The lysosomal storage disorders (LSDs) are a heterogeneous group of inherited metabolic diseases that result from a deficiency in one or more of the 80 enzymes or transporters that normally reside within the lysosomal compartment (1–3). A reduction or loss of one or more of these activities results in the progressive and relentless accumulation of undegraded macromolecules, a consequent derangement of proper lysosomal and autophagosomal functions, and ultimately, cellular demise. However, the precise mechanistic links between these altered biochemical and cellular states and disease pathophysiology and pathogenesis remain unclear. Typically, disease severity is correlated with the level of residual activity, with individuals harboring lower levels presenting with more aggressive and severe disease manifestations. A large percentage of individuals, particularly those with significantly diminished lysosomal function, also exhibit CNS involvement, the extent of which is dependent on the nature of the specific storage metabolites and the differential sensitivity of the resident cell types to the accumulated substrates. Although individually each LSD may be relatively rare, as a group, these disorders have an incidence of approximately 1 per 5,000 live births (4, 5).

Of the approximately 50 LSDs that have been identified thus far, the majority (greater than 70%) exhibit significant CNS involvement (6). This finding is not surprising given that lysosomes and related organelles are ubiquitously present in most cell types and are involved not only in recycling cellular debris but also in maintaining cellular homeostasis (7–9). Irrespective of the LSD, these CNS diseases are typically characterized by generalized inflammation and neurodegeneration in multiple brain regions. In a subset of the diseases, altered calcium homeostasis and oxidative stress may also contribute to the overall pathology (10). Moreover, each specific disease may initially present with a unique temporal and spatial alteration that is dictated by the nature of the storage metabolites prior to the development of a more generalized global derangement (11). In some diseases, the symptoms are evident in neonates, whereas in others, they become apparent only in early childhood. Consequently, some LSDs may require early therapeutic intervention to affect broad and potentially all regions of the CNS.

Gene therapy for the neurological manifestations in lysosomal storage disorders

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Abstract Over the past several years, considerable progress has been made in the development of gene therapy as a therapeutic strategy for a variety of inherited metabolic diseases, including neuropathic lysosomal storage disorders (LSDs). The premise of gene therapy for this group of diseases is borne of findings that genetic modification of a subset of cells can provide a more global benefit by virtue of the ability of the secreted lysosomal enzymes to effect cross-correction of adjacent and distal cells. Preclinical studies in small and large animal models of these disorders support the application of either a direct in vivo approach using recombinant adeno-associated viral vectors or an ex vivo strategy using lentiviral vector-modified hematopoietic stem cells to correct the neurological component of these diseases. Early clinical studies utilizing both approaches have begun or are in late-stage planning for a small number of neuropathic LSDs. Although initial indications from these studies are encouraging, it is evident that second-generation vectors that exhibit a greater safety profile and transduction activity may be required before this optimism can be fully realized. Here, I review recent progress and the remaining challenges to treat the neurological aspects of various LSDs using this therapeutic paradigm. —Cheng, S. H. Gene therapy for the neurological manifestations in lysosomal storage disorders. J. Lipid Res. 2014. 55: 1827–1838.

Supplementary key words neurodegenerative diseases • adeno-associated virus • lentivirus • hematopoietic stem cells • inherited metabolic diseases

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The seminal observation that small amounts of lysosomal enzymes are secreted and can be recaptured by adjacent and distal cells (12) has led to the development of enzyme augmentation therapy for an increasing number of LSDs (13, 14). This ability of lysosomal enzymes to cross-correct cells is facilitated by the cation-independent mannose 6-phosphate receptor that is present, albeit in different abundances, on the surface of virtually all cells (15, 16) or by the mannose receptor, which is primarily located on cells of the reticuloendothelial system (17). Because lysosomal enzymes are invariably decorated with oligosaccharide side chains that bear mannose 6-phosphate residues or can be modified to expose the core mannose structures, systemic delivery of recombinant enzymes has been shown to confer widespread complementation and subsequent reversal of tissue pathology in several LSDs (13, 14, 18–23). This therapeutic concept is also applicable in the context of the emerging technology referred to as gene therapy. Genetic modification of a subset of cells in the liver, muscle, or lung to produce and continuously secrete the deficient enzymes into systemic circulation has been shown to be efficacious in several animal models of these diseases (24–27). However, although these strategies have adequately addressed many of the visceral manifestations associated with LSDs, because the enzymes (and gene delivery vectors) in systemic circulation are unable to efficiently traverse the blood-brain barrier, they do not adequately treat the disease in the CNS.

