The childhood leukodystrophies are characterized by neonatal or childhood deficiencies in myelin production or maintenance; these may be due to hereditary defects in genes for myelin maintenance, as in Pelizaeus-Merzbacher disease, or to enzymatic deficiencies resulting in substrate misaccumulation or misprocessing, as in the lysosomal storage disorders. Regardless of their respective etiologies, these disorders are essentially all manifested by a profound deterioration in neurological function with age. A congenital deficit in forebrain myelination is also noted in children with the periventricular leukomalacia of cerebral palsy, which yields a more static morbidity. In light of the wide range of disorders to which congenital hypomyelination or postnatal demyelination may contribute, and the relative homogeneity of oligodendrocytes and their progenitors, the leukodystrophies may be especially attractive targets for cell-based therapeutic strategies. As a result, glial progenitor cells, which can give rise to new myelinogenic oligodendrocytes, have become of great interest as potential vectors for the restoration of myelin to the dysmyelinated brain and spinal cord. In addition, by distributing throughout the neuraxis after perinatal graft, and giving rise to astrocytes as well as oligodendrocytes, glial progenitor cells may be of great utility in rectifying the dysmyelination-associated enzymatic deficiencies of the lysosomal storage disorders.

Oligodendrocytes produce myelin in the postnatal central nervous system (CNS), and their loss or dysfunction is at the heart of a wide variety of diseases of both children and adults, designated the leukodystrophies. Since neurological dysfunction in the leukodystrophies is typically a direct function of myelin absence or loss, a number of cell therapeutic strategies have been developed, intended to either directly restore lost myelin by replacing diseased cells with new oligodendrocytes and their progenitors or to support their viability by introducing other cell types able to restore missing enzymes to an otherwise deficient environment. To accomplish these goals, both neural stem cells and their derived glial progenitor cells (GPCs) have been assessed as potential therapeutics for the treatment of a variety of hereditary metabolic disorders of the brain and spinal cord. These largely pediatric conditions, which include disorders of myelin formation and maintenance as well as those reflecting congenital enzymatic deficiency, compose just a fraction of the diseases of the CNS for which neural stem and progenitor cell therapy are now being evaluated. However, rather than treat that broader topic lightly, I will instead focus herein on the pediatric leukodystrophies and refer to several recent reviews on the larger issues of stem cell–based therapy and modeling of neurological disease. This is hardly an arbitrary choice; the pediatric disorders of myelin may be especially amenable targets for neural stem and progenitor cell transplant and as such stand to be in the vanguard of cell-based therapeutic development.
NEURAL AND NEUROGLIAL PROGENITOR CELLS FOR CELLULAR THERAPY

Neural stem cells, defined as the self-renewing and multilineage-competent derivatives of the early neuroepithelium, are most prevalent in the developing CNS, yet remain within the ventricular subependyma of all adult vertebrates that have been studied. As such, neural stem cells can be isolated to purity from both the fetal and adult human forebrain. While neural stem cells can give rise to neuronal and glial populations alike, a large body of studies have focused on their ability to generate GPCs of the brain and spinal cord. Glial progenitor cells may be generated from both tissue- and embryonic stem cell-derived neural stem cells, but they may also be isolated directly from tissue, including from both the fetal and adult human brain. In the normal adult brain, GPCs disperse and persist widely throughout the parenchyma, within which they reside as relatively primitive neural precursors; when removed from the local tissue environment and raised in vitro, they are able to generate neurons as well as both astrocytes and oligodendrocytes. Yet, in vivo, they appear restricted to a glial fate and appear to generate either or both astrocytes and oligodendrocytes depending on their local signal environment. As such, GPCs may serve as transit-amplifying intermediates between the ventricular zone neural stem cells and their terminally differentiated glial daughters. In vitro, both fetal- and adult-derived GPCs are able to give rise to both astrocytes and oligodendrocytes, but adult GPCs differ markedly from their fetal counterparts in their slower turnover and greater ease of oligodendrocyte maturation and myelination.

