Stem Cell Therapy for the Central Nervous System in Lysosomal Storage Diseases

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Neurological diseases with genetic etiologies result in the loss or dysfunction of neural cells throughout the CNS. At present, few treatment options exist for the majority of neurogenetic diseases. Stem cell transplantation (SCT) into the CNS has the potential to be an effective treatment modality because progenitor cells may replace lost cells in the diseased brain, provide multiple trophic factors, or deliver missing proteins. This review focuses on the use of SCT in lysosomal storage diseases (LSDs), a large group of monogenic disorders with prominent CNS disease. In most patients the CNS disease results in intellectual disability that is refractory to current standard-of-care treatment. A large amount of pre-clinical work on brain-directed SCT has been performed in rodent LSD models. Cell types that have been used for direct delivery into the CNS include neural stem cells, embryonic and induced pluripotent stem cells, and mesenchymal stem cells. Hematopoietic stem cells have been an effective therapy for the CNS in a few LSDs and may be augmented by overexpression of the missing gene. Current barriers and potential strategies to improve SCT for translation into effective patient therapies are discussed.

INTRODUCTION

MONOGENIC NEURODEGENERATIVE DISORDERS include a wide range of diseases characterized by various neuronal dysfunctions. The neurological symptoms can include cognitive decline, mental retardation, epileptiform activity, ataxia, chorea, and early death. The disorders may result in degeneration of specific neuronal subtypes, loss of myelinating oligodendrocytes, activation of endogenous inflammatory responses, and progressive degradation of cellular structure. There are few effective treatments for rare diseases and thus a great need to develop new therapeutic approaches.

Current treatment options being investigated include gene therapy to replace dysfunctional proteins, knockdown of dominant negative genes, injection of purified proteins (e.g., enzyme replacement therapy), substrate reduction to reduce accumulated toxic molecules, or hematopoietic stem cell transplantation to provide the missing protein.¹ However, treatment of the CNS component poses a unique challenge, due to the inability of most macromolecules to cross the blood–brain barrier and the

limited entry of hematopoietic cells into the CNS. Stem cell engraftment into the CNS appears promising for reducing or reversing neurodegenerative disease pathology. However, there are significant limitations to engraftment and gene delivery to the sites of pathology that require further investigation.

A large number of monogenic diseases that affect the CNS are lysosomal storage diseases (LSDs), which have been shown in rodent models to be potential candidates for stem cell-based treatments. Lysosomes are the organelles responsible for degradation of macromolecules, and mutations in specific lysosomal hydrolase or regulatory protein genes result in accumulation of undegraded substrates. This results in secondary alterations in numerous genes and proteins in the brain, with subsequent pathologic changes.² Rodent models have served as test systems for neural stem cells (NSCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and hematopoietic stem cell (HSC) transplants.

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GOALS AND CHALLENGES OF STEM CELL TRANSPLANTATION

Despite the expanding understanding of stem cell biology, only HSC transplantation is considered the standard-of-care for most LSDs, with enzyme replacement therapy (ERT) available for some.³ Other types of stem cell transplantation (SCT) have yet to advance into clinical treatments. Several types of stem cells can potentially be used to treat neurological diseases. Cell lines have the advantage of being selected or engineered for positive engraftment properties, but are allogeneic to individual patients. The advent of reprogramming somatic cells into pluripotent stem cells and other types of progenitors makes it possible to harvest cells from patients, correct the defect by gene replacement or repair, reprogram them into stem cells, and reintroduce them into the patient for disease mitigation. In the CNS, stem cells may replace diseased neuronal lineages, rescue demyelination, provide trophic support, induce axonal growth and connectivity, or secrete therapeutic macromolecules to metabolize toxic substrates.^{4–6}

Several barriers need to be surmounted to translate SCT into effective therapeutic uses: (1) matching an ideal donor stem cell subtype with the pathophysiological requirements of an individual disease; (2) providing a permissive host brain environment for increasing donor cell survival; (3) achieving distal migration from sites of intracranial injection; (4) managing the immunological impact of allografts or xenografts on macrophages and resident microglia; (5) prevention of tumor formation from donor stem cells due to incomplete differentiation; and (6) inducing neuroprotective and trophic effects on chronically diseased host brain tissue to prevent further deterioration.⁷

NEURAL STEM CELLS

NSCs include several subpopulations of CNS-originating progenitor cells with multiple fates. During development NSCs arise from embryonic germinal zones, such as those located at the developing neural tube⁸ or neuroepithelium lining the walls of the lateral ventricles.⁹ NSCs continue to be present in the adult brain, but only in specific neurogenic niches, including the subventricular zone of the cerebrum, the dentate gyrus of the hippocampus, the olfactory bulbs, and for a limited period of time the external granule layer of the cerebellum.^{9,10} Neural progenitor subtypes may generate neurons, astrocytes, or oligodendrocytes, depending on the developmental context. The types of NSCs that have been used for experimental SCT include

primary NSCs isolated from neurogenic regions of the brain, glial progenitors, immortalized clonal cell lines, and NSCs differentiated from ESCs or iPSCs.¹¹ Preclinical studies have shown therapeutic promise in several rodent disease models.