To address the neuropathic disease associated with LSDs, several initiatives are under consideration. These approaches include: i) stem cell transplantation (hematopoietic and neural origin), ii) direct intracranial or intrathecal injection of the enzyme or gene transfer vector, iii) substrate reduction therapy, iv) nonsense mutation readthrough therapy, v) chaperone therapy, and vi) anti-inflammatory therapy (14, 28, 29). Although several of these experimental therapies are currently under clinical evaluation, only allogenic bone marrow transplantation (30) and substrate reduction therapy using miglustat (31, 32) have been approved as treatments for LSDs. Here, I will review recent accomplishments and emerging developments using gene therapy and their prospects for the treatment of lysosomal storage disease in patients with significant neurological involvement.

CONSIDERATIONS FOR GENE THERAPY OF LSDS

Several features of neuropathic LSDs make them particularly attractive candidates for intervention using gene therapy. For example, the underlying molecular bases for most of these monogenic disorders are well understood. Importantly, genetic correction of a small subset of neural cells may suffice to correct large regions of the CNS by virtue of the ability of the enzymes to diffuse and affect cross-correction of adjacent and distal cells (33–38). Diffusion of the enzymes may occur via the ventricular system or may be facilitated by axonal transport from the site of production to distal sites (39). The amount of enzyme required for therapeutic correction will vary with each disease but may be less than 10% of normal levels based on observed enzyme levels in individuals with attenuated forms of the disease. Hence, the level of gene expression that is necessary for therapeutic efficacy for this group of disorders may be relatively modest. Tight regulation of enzyme levels is not anticipated because preclinical studies have not revealed overt deleterious effects associated with the expression of supraphysiological levels of many of the acidic hydrolases. Moreover, several murine models, as well as naturally occurring large animal models, of LSDs are available, which allows for the assessment of these predictions (40, 41). Finally, because the therapeutic delivery into the CNS will likely be invasive, the use of gene therapy offers the potential of infrequent treatments by virtue of its ability to confer prolonged production of the deficient enzymes. Indeed, a recent clinical study that used a recombinant adeno-associated virus (AAV)-based vector in the brains of Parkinson’s disease patients indicated that transgene expression could be sustained for greater than 4 years following a single administration (42).

To effect gene therapy of neuropathic LSDs, several gene delivery systems have been evaluated and shown to provide some measure of correction of the underlying pathology, as well as the motor and behavioral aberrations in animal models. Recombinant viral- and nonviral-based gene transfer vectors have been employed that were delivered either directly into the CNS or into the systemic circulation (43–46). Transplantation of stem cells that were genetically modified ex vivo has also shown promise. Each system is characterized by its i) cellular tropism, ii) genome integration activity, iii) durability of expression, iv) ease of manufacturing at scale, v) safety, vi) transduction activity of dividing and nondividing cells, and vii) insert size packaging capacity. Although all of these systems are meritorious and worthy of continued investigation, this review will focus on the platforms that have received the most attention and that are maturing in the clinical setting. In particular, I will emphasize the potential of in vivo gene therapy using recombinant AAV-based vectors and ex vivo strategies using lentiviral vectors.

In addition to selecting a gene transfer system that affords optimal reconstitution of the deficient enzyme in the CNS, the route of delivery must also be carefully considered. Noninvasive delivery of vectors (e.g., following intravenous delivery) to the CNS is clearly preferred. However, at least in the context of AAV vectors, because most serotypes described to date do not efficiently traverse the blood-brain barrier, considerable effort has been centered on direct injection into the brain parenchyma or the cerebroventricular or intrathecal space (35, 36, 47–49). Depending on the specific LSD, delivery to select anatomical regions in the brain may result in some improvement; however, global coverage of the CNS is likely necessary for maximal benefit.

The timing of therapeutic intervention is yet another important consideration (50). In some LSDs, neural derangement occurs early in development, which may require treatment immediately following diagnosis and even
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paroviruses are devoid of viral genes and are nonpathogenic
in humans (55) because they require coinfection with a
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these viruses are able to infect nondividing cells and confer
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ture reduces the potential for insertional mutagenesis and
oncogenesis; however, an increased susceptibility to develop
hepatic tumors was noted in mice systemically injected
at birth with an AAV vector expressing β-glucuronidase
(58, 59). Recombinant AAV vectors are capable of accom-
modating approximately 4.5 kb of foreign DNA between
the flanking inverted terminal repeats and as such, should
package most lysosomal cDNAs (including their transcrip-
tional regulatory elements).