Because GPCs can give rise to both oligodendrocytes, the sole myelinating cell type of the adult CNS, and astrocytes, the most prevalent cell type of the adult human CNS and a key regulator of brain metabolic homeostasis, they have been assessed as potential therapeutic vectors in a variety of diseases with prominent glial involvement, especially in the congenitally hypomyelinating and lysosomal storage disorders. Glial progenitor cells are competent to differentiate as myelogenic oligodendrocytes after transplant as a result of which they have been tested extensively in models of acquired adult demyelination, including both experimental allergic encephalomyelitis and spinal cord injury. However, their more immediate value may be in mediating the myelination of congenitally dysmyelinated hosts, since central oligodendrocytes are the primary, and often sole, victims of the underlying disease process. Indeed, given the relative availability and homogeneity of human oligodendrocyte progenitor cells, the disorders of myelin formation and maintenance may be especially compelling targets for cell-based neurological therapy. In addition, by distributing themselves throughout the deficient host neuraxis after perinatal allotransplantation, both neural stem cells and GPCs appear to be of potentially great utility in rectifying enzymatic deficiencies.

GPC TRANSPLANT FOR THE PEDIATRIC MYELIN DISORDERS

The pediatric leukodystrophies are especially attractive targets for a progenitor cell–based treatment strategy. Children have a variety of hereditary diseases of myelin failure or loss that include (1) the hypomyelinating diseases, such as Pelizaeus-Merzbacher disease and hereditary spastic paraplegia 2, and X-linked disorders of proteolipid protein 1 production, which represent primary disorders of myelin; (2) the metabolic demyelinations and lysosomal storage disorders, such as metachromatic leukodystrophy and Tay-Sachs, Sandhoff, and Krabbe diseases, as well as adrenoleukodystrophy and the mucopoly saccharidoses; and (3) disorders of gross tissue loss, such as Canavan disease, vanishing white matter disease, megalencephalic leukoencephalopathy with subcortical cysts, and Alexander disease, all primary glial disorders in which oligodendrocytes are early targets. In addition, a variety of hereditary metabolic disorders that are manifested by early neuronal loss, such as the organic acidurias and neuronal ceroid lipofuscinoses, are accompanied by early oligodendrocyte loss.

Besides these genetic disorders of myelin, periventricular leukomalacia, the most common single form of cerebral palsy, may also be due in part to a perinatal loss of oligodendrocytes and their precursors. As such, cerebral palsy may also be an attractive target for cell-based myelin replacement. Similarly, monotononous forms of childhood acute disseminated encephalomyelitis may prove attractive targets for transplant-based GPC repopulation and remyelination. Indeed, their mechanistic heterogeneity notwithstanding, all of these conditions include the prominent loss of oligodendrocytes and central myelin, highlighting the potential importance of restoring oligodendrocytes and their progenitor cells throughout this wide spectrum of pediatric disorders.

