed 12 weeks later to assess the migration and expansion potential of human cells in both the uninjured brain (*top*), and in the lysolecithin-lesioned brain, which harbors focally demyelinated lesions (*lower panel*, pink areas rostral and caudal). Reconstruction of the rat brain performed using 20 μ m coronal sections 1 mm apart. Human cells were identified and mapped by immunolabeling for human nuclear antigen. Cells migrated predominantly within the major white matter tracts and reached a typical maximal radius of 10 mm from the point of administration. The presence of demyelinated lesions distant from the injection site did not evidently affect the migration or expansion patterns of the donor cells.

Figure 3. iPSC-derived hGPCs are as effective as tissue-derived hGPCs in myelinating host brain Human fetal-derived and pluripotent cell-derived GPCs are capable of whole neuraxis myelination. Dot maps indicating the distribution of human donor cells (*red*), immunolabeled using an anti-human nuclear antibody in 14 μ m sagittal sections, 12 months (A1) and 7 months (B1) after neonatal transplant into the shiverer mouse brain. Human GPC-derived myelination in the shiverer forebrain at these same time-points (A2, B2), as identified by myelin basic protein-immunoreactivity (MBP; *green*). Both fetal and iPSC-derived human oligodendroglia myelinated host axons (A3, A4 and B3: MBP, *green*; neurofilament, *red*; human nuclei, *blue*), formed myelin with normal ultrastructure (A5, B4), and reconstituted nodes of Ranvier (A6 and B5: β IV spectrin, *green*; Caspr in *red*).