To date, many naturally occurring AAV serotypes from
different species and over 100 AAV variants have been
identifi ed (60–64). These isolates exhibit a broad host
range, as well as different tissue tropisms, including the
CNS. Notably, AAV1, -5, -9, and -rh.10 have been shown to
be particularly eff ective at transducing cells in the CNS of
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In addition to the growing library of new AAV variants,
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pects may be important, particularly for LSDs that present
early in life and that are rapidly progressive. However,
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approximately 2.3 kb; this limitation can be overcome
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tional regulatory elements (72). Because lysosomes
are a key component of most cell types in the CNS, the
utilization of strong and ubiquitous promoters is favored
to ensure widespread transgene expression and correction
of the pathology throughout this organ. In this regard,
promoters that have demonstrated adequate performance
in the CNS of animal models of LSDs include those of
chicken β-actin, cytomegalovirus, β-glucuronidase, and
ubiquitin C (33, 38, 73). The incorporation of a protein
transduction domain onto the lysosomal proteins, such as
that of HIV Tat, can also serve to further disperse the se-
creted therapeutically active motifs onto the lysosomal enzymes to encour-
age effective and safe delivery of recombinant AAV vectors to the CNS.

Recombinant AAV vectors

Recombinant AAV vectors are emerging as the in vivo
gene delivery platform of choice for several neurological
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β-glucuronidase into MPS VII mice did not provide any CNS benefit, presumably because these mice harbored elevated CNS levels of sialic acid, which is an inhibitor of the vector (86). Nevertheless, given the encouraging preclinical results in certain LSDs and the recent regulatory approval of systemic gene therapy using AAV9 to potentially address the CNS aspects of spinal muscular atrophy patients (87), this line of research will likely continue in earnest over the next several years.

An additional approach to enhance the delivery of systemically delivered AAV to the CNS is to modify the viral capsids such that they are either detargeted from non-CNS tissues or, preferably, preferentially targeted to the brain vascular endothelium (88, 89). Because the vast brain vasculature forms an intimate network with neural cells, the AAV-transduced endothelial lining is expected to then serve as a depot for the production of lysosomal enzymes to the diseased brain. CNS-targeting entities identified through biopanning using phage libraries are typically engineered onto the viral capsids (e.g., VP3 of AAV) such that these entities (i.e., short peptides) are displayed at the virion surface. Evaluation of these AAV pseudo-variants in the identical species from which the epitopes were identified demonstrated the anticipated preferential targeting to the brain. Chen, Chang, and Davidson (90) have elegantly extended this concept to include peptide sequences that are highly specific for the diseased brain. Using in vivo screening of a phage library in the murine models of MPS VII and late-infantile neuronal ceroid lipofuscinosis (LINCL), these authors identified distinct brain-specific epitopes for each disease. Additionally, these motifs differed from those in normal mice, suggesting that the diseased state imparted a unique vascular molecular signature. Engineering these epitopes into the AAV2 capsid and their evaluation in the respective lysosomal storage disease mouse models demonstrated the anticipated brain tropism and global correction of the neuropathology (90). However, whether these attributes are translatable to other species, and, importantly, to human patients, remains to be determined. Interestingly, the brain-targeting epitopes identified in normal mice are reportedly different from those in normal rats (88). Nevertheless, these findings suggest that AAV vectors can be conferred the appropriate tropism and that genetic modification of the brain vasculature to express the deficient lysosomal enzyme can result in significant cross-correction of adjacent diseased neural cells.

The availability of engineered AAV vectors that preferentially target the CNS via the vasculature has clear therapeutic applications beyond LSDs. However, several challenges remain with this strategy that will require further consideration prior to their application for treatment of LSDs. Foremost is the dose of virus required for systemic administration to effect a therapeutic outcome. For example, in the context of spinal muscular atrophy, the proposed clinical study plans to use approximately $10^{14}$ vg of AAV9/kg which will likely tax the capacity of current scalable manufacturing processes (91). Individuals with pre-existing immunity to the virus may require higher doses, which may evoke safety concerns as illustrated in hemophilia trials (92). Additionally, due to differences in biology, anatomy, and size, the encouraging results in mice may not translate to larger animal models with the latter providing less salutary outcomes (93, 94). Nevertheless, there is a planned clinical study in galactosialidosis using a systemically delivered recombinant scAAV8 vector encoding protective protein cathepsin A (Table 1).