MYELIN RESTORATION IN ANIMAL MODELS OF CONGENITAL HYPOMYELINATION

Cell-based treatments for congenital dysmyelination have now been assessed in a number of genetic models of hypomyelination. The most commonly investigated among these has been the shiverer mouse, a dysmyelinated mouse deficient in myelin basic protein, which was the first hypomyelinated model in which some degree of remyelination could be accomplished through a cell transplant–based strategy. Whereas these first attempts used fetal brain tissues and dissociates thereof, later efforts were directed at using defined donor cell populations for this purpose. Yandava and colleagues reported first reported context-dependent differentiation and myelination of myotransduced murine neural stem cells in shiverer mice, while Mitome and colleagues subsequently reported the widespread dispersal and myelin production by epidermal growth factor–expanded neural stem cells. Following the isolation of adult human GPCs by Roy and colleagues, Windrem and colleagues then transplanted enriched populations of human GPCs, of both fetal and adult origin, into newborn shiverer mice. In these experiments, fetal GPCs were extracted from the late second-trimester forebrain and adult GPCs, from surgically resected subcortical white matter, by either fluorescence-activated or immunomagnetic sorting based on the antigenic phenotype A2B5+/PSA-NCAM, which identifies human GPCs with reasonable specificity and sensi-
tivity. When introduced as highly enriched isolates, both fetal and adult-derived donor GPCs spread widely throughout the white matter, ensheathed resident mouse axons, and formed antigenically and ultrastructurally compact myelin. Fetal GPCs in particular dispersed widely throughout the shiverer forebrain white matter, such that single neonatal injections of GPCs into the lateral ventricles and callosum yielded abundant infiltration of the entire corpus callosum, fimbria, and internal and external capsules, as well as the deep subcapsular white matter to the level of the cerebral peduncles. These traits recalled previously reported differences between postnatal and adult rodent GPCs, which differed in their cell cycle durations and expansion competence as well. The 2 phenotypes also differed in terms of their lineage competence: adult-derived GPCs are multilineage competent in vitro, but in vivo, these cells generated only oligodendrocytes or additional GPCs. In contrast, fetal-derived GPCs generated either astrocytes and oligodendrocytes in vivo in a context-dependent fashion: those donor cells that engulfed presumptive white matter developed as oligodendrocytes or remained as parenchymal progenitors, while those invading cortical or subcortical gray matter developed largely as astrocytes. Thus, fetal and adult GPCs differ in their migration competence, expansion capability, lineage restriction, and maturation rate, all of which need to be considered in the choice of any cell therapeutic intended for use in remyelination.

ENGrafted Fetal HUMAN GPCs CAN ACHIEVE WHOLE-NEURAXIS MYELINATION

As a result of the biological distinctions between fetal and adult GPCs, fetal and adult GPCs may differ in their relative utility in different disease phenotypes and, hence, in their optimal disease targets. Adult GPCs, or fetal cells induced to mature to an adult phenotype in vitro, may be more efficient at the rapid remyelination of acutely demyelinated tissue and as such may prove superior vectors for remyelinating focally demyelinated lesions. In contrast, fetal-derived GPCs, by virtue of their broader migration and expansion potential, might be more appropriate for treating the hereditary and metabolic disorders of myelin, the widespread pathology of which mandates whole-neuraxis dispersal and myelination by any putative cellular therapy. On that basis, my colleagues and I next assessed the specific utility of fetal human GPCs as cellular vectors for the myelination of the congenitally hypomyelinated shiverer brain and spinal cord. This study included a multisite injection protocol that incorporated neonatal cell injections into the cerebellar and brainstem white matter as well as the corpus callosum and internal capsules to ensure that implanted GPCs had ready access to the brainstem and spinal cord and that they could migrate within the major white matter tracts without having to traverse intervening gray matter. This protocol indeed proved sufficient to allow cell dispersal, and ultimately donor-derived myelination, throughout the entire brain, brainstem, cerebellum, and spinal cord and roots of these recipient mice; effectively, their entire CNS was infiltrated and myelinated by neonatally delivered donor cells (Figure, A and B). The donor-derived myelin effectively ensheathed and enwrapped host axons, exhibited normal myelin compaction and ultrastructure (Figure, C-E), and restored both normal transcallosal conduction velocities and nodes of Ranvier (Figure, F) in recipients who underwent transplant.

Most importantly, these animals exhibited prolonged survival relative to untreated shiverers (Figure, G). Whereas untreated shiverer mice typically die by 4 months of age, most mice who underwent transplant enjoyed significantly longer survival: more than a quarter of the graft recipients were frankly rescued, with restoration of the normal lifespan. In our survival series, the mice lived well over a year and seemed destined for normal lifespans until most were killed for histological analysis; a small cohort was allowed to live beyond that point and did so until they were killed at 2 years of age. Many of these older mice exhibited considerable phenotypic improvement as well, with a restitution of substantially normal neurological function. Histologically, the mice manifested progressively denser and more complete axonal ensheathment and myelination with time; indeed, myelination did not become asymptotic throughout the CNS of these animals until roughly 9 months postgraft. In light of the widespread dispersal of donor GPCs, their high-density engraftment and myelination, and their architecturally appropriate and quantitatively significant ensheathment of host axons, these results indicated the feasibility of neonatal progenitor cell implantation as a means of treating, and frankly rescuing the recipients with, the congenital disorders of myelin.