Proof-of-principle for NSC transplantation for the treatment of global neurogenetic lesions was established by transplanting the murine NSC line C17.2 into the ventricles of neonatal mice with mucopolysaccharidosis (MPS) type VII, an LSD.¹² The cells migrated into the parenchyma, were distributed throughout the adult brain, and stably engrafted for the lifetime of the animals. This was also the first experiment to use a stem cell from a solid organ as a treatment approach. The largest number of preclinical animal studies on NSC-based transplantation for genetic diseases has been in LSDs.

LSDs are leading candidates for SCT because most of them are caused by genetic deficiencies of specific enzymes, which can be secreted by wild-type or gene-corrected cells and then taken up by surrounding cells to restore the blocked metabolic pathway (a process known as *cross-correction*). NSCs have been used to mediate partial correction of the pathological lesions in the brain in a number of mouse models of LSD, including Batten (infantile neuronal ceroid lipofuscinosis),¹³ Krabbe,^{14–22} Niemann-Pick A/B,^{23,24} metachromatic leukodystrophy (arylsulfatase A deficiency),^{25–28} Sandhoff,^{29–33} Sanfilippo A (MPS IIIA),³⁴ and Sly (MPS VII)^{12,35–37} diseases.

A few LSDs are caused by mutations in genes that regulate biosynthesis or intracellular trafficking of the enzymes or substrates. NSC grafts have been tested in Niemann-Pick type C mice, which have a mutation in a transmembrane cholesterol transporter.^{38,39} NSCs have also been evaluated in a few non-LSD neurogenetic diseases, including the “*shiverer*” mouse with a mutation in myelin basic protein⁴⁰ and in monogenic expanded trinucleotide repeat diseases, such as Huntington disease⁴¹ and spinocerebellar ataxia types 1 and 3.^{42–44}

An important finding from the studies using NSC lines is that donor cells can migrate widely when injected into the developing brain, when signals for migration and differentiation are present,^{12,15,23,25,29,30,34,40} but are greatly restricted when injected into adult brains.^{31,32,35,41,45} Another finding is that the number of cells surviving for long-term engraftment is significantly less with primary NSCs,^{16,17,24,26,28,36} even when the donor cells are selectively enriched for the earliest progenitor stage.⁴⁶ ESC- and iPSC-derived NSCs engraft at levels similar to primary NSCs.^{27,33,37,47} The significantly greater engraftment of the cell lines may be due to their preferential selection among

other clones for their migratory ability.^{33,48,49} Despite the limited engraftment from primary or iPSC-derived NSCs, reversal of lysosomal storage and biomarkers of disease in brain tissue surrounding the grafts have been documented in metachromatic leukodystrophy (arylsulfatase A deficiency),^{24,26,27} Niemann-Pick A/B,²⁴ Krabbe,^{16,17} and Sly (MPS VII)^{36,37,47} diseases.

The limited engraftment in rodent experiments represents a significant barrier to translating this modality into clinical trials for LSDs, as illustrated in large-animal experiments. Transplants of canine cerebellar-derived NSCs formed grafts when injected into the parenchyma of neonatal MPS VII dog brains and could be identified *in vivo* by magnetic resonance imaging detection of the paramagnetic particle-labeled donor NSCs.⁵⁰ However, the donor cells remained near the injection site.⁵⁰ In a nonhuman primate model, autologous iPSC-derived neurons also formed stable grafts, but the number of cells appeared to be relatively small.⁵¹

MESENCHYMAL STEM CELLS

MSCs are a multipotent stem cell population that give rise to mesodermal lineage cells including osteoblasts, tendons, fibroblasts, adipocytes, cartilage, and myocytes.⁵² The most primordial MSCs are derived from bone marrow stroma, but MSCs have also been isolated from adipose tissue, blood, umbilical cord, placenta, muscle tissue, and dental pulp.^{53,54} MSCs are morphologically fibroblastic, adherent under culture conditions, clonally expansive, and transducible; they retain stable karyotypes and differentiate into multiple mesenchymal lineages.⁵²