### Efficacy of intracranial delivery of AAV vectors for neuropathic LSDs

An alternative and more direct, albeit more invasive, approach to treat neuropathic LSDs is to deliver the recombinant virus directly into the CNS. This approach can be accomplished by administering: i) multiple injections into the brain parenchyma to affect the correction of large regions of the brain, ii) injections into select brain regions of high interconnectivity with efferent and afferent projections to facilitate broad distribution of the vector and enzyme, iii) injections into the cerebrospinal fluid (CSF) (via the ventricles, cisterna magna, or spinal cord) to transduce the ependymal and underlying cells, and iv) a combination of these different approaches (43, 46). This localized approach offers the potential advantages of requiring lower doses of vector for efficacy and a smaller impact from preexisting immunity to the viral vectors (95). Additionally, this approach exploits the property of the tropism for neural cells of select AAV serotypes and their ability to undergo axonal transport, thereby potentially distributing

### TABLE 1. Summary of gene therapy clinical trials for neuropathic LSDs

| Disease       | Phase | Vector    | Route            |
|---------------|-------|-----------|------------------|
| LINCL         | 1     | AAV2      | Intracranial     |
| LINCL         | 1/2   | AAVrh.10  | Intracranial     |
| Canavan       | 1     | AAV2      | Intracranial     |
| MLD           | 1/2   | Lentivirus| HSCT             |
| MLD           | 1     | AAVrh.10  | Intracranial     |
| MPS IIIA      | 1     | AAVrh.10  | Intracranial     |
| MPS I         | 1     | Retrovirus| HSCT             |
| MPS II        | 1     | Retrovirus| HSCT             |
| MPS VII       | 1     | Lentivirus| HSCT             |
| Galactosialidosis | 1   | scAAV8    | Intravenous      |
| Tay-Sachs     | 1     | AAV1      | Intracranial and intrathecal |

MLD, metachromatic leukodystrophy; HSCT, hematopoietic stem cell transplantation.
the therapeutic agent to regions that are distal to the sites of the injections (33, 34, 39, 96).

**Intrathecal route.** As with the systemic route, the consideration of intrathecal injection of AAV to treat the CNS disease was supported by early studies using recombinant enzymes. Periodic injections of several different lysosomal enzymes into the intrathecal space or ventricles of mouse, cat, and dog disease models resulted in efficient clearance of the storage metabolites in the CNS and the consequent correction of motor function (97–101). Based on these preclinical findings, clinical studies using intrathecal injections of the respective lysosomal enzymes to treat the CNS diseases of MPS I (NCT00852358), MPS II (NCT00920647), and MPS IIIA (NCT01155778) have been initiated.

Injection of AAV vectors into the CSF seeks to transduce the cellular components of the ventricular system, such as the ependymal cell lining, the choroid plexus, and the spinal cord central canal. Broad distribution of the therapeutic payload throughout the CNS would then be facilitated by the subsequent expression and secretion of the enzymes into the adjacent cells and the CSF. Because the CSF is continuously produced by the choroid plexus and flows throughout the CNS, this approach is anticipated to generate a constant source of high concentrations of enzyme that then bathes the organ. Moreover, because the CSF drains into the systemic venous circulation, it also has the potential to address the disease in the visceral organs (102). Intrathecal delivery of therapeutics via a lumbar puncture or injection into the cisterna magna is routine clinical practice.

Proof-of-concept for CSF delivery of recombinant AAV vectors to treat the CNS disease has been demonstrated in several animal models of LSDs. For example, intrathecal or intraventricular injections of recombinant AAV1 or AAV2 vectors encoding β-glucuronidase resulted in the expression of the enzyme in broad regions of the CNS and the consequent elimination of the lysosomal storage throughout the brains of MPS VII mice (103, 104). Similar observations were reported using an intraventricular injection of an AAV4-based vector that primarily transduced the ependymal cell lining in MPS VII mice (36). Evidence of efficacy has also been demonstrated following CSF administration of AAV vectors (serotypes 1, 2, 5, and 9) in MPS I, MPS III, and GM1-gangliosidosis mice (102, 106, 107). Importantly, the translatability of this route of delivery in the CNS of larger animals such as dogs and primates has been verified (49, 84, 102). However, because the ependymal cell lining has a turnover rate of approximately 130 days (109) and because readministration of the identical AAV serotype vector is currently untenable, it is unclear whether this approach will provide long-term benefits.