OPTIMIZING CELLULAR AGENTS FOR TREATING THE CONGENITAL MYELIN DISORDERS

Cell transplant–based strategies for treating the dysmyelinating diseases require the acquisition of human neural cells and GPCs in both high purity and high yield. Many of these disorders require whole-neuraxis myelination or remyelination, mandating the introduction of large numbers of progenitor cells biased to oligodendrocyte differentiation and myelogenesis. Whether derived directly from tissue, or from propagated lines of multipotential neural stem cells or pluripotent embryonic stem or induced pluripotent stem (iPS) cells, competent myelogenic cells of reproducible and uniform phenotype must be deliverable in both reliable purity and significant quantity. To address this need, several antibody-based methods for isolating GPCs from mixed cell populations have been developed. In particular, the selective isolation and purification of both fetal and adult human GPCs, by both surface antigen-based fluorescence-activated cell sorting and magnetic cell sorting, have allowed the assessment of these cells in a variety of animal
Figure. Myelination of a leukodystrophic brain by engrafted human glial progenitor cells. Implanted human fetal glial progenitor cells myelinated extensive regions of a shiverer mouse forebrain. These images are taken from either 1-year-old (A and B), 12-week-old (C and D), or 35-week-old (E and F) immunodeficient and myelin-deficient shiverer mice (shi/shi x rag2−/−), implanted at birth with A2B5+/PSA-NCAM−–sorted human glial progenitor cells. A, Low-magnification coronal image of the shiverer forebrain after transplant, immunostained for myelin basic protein (MBP) (green); because these mice are MBP deficient, all observed MBP immunoreactivity is of human origin. B, Higher-magnification view of the engrafted shiverer cerebellum, illustrating the high-efficiency myelination of the cerebellar white matter (MBP, green) by human glial progenitor cells (red) (4',6-diamidino-2-phenylindole counterstain of mouse cells, blue). C, Individual myelinated human oligodendrocytes (human nuclear antigen, red; MBP, green) in the callosum of a 12-week-old shiverer who underwent neonatal transplant. D, Confocal image showing the donor myelin (MBP, red)–ensheathed host axons (neurofilament, green) imaged in the cervical spinal cord of a 1-year-old shiverer x rag2−/− mouse who underwent transplant. E, Optical section through the cerebellar white matter of the 35-week-old shiverer who underwent transplant, manifesting the normal nodal organization of its donor-myelinated axons. Caspr (a paranodal protein), red; Caspr2 (a juxtaparanodal marker), green. F, Electron micrograph of a myelinated fiber in the callosum of a shiverer brain, 12 weeks after neonatal graft, showing the normal major dense lines and myelin lamellae of the donor cell–myelinated recipient axon. G, Kaplan-Meier plot showing the survival curves of shiverer mice who were untreated, were treated with a saline control, or underwent human glial progenitor cell transplant. Whereas all untreated shiverer mice invariably died by 21 weeks of age, a little less than a fourth of mice who received neonatal xenograft lived, some for as long as 2 years after birth, with substantial recovery of lost neurological function. Adapted from Windrem et al.©2011 American Medical Association. All rights reserved.
models of congenital dysmyelination. In shiverer mice, fetal and adult-derived GPCs behaved quite differently after neonatal xenograft. Isolates of human GPCs derived from adult white matter myelinated the recipient brain much more rapidly than did fetal GPCs; adult-derived progenitor cells achieved widespread myelination by just 4 weeks after graft, while cells derived from late second-trimester fetuses took more than 3 months to do so. The adult GPCs also generated oligodendrocytes more efficiently than fetal GPCs and ensheathed more axons per donor cell. In contrast, fetal GPCs emigrated more widely and engrafted more efficiently, differentiating as astrocytes in gray matter regions and oligodendrocytes in white matter.