MSCs share some attributes with NSCs that may influence migratory behavior. For instance, donor MSCs express surface adhesion molecules and respond to chemotactic signals such as SLIT/ROBO, netrin, and neuropilin signaling, which have been implicated in MSC motility as well as neuroblast migration.⁵⁵ As with NSCs, if migration within the CNS can be increased it would be useful for distribution of therapeutic molecules to the global brain lesions in genetic diseases. Direct transplantation of MSCs into the brain in the mouse model of Krabbe disease (globoid cell leukodystrophy) showed evidence of improved neuropathology, including increased myelination and reduced inflammation, which was accompanied by improved motor functions.^{56,57} Mouse MSCs have also been reported to migrate into multiple organs after intravenous or intraperitoneal injection, but only small numbers reach the brain, limiting MSC transplants as a therapy for reversing CNS disease pathology.^{52,53}

MSCs may also serve as an adjuvant therapy to NSCs because they appear to have roles in maximizing cell engraftment of cotransplanted NSCs and in preservation of degenerating host cells. In a rat model of Huntington disease, transplantation of MSCs into the striatum resulted in a reduced immunological response compared with engrafted NSCs alone.⁵⁸ Furthermore, cotransplanting MSCs with NSCs resulted in increased survival of the NSC population, resulting in an improvement in motor function.⁵⁸ The immunomodulatory effect of the MSCs appeared to improve survival and efficacy of the transplanted NSCs.

Evidence that MSC grafts directly support host neuronal survival *in vivo* is seen in Niemann-Pick A/B and type C animal models, where MSC transplants have a neuroprotective effect on endogenous Purkinje cells and promote cell survival.⁵⁹ Donor MSCs delayed overall neurodegeneration, with an improvement in host circuit activity.^{60,61} In Niemann-Pick type C, MSCs can restore diminished vascular endothelial cell growth factor signaling required for proper neuronal function of Purkinje cells, thus providing the trophic support required for proper cerebellar function, which reduced disease pathology.⁶²

Other studies suggest a fusion-like event may occur between bone marrow-derived MSCs and Purkinje neurons.^{61,63,64} This cellular interaction may increase survival of host Purkinje cells and result in improved physiological activity and subsequent rescue of motor function.^{61,63,64} In a mouse model of spinocerebellar ataxia type 1, intrathecal injection of MSCs resulted in improved organization of the Purkinje cell layer of the cerebellum.⁶⁵ Taken together, these data suggest that MSC engraftment may provide indirect trophic support through the release of paracrine factors⁶⁶ and suppression of the host immune response⁶¹ or by direct physical interaction with host cells⁶³ to improve disease pathology. These preclinical studies suggest MSCs possess biological properties that make them well suited for SCT, particularly in hereditary neurodegenerative diseases with demyelination and motor dysfunction.

CLINICAL STUDIES

Although SCT has been tested in relatively few neurologic diseases, these include Parkinson disease,^{67,68} CNS injury,⁶⁹ stroke,⁷⁰ cerebral palsy,⁷¹ and amyotrophic lateral sclerosis,^{72,73} for which multiple reviews are available.^{74–77} Clinical trials of SCT in monogenic neurological diseases have been performed in LSDs, leukodystrophies, and trinucleotide repeat diseases (Table 1^{78–109}).

Table 1. Human clinical studies in neurogenetic diseases, using hematopoietic stem cell, mesenchymal stem cell, or neural stem cell grafts