**Intraparenchymal route.** A more direct, albeit intrusive, procedure is to administer the AAV vector into the brain parenchyma. Despite the invasiveness of this approach, there is significant interest in this strategy, as illustrated by the increasing number of ongoing and planned clinical studies in patients with LSDs (Table 1). The premise is based on the ability of locally transduced cells to effect the secretion and cross-correction of nearby, as well as distal, cells through diffusion and axonal transport of both the enzyme and the AAV vector. AAV vectors transported to distal sites in this manner could, in turn, correct additional areas of the brain, thereby providing broader therapeutic coverage than may be expected from a localized injection (39, 110–113). Despite this phenomenon, efficacy in animal models of LSDs has only been demonstrated using multiple bilateral intracranial injections of the AAV vectors (38, 46). However, the number of injections may be reduced through injection of structures that harbor high levels of neural interconnectivity (34, 96, 114) or the utilization of AAV serotype vectors, such as AAVrh.10, that exhibit improved neural tropism (115).

The viability of providing multiple injections of recombinant AAV vectors to treat the CNS disease has been illustrated in both small and large animal models of several LSDs. The efficacy of gene transfer using different AAV serotypes has been demonstrated in mouse models of infantile neuronal ceroid lipofuscinosis, LINCL, Niemann-Pick type A, metachromatic leukodystrophy, globoid cell leukodystrophy, Sandhoff, and MPS I, IIIB and VII diseases (34, 37, 47, 48, 103, 111, 115–123). Treatment was associated with a clear reduction in the overall levels of the accumulated substrate in large regions of the mouse brain and was accompanied by measurable improvements in neuropathology and motor function. Importantly, AAV-mediated reconstitution of the respective deficient enzymes in the CNS of these mice also significantly extended the lifespans of the animals. Efficacy was observed when the treatment was administered into both presymptomatic and symptomatic animals, with greater benefits observed when the therapeutic interventions were introduced in younger animals. The introduction of AAV vectors into neonates extended their lifespans to comparable lifespans of healthy control animals. Based on these observations, early intervention would appear to be critical to minimize the relentlessly progressive and irreversible neurodegenerative disease associated with LSDs.

An indication of efficacy from focal intraparenchymal injections has also been observed in large animal models (e.g., cats and dogs) of various LSDs (119, 124–127). However, despite these encouraging results, scaling this strategy to an infant brain is estimated to nevertheless require between 50 and 350 injection tracts, which is arguably impractical (124, 127). A potential approach to reduce the number of injections and yet deliver the enzymes to large areas of the brain is to inject structures with divergent connections such as the deep cerebellar nuclei, the thalamus, and the ventral tegmental area (34, 48, 96, 114). Evidence of broader therapeutic coverage of brain structures with the enzyme was observed when these sites were included as part of the compendium of intracranial injections. Over the past few years, there have been significant improvements in imaging methodologies that may support the deposition of viral vectors to precise locations of interest thereby imparting greater specificity and safety for this procedure (114, 128). The number of intracranial injections may also
be further minimized by favoring AAV serotypes that demonstrate a higher tropism for neural cells or a propensity to undergo axonal transport (e.g., AAV1, -5, -9, and -rh.10). Finally, these strategies may also be combined with the use of convection-enhanced delivery to further physically disseminate the vectors (114, 128).

Despite reservations regarding the ability of localized intraparenchymal injections to provide sufficiently broad coverage throughout the brain, clinical studies have proceeded in patients with LINCL, Canavan, metachromatic leukodystrophy and MPS IIIA disease. Early phase 1 safety studies using first-generation AAV2-based vectors in Canavan (129, 130) and LINCL (131) patients were not informative regarding efficacy, in part because some of the patients had advanced disease at the time of intervention and the number of enrolled patients was small. No evidence of serious adverse events was attributed to vector administration (using six image-guided tracks and two deposits per track); although there was a suggestion of a slowing of neurological progression in treated LINCL children, the number of individuals in the study was too small to be significant (132). Nevertheless, additional phase 1/2 trials are planned to evaluate the newer AAVrh.10-based vector that may confer greater enzyme distribution within the brain of LINCL patients (NCT01414985, NCT01161576). Long-term follow-up of the small number of Canavan patients treated with an AAV2-based vector also indicated a decrease in substrate levels in the brain and the stabilization of clinical disease (133). Additionally, a phase 1/II clinical trial for MPS IIIA using AAVrh.10 to express the deficient sulfatase enzyme and the sulfatase-modifying enzyme factor 1 (to increase enzymatic activity) is ongoing (NCT01474343) as is a phase 1 study using AAVrh.10 for metachromatic leukodystrophy (NCT01801709). A planned phase 1 study using 12 intraparenchymal deposits and a supplementary infusion of an AAV vector encoding hexosaminidase A and B into the CSF for Tay-Sachs disease is also under consideration in the UK (ISRCTN57061190). Whether these clinical protocols with the ascribed number of intracranial injections will be sufficient to confer clinical improvements or whether substantially more vector is required for efficacy should be forthcoming from these studies.