The divergent behavior of fetal and adult-derived GPCs suggests their respective use for different disease targets. Fetal GPCs may prove more effective for treating disorders of dysmyelination due to enzymatic deficiency, such as occur in lysosomal storage disorders, since the extensive migration of fetal progenitor cells better assures their uniform and widespread dispersal, while their astrocytic differentiation and invasion of gray matter may offer the correction of enzymatic deficits in deficient cortex. In contrast, adult oligodendrocyte progenitor cells, by virtue of their oligodendrocytic bias and rapid myelination, may be most appropriate for diseases of acute oligodendrocytic loss, such as postinflammatory demyelinated lesions and postischemic subcortical demyelinated loci.

NEURAL PROGENITOR CELL–BASED STRATEGIES FOR TREATING METABOLIC AND STORAGE DISORDERS

In the metabolic disorders of myelin, such as Krabbe and Canavan diseases, oligodendrocytes are essentially bystanders, killed by toxic metabolites emanating from cells deficient in 1 or more critical enzymes. Because the engraftment of GPCs is associated with astrocytic as well as oligodendrocytic production, and because both the subcortical and cortical gray matter are infiltrated with donor-derived astrocytes after early implantation, fetal GPCs would seem an especially promising vehicle for the distribution of enzyme-producing cells throughout otherwise deficient brain parenchyma. On that basis, several groups have begun to assess the ability of enzymatically competent, effectively wild-type GPCs to delay or ameliorate the signs and symptoms of the central storage disorders and other metabolic leukodystrophies. Indeed, perinatal grafts of fetal progenitor cells might prove a means of simultaneously myelinating and correcting enzymatic deficiencies in the pediatric leukodystrophies. The lysosomal storage disorders present especially attractive targets in this regard, because wild-type lysosomal enzymes may be released by integrated donor cells and taken up by deficient host cells by neural stem cell implantation was first noted in a mouse model of Sly disease (MPS-VII), in which myc-transduced neural stem cells were implanted neonatally and observed to migrate widely and restore lost enzymatic function broadly in the recipient forebrain. Lactorazza and colleagues subsequently reported expression of β-hexosaminidase on engraftment of transduced neural stem cells into recipient mice. More recently, the same group assessed the utility of human neural stem cells in the neonatal β-hexosaminidase–deficient Sandhoff mouse. These grafts yielded significant engraftment-associated enzyme expression and a corresponding functional and survival benefit to the hosts who received the grafts. Similarly, Pellegrato and colleagues recently transplanted in twitcher mice, a murine model of Krabbe globoid cell leukodystrophy, cultured neural stem cells transduced to overexpress galactocerebrosidase, the enzyme deficient in Krabbe disease. Although the engrafted cells did not survive well in the highly inflammatory environment of the twitcher brain, they migrated appropriately to active sites of demyelination, in a manner akin to that noted by Pluchino et al in adults with experimental allergic encephalomyelitis. Similarly, neural stem cells engineered to overexpress acid sphingomyelinase–deficient Niemann-Pick type A mice efficiently infiltrated regions of forebrain pathology and yielded substantial reductions in misaccumulated lysosomal sphingomyelin. Taken as a group, these experiments lend considerable optimism to the prospect of neural stem cell–based treatment for the relief of the central storage disorders. Particularly in recipients immunosuppressed to reduce both local inflammation and donor cell rejection, trials may be needed to assess the capacity of engrafted neural stem or progenitor cells to delay disease progression, restore lost function, and extend meaningful survival in these and other lysosomal storage disorders.