Disease type and deficiency	Eponym and subtype	Defective gene	Inheritance pattern	Disease prevalence	CNS pathology	Major clinical findings using HSC, MSC, and NSC grafts
LSD (soluble enzyme)	Hurler disease; mucopolysaccharidosis type I (MPS I)	α -L-Iduronidase (IDUA)	Autosomal recessive	1 in 100,000	Mental retardation macrocephaly, spinal stenosis	<ul style="list-style-type: none"> Modified HSCT graft resulted in reduced toxicity, high donor chimerism, and stabilized neurological performance⁷⁹⁻⁸⁰ Combination of ERT plus HSCT was well tolerated and provided increased efficacy when compared with single therapy⁸¹⁻⁸⁴ MSC infusion improved bone mineral density observed in spinal lumbar region in some patients over time⁸⁵
LSD (soluble enzyme)	Batten disease; infantile neuronal ceroid lipofuscinoses (INCLs)	Palmitoyl-protein thioesterase (PPT1)	Autosomal recessive	1 in 100,000	Epilepsy, mental retardation microcephaly, myoclonus, ataxia	<ul style="list-style-type: none"> HSCT infusion resulted in transient benefit with no therapeutic benefit to CNS⁸⁶ NSC transplants into patient brains were well tolerated, remained proximal to the needle track, and did not change neurological decline⁸⁷
LSD (soluble enzyme)	Metachromatic leukodystrophy (MLD)	Arylsulfatase A (ARSA)	Autosomal recessive	1 in 160,000	Demyelination, mental retardation	<ul style="list-style-type: none"> Intravenous infusion of bone marrow-derived MSCs improved nerve conduction in peripheral nerves in majority of patients with MLD⁸⁸ HSCT delayed disease onset and progression as determined by neuropsychological, physiological, radiological, and IQ examination⁸⁸ Lentivirus-corrected HSCs substantially increased enzyme activity in CSF with low toxicity, demonstrated long-term engraftment with reduction in disease pathology, and maintained normal cognition in some patients⁸⁹
LSD (soluble enzyme)	Krabbe disease; globoid cell leukodystrophy (GCL)	Galactosyl-ceramidase (GALC)	Autosomal recessive	1 in 100,000	Demyelination, mental retardation, presence of globoid cells	<ul style="list-style-type: none"> Reversal of disease pathology and substantial benefit to CNS when umbilical cord blood was transplanted into asymptomatic patients^{90,91} However, over an extended period of time the patients began to deteriorate⁹² HSCT did not increase overall patient survival and was of no benefit to CNS⁹³
Leukodystrophy (oligodendro-cyte function)	Pelizaeus-Merzbacher disease (PMD)	Proteolipid protein I (PLP1)	X-linked recessive	1 in 200,000	Nystagmus, hypotonia, hypomyelination, axonopathy	<ul style="list-style-type: none"> NSC allografts injected into cerebral white matter of patients are well tolerated and show modest gains in neuropsychological examinations, motor tests, and radiological imaging⁹⁴
Trinucleotide repeat (multiple neuronal functions)	Huntington disease (HD)	huntingtin-protein (HTT)	Autosomal dominant	3-7 per 100,000	Chorea, personality changes, astrogliosis, loss of medium spiny neurons	<ul style="list-style-type: none"> Injections of fetal neural cells into host striatum show donor gray matter nodule formation, lack of rejection or neoplasm, variable D₂ receptor binding, and some disease stabilization in neurological tests⁹⁵⁻¹⁰¹ <i>Post-mortem</i> tissue histology showed that grafted fetal neural cells differentiate into striatal neurons in host striatum but have limited host connectivity, occupy minimal striatal volume, and acquire host disease-like pathology¹⁰²⁻¹⁰⁴ Long-term patient outcomes after fetal neural cell grafts are inconsistent as shown by neurological and motor tests, or PET imaging with no major functional impact compared with control subjects^{105,106}
Trinucleotide repeat (unknown neuronal function)	Spinocerebellar ataxia type I (SCA I)	Ataxin-1 protein (ATXN1)	Autosomal dominant	3 in 100,000 (all SCA subtypes)	Ataxia, chorea, dystonia, loss of Purkinje cells, granule cells and parallel fibers	<ul style="list-style-type: none"> Patients with SCA who received intravenous and intrathecal infusion of umbilical cord MSCs were evaluated on the basis of balance and ataxia rating scales and showed slight immediate improvement with no long-term functional benefit^{107,108} Single-patient study showed multifocal donor-derived tumor in CNS from transplanted NSCs as confirmed by histology, mass spectrometry, PCR, and <i>in situ</i> hybridization¹⁰⁹

CSF, cerebrospinal fluid; ERT, enzyme replacement therapy; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplantation; MSC, mesenchymal stem cell; NSC, neural stem cell; PET, positron emission tomography.

Most of the trials in LSDs have used HSCs, with or without viral vector modification. NSCs have been used in only one trial in an LSD,⁸⁷ where they were injected into subjects with either of two forms of Batten disease, using multiple injection sites combined with immunosuppression in an attempt to delay disease progression. The subjects tolerated the donor NSCs but engraftment was low and there was little migration away from the transplant sites. In contrast, human NSC transplants in a mouse model of Batten disease (infantile neuronal ceroid lipofuscinosis) engrafted, migrated, differentiated into neural lineages, and produced therapeutic levels of palmitoyl-protein thioesterase 1 enzyme to mitigate host brain pathology.¹³ However, the mouse study used multiple injections over time with increasing NSC doses, whereas the human trial used a single time point with injection into multiple sites, which may account for the differences in outcome.