MESENCHYMAL AND UMBILICAL CORD STEM CELL–BASED TREATMENT OF THE STORAGE DISORDERS

As an alternative to the use of neural cells or GPCs for enzymatic replacement in the CNS, systemically administered hematopoietic and mesenchymal cells with broad distribution may be used as effective vehicles for the delivery of either wild-type or overexpressed protein to central sites of need. Escolar and colleagues first reported clinical benefit in infants with Krabbe disease who received allogeneic umbilical cord blood stem cells. Patients with asymptomatic Krabbe disease receiving these cell grafts exhibited slower disease progression than both controls who did not undergo transplant and those who underwent transplant after symptom onset. In contrast, the benefits of transplant in children after symptomatic impairment seemed minimal. Indeed, the appreciable differences in outcome noted between patients who underwent transplant before and after symptom onset strongly suggest the wisdom of initiating treatment as early as possible after genetic diagnosis in these children; this may prove to be the case with GPCs as well as with umbilical and hematopoietic cell sources, at least when the therapeutic intent is enzyme replacement.

Despite the promise of using non–neural cell grafts in some enzyme deficiency–associated demyelinating dis-
cases, many of these disorders require replacement of enzymes only expressed by neural and glial cells, with unclear transport characteristics within the brain interstitium; treatment of these disorders will likely require neural cell grafts. By way of example, metachromatic leukodystrophy is characterized by deficient expression of arylsulfatase A, which results in sulfatide misaccumulation and oligodendrocyte loss. Mesenchymal and hematopoietic stem cell grafts have proven unable to correct the CNS manifestations of this disorder, yet experimental models of metachromatic leukodystrophy have responded well to GPC grafts. Similarly, the neuronal ceroid lipofuscinoses will likely require neural cell grafts for their cell-based treatment, because the enzymes deficient in this class of disorders are largely neural in their normal expression. In this regard, recent trials to assess the use of human neural stem cell allografts in treating Batten disease (discussed later) speak to the efforts that may be anticipated in developing the use of engrafted neural stem cells and GPCs as vehicles for intracerebral enzyme replacement, in both the lysosomal storage disorders as well as other genetic disorders of brain metabolism characterized by substrate misaccumulation or aberrant catabolism.

Newer approaches to the use of mesenchymal stem cells for enzymatic repletion include microRNA-specified expression of therapeutic genes; the development of such approaches portends the increasing use of therapeutic strategies that combine gene therapies with cell therapy to achieve increasing levels of control of both transgene expression levels and geographic distribution in the recipient brain and spinal cord.

**CHALLENGES FOR THE USE OF GPC GRAFTS IN THE PEDIATRIC LEUKODYSTROPHIES**

One might hope that in recipients immunosuppressed to reduce donor cell rejection, engrafted progenitor cells may indeed prove competent to prevent progressive demyelination in the lysosomal storage disorders and metabolic leukodystrophies. However, few data currently exist with regard to the number or proportion of wild-type cells required to achieve local correction of enzymatic activity and substrate clearance in any storage disorder, and these values will likely need to be obtained for each disease target. Similarly, effective cell doses, delivery sites, and time frames will need to be established in models of congenital hypomyelination before clinical trials of progenitor-based therapy can be contemplated. Moreover, the efficiency of myelination required for significant benefit remains undecided, because functional improvement may require remyelination over much if not the entire linear extent of each recipient axon. These caveats notwithstanding, there are considerable grounds for optimism that cell-based therapy of the pediatric myelin disorders, in particular for primary dysmyelinations such as Pelizaeus-Merzbacher disease, vanishing white matter disease, and spastic diplegic forms of cerebral palsy, may prove both feasible and effective.

**CURRENT TRIALS USING NEURAL STEM AND PROGENITOR CELLS TO TREAT MYELIN DISORDERS**

Neural stem and progenitor cells have already been introduced to the clinic in several early-stage trials. Neural stem cells, derived from the fetal human brain and maintained as uncommitted populations of cells able to give rise to neurons or glia, are currently being assessed in phase 1 trials in Batten disease, specifically in both the infantile and late-infantile forms of neuronal ceroid lipofuscinoses (NCL), as well as in Pelizaeus-Merzbacher disease.

In the NCLs, neural stem cells are essentially being assessed as delivery vehicles for the respective enzymes deficient in infantile and late-infantile NCL, palmityl protein thioesterase 1 and tripeptidyl peptidase 1. Importantly, while propagated neural stem cells have broad differentiation competence, they largely mature as astrocytes and neurons when introduced into adult brain tissue in vivo. Indeed, many investigators have reported that transplanted neural stem cells are biased to generate both neuronal and astrocytic phenotypes in vivo, to the relative detriment of oligodendrocytic differentiation. As such, they may be promising vectors for some metabolic and storage disorders. In that respect, the NCLs are feasible targets for neural stem cell–based cell therapy, and a mouse model of infantile NCL manifested considerable attenuation in both neuronal pathology and lipofuscin accumulation, as well as delayed disease progression, after neonatal neural cell engraftment. Nonetheless, the highly inflammatory nature of the Batten brain, and its early and severe neuronal loss, conspires to make it an especially difficult target for any cell-based therapeutic approach. That being said, the poor prognosis of infants with NCL, and the absence of alternative disease-modifying treatment options, suggested the NCLs as early targets for assessing the therapeutic potential of implanted neural stem cells. A successful phase 1 safety trial was completed in 2008, and a follow-up phase 1b is now under way (http://clinicaltrials.gov; identifier NCT01238315).

As noted, neural stem cells are broader in their lineage potential than are GPCs, which are phenotypically biased to an oligodendrocytic lineage. As such, GPCs will likely prove preferable cellular agents for remyelination than unrestricted neural stem cells. Nonetheless, transplanted neural stem cells have been reported to be capable of oligodendrocytic production and myelination in vivo. On that basis, a phase 1 trial has recently been initiated to assess the safety and potential utility of implanted neural stem cells in connalal Pelizaeus-Merzbacher disease (http://clinicaltrials.gov; identifier NCT01005004). Although it would seem unlikely that propagated neural stem cells may prove as efficient at myelinating hypomyelinated tissue as GPCs, few direct comparative data are available to address this point. Moreover, their relative performance notwithstanding, neural stem cells may nonetheless prove sufficient to establish clinical benefit and have the advantage of reader scalability than GPCs, the long-term propagation and expansion methods for which are still under development. In a similar vein, whether neural stem cells have the broad migration potential exhibited by GPCs is un-
clear, but few actual comparative data are available to that point. Furthermore, we are unaware of any studies that have yet assessed the migration competence of either of these cell types as allografts into human hosts; virtually all available information as to the dispersal of these cells after transplant has been acquired in experimental models, with human cells xenografted into either rodent or canine recipients. Thus, until the initial clinical grafts of these cells are assessed histologically, one can only speculate on the migration and fate of allografted neural and glial precursor cells in the environment of the postnatal human brain, belying any attempt at predicting the best cellular vectors for any given therapeutic indication. As critically important as such determinations are, more data obtained from patients who have undergone transplant will have to be acquired before transplantable cellular phenotypes and their most appropriate disease targets can be optimally paired.

**HUMAN EMBRYONIC STEM- AND iPS-DERIVED NEURAL PROGENITOR CELLS AS CELL THERAPEUTICS**

The practical limitations on both fetal and adult cell acquisition for human allograft have driven research on deriving tissue-specific progenitor cells from both human embryonic stem (hES) cells and iPS cells. Oligodendrocytes derived from hES cells were recently shown to myelinate demyelinated foci in spinal cord contusions. However, neither of these studies followed up animals for the long periods required to ensure the long-term survival and phenotypic stability of the engrafted cells. These are notable deficiencies, in that hES-based approaches may prove limited by the potential for tumorigenesis by any undifferentiated embryonic stem cells in the donor pool, which might yield either teratomas or undifferentiated neuroepithelial tumors after implantation. Although protocols have been reported that appear to minimize the possibility of tumorigenesis—one of which has already been approved for a phase 1 clinical trial in subacute spinal cord injury (http://clinicaltrials.gov, identifier NCT01217008)—one must exercise caution in using unpurified hES progeny as clinical vectors, given the persistent risk of including undifferentiated cells in the transplant pool.