HSC and MSC systemic transplants have been used in LSDs but have been relatively ineffective in mitigating CNS disease progression.³ There is significant phenotypic variation between diseases and among patients with any specific disorder. However, some LSDs have responded to augmented HSC transplants. Presymptomatic neonatal cord blood HSC transplants (CBT) in Krabbe disease, which requires prenatal diagnosis, initially improved mentation in patients compared with those receiving CBT postsymptomatically.⁹⁰ However, over an extended period of time the patients began to deteriorate.⁹² In metachromatic leukodystrophy (arylsulfatase A deficiency) lentiviral vector transduction of HSCs to overexpress the deficient enzyme improved brain pathology over time in mice and patients.^{89,110} Similarly, lentiviral vector correction of HSCs in the peroxisomal disorder adrenoleukodystrophy also improved the CNS in human patients.¹¹¹ Given the extensive history of little or variable effect on the CNS after bone marrow transplantation in many LSDs, the success of HSC treatment may be limited to certain disease subtypes. Although analysis of *post-mortem* tissue is limited in human studies by the unavailability of tissue samples, the LSD types that may respond to HSC infusion may be diseases where the CNS is more permissive to HSC infiltration or where low levels of enzymatic activity may be sufficient to slow disease progression.

SAFETY CONSIDERATIONS

One potential barrier to stem cell grafts is abnormal growth of the donor cells, although it

appears to be a rare event in the CNS. Hyperplasia of transplanted astrocytes has been documented in mice¹¹²; one patient with hereditary ataxia telangiectasia who received human fetal NSC allografts developed multifocal tumors several years after transplantation,¹⁰⁹ and a clone of an iPSC-derived NSC line has produced abnormal growth in a mouse spinal cord injury model.¹¹³ In the iPSC study, the transplanted cells reactivated OCT4, and thus differentiation into tissue-specific NSCs after reprogramming appeared to be incomplete, which is a concern with therapeutic uses of any reprogrammed cell. Another concern is that high levels of donor NSC engraftment can interfere with neuronal circuits; however, the level required to interfere with function appears to be well above that needed for therapy.¹¹⁴

IMPROVING TRANSLATIONAL FEASIBILITY OF SCT FOR THE CNS

NSCs represent a promising approach for treating the neurological component of LSDs. Because the pathological lesions are widely distributed in the brain due to the inherited deficiency in metabolism affecting all cells, NSC therapy requires widespread dissemination of the donor cells. The transfer of wild-type enzyme from donor cells to surrounding host cells by cross-correction amplifies the therapeutic effect of each donor cell. Proof-of-principle for widespread dissemination has been established in neonatal mouse models of LSDs but is much more limited in adult transplants. In humans, most LSDs are not diagnosed until a child begins to show a constellation of developmental delays, by which time the pathology is already advanced. The human brain is more developmentally mature at birth than the neonatal mouse brain, and thus the demand for donor NSC allografts will be more similar to that of adult transplants in mice.

The greatest engraftment in mouse brains has been achieved with NSC lines because they were selected for their ability to migrate, whereas the engraftment of primary NSCs or those derived from pluripotent stem cells is far less robust. Studies on primary NSC grafts show they tend to spread along white matter tracts with some regional differences,^{17,46,112,115–117} as do iPSC-derived NSCs,³⁷ but are much more constrained within brain parenchymal structures.^{37,45} However, in LSDs even a small graft secreting normal enzyme into the surrounding parenchyma may mitigate disease pathology in a much larger volume of brain tissue surrounding the engrafted donor cells.^{17,35,37} The restricted engraftment of

NSCs in the postnatal brain of rodent models as well as in large-animal and human studies^{50,87} indicates that significant improvements in NSC engineering will be needed to develop them into an efficacious modality for clinical use.

Studies on the minimum density of donor cells that can mediate correction of a specific volume of surrounding brain tissue would provide a goal that NSC engraftment would need to achieve. Advances are also needed in understanding the mechanisms of NSC migration and their differentiation into mature cells in order to engineer NSCs to optimize their therapeutic capacity in the LSD brain. The best evidence for distal migration from injection sites *in vivo* has occurred with medial ganglionic eminence (MGE) GABAergic cell precursors, which

migrate extensively through parenchyma during brain development, and have been used as transplants to suppress host hyperexcitability.^{118–120} Adopting mechanisms required for MGE migration into other NSC populations may allow NSCs to be used to treat other neurodegenerative disease with widely disseminated lesions.

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