Broad enthusiasm has recently developed for the potential use of iPS cells as a source of new oligodendrocytes for myelin repair. Induced pluripotent stem cells are pluripotent cells that have been generated by the reprogramming of somatic cells to a less phenotypically committed stem cell ground state, through the concurrent forced expression of a small set of transcription factors critical to maintenance of the self-renewing stem cell phenotype. Most typically, iPS cells have been generated from dermal fibroblasts, cotransduced with a number of stem cell–associated transcription factors, including POU5F1 (OCT3/4), SOX2, MYC, KLF4, and/or NANOG. Induced pluripotent stem cells are pluripotential, as defined by their ability to generate cells of all major germ layers and teratomas in vivo. Induced pluripotent stem cells were first generated from mouse and human fibroblasts and have since been generated from a variety of cell types and differentiated into an even greater variety of committed progenitor cells and somatic phenotypes. Most notably among these, the production of dopaminergic neurons from iPS cells validated their ability to generate postmitotic neuronal derivatives. Induced pluripotent stem cells have the decided advantage over hES cells of being readily derived from adult somatic cells, such as dermal fibroblasts or marrow stromal cells. Once so derived, they may be used to produce cell types of interest that may be transplanted as autologous grafts back to the very patients from whom they were generated, thereby obviating the need for posttransplant immune modulation. Yet, to date, no terminally differentiated myelinogenic oligodendrocytes have yet been reported from human iPS cells. Once this important milestone is reached, we may begin to explore the potential for generating populations of iPS-derived oligodendrocytes for autologous grafting in the myelin disorders. That being said, the hurdles that will need to be overcome are similar to those facing hES-derived GPCs and oligodendrocytes: GPCs derived from iPS cells share the same risks as those derived from hES cells, in terms of both unintended differentiation of unrestricted contaminants, as well as frank tumorigenesis. Just as with the use of GPCs derived from hES cells, those generated from iPS cells will need to be purified before use, so as to minimize the risk of any potentially tumorogenic contaminants accompanying the transplanted cell populations. That being said, this risk should be obviated by the many fluorescence- and magnetic-activated cell sorting techniques now available for enriching neural and GPCs to clinically appropriate purity. As a result of these considerations, future studies will need to consider the stringent selection for committed GPCs before any attempt at hES or iPS cell-based therapy.

Taken together, these data suggest the great promise of embryonic stem– and iPS-based production of potentially myelinogenic donor cells. Yet, they also argue that before these promising embryonic stem– or iPS-based strategies may be translated to the clinic, stringent differentiation and isolation of committed GPCs will have to be achieved, so as to ensure both the safety and efficacy of implanted donor cell pools. Until that time, the implantation of tissue-derived GPCs will necessarily be the more clinically feasible option for treatment of the pediatric leukodystrophies and allied myelin disorders.

**OVERVIEW**

In most developmental disorders of myelination, resident GPCs are either lost, as in prenatal stroke and cerebral palsy, or diseased, as in the hereditary and metabolic leukodystrophies. In these disorders, progenitor cell transplants, which can efficiently disperse and myeline the otherwise dysmyelinated CNS, may offer an effective means for treating both infants and children with congenital disorders of myelin. Over the past several years, a number of neural and GPC phenotypes have been identified and isolated that are capable of efficient myelina-
tion of the congenitally dysmyelinated brain and spinal cord, in a variety of experimental models. These cells may now be derived from sources that include fetal and adult human tissue, as well as from hES cells, and it seems likely that transplantable autologous progenitor cells may soon be developed from human iPSC cells as well. It thus seems reasonable to predict that in the next few years, disorders of myelin formation, such as periventricular leukomalacia and Pelizaeus-Merzbacher disease; myelin maintenance, such as in vanishing white matter disease; and postnatal demyelination, such as occurs in the lysosomal storage disorders, may become feasible targets of GPC-based therapeutic trials.

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