PROSPECTS FOR EX VIVO GENE THERAPY USING RETROVIRAL VECTORS

The concept of gene-modified autologous hematopoietic stem cells as an approach to treat the CNS manifestations of LSDs originated from the positive results using allogenic hematopoietic stem cell transplantation with a subset of the diseases (134–136). Enzyme delivery was facilitated by the ability of activated lymphocytes, monocytes, and microglial precursors to cross the blood-brain barrier (137–139) to form perivascular and parenchymal microglia, which then became a source of enzymes to affect cross-correction of adjacent resident cells. However, efficacy was limited to individuals with less aggressive neuropathic LSDs (140) and who were presymptomatically treated prior to the onset of irreversible damage. Other undesirable aspects of allogenic bone marrow transplantation included the low availability of matched donors and the risk of graft failure or graft-versus-host disease. For these reasons, current efforts are primarily focused on the transplantation of gene-modified autologous hematopoietic stem cells. In addition to offering a lower morbidity profile, cells engineered to overexpress the enzymes are expected to be more effective than normal (unmodified) donor cells at addressing the neuropathology because a clear dose-response relationship has been demonstrated in murine models of LSDs, in which animals transplanted with cells expressing higher levels of the enzyme exhibited a correspondingly greater correction of the disease manifestations in the CNS (141, 142).

Recombinant γ-retroviral vectors are frequently used to modify the stem cells ex vivo because of their ability to integrate into the host chromosome and facilitate stable transgene expression. Replication-defective lentiviral vectors derived from the human immunodeficiency virus or equine immunodefected virus are becoming particularly prominent because of their ability to transduce nonproliferating cells (143–145). Although insertional mutagenesis resulting from vector integration presents a risk, safety concerns continue to be allayed through the use of modified vectors that harbor self-inactivating mutations in their long terminal repeat to minimize the activation of cellular oncogenes or that impart preferences for integration at specific sites, thereby limiting the spectrum of genomic target sites (146–149).

The potential of gene-modified autologous hematopoietic stem cells to treat the neuropathic aspects of LSDs has been illustrated in several small animal models. The most extensive studies have been performed in a model of metachromatic leukodystrophy and initially used donor cells modified by first-generation γ-retroviral vectors which demonstrated a modest correction of the neuropathology in the mouse model (150). Subsequent studies using more efficient lentiviral-based vectors that transduced a greater number of the donor hematopoietic stem cells and that resulted in the secretion of higher levels of the enzyme (i.e., arylsulfatase A) completely prevented the development of neuropathology and behavioral abnormalities in mice (141, 151). Similar encouraging results were obtained in mouse models of other LSDs including MPS I, MPS IIIA, globoid cell leukodystrophy, and GM1 gangliosidosis (142, 152–155). In addition to the correction of the neurological disease, transplantation of gene-modified donor cells also provided an additional benefit of addressing the visceral components of the disease. In general, more favorable biochemical and clinical outcomes in the mice were obtained using donor cells that had been engineered to express higher levels of the respective lysosomal enzymes. For example, in the study using MPS IIIA mice, the utilization of a strong, myeloid-restricted promoter to enhance transgene expression by microglia in the CNS was necessary for robust efficacy (155). For LSDs that are primarily characterized by neurological disease, restriction of the expression to the brain using selective promoters may...
also offer a safety advantage. Indeed, in the mouse model of globoid cell leukodystrophy, expression of the enzyme (i.e., galactocerebrosidase) was toxic to hematopoietic stem cells and early progenitors, requiring a microRNA-regulated approach to therapy (154). As observed in studies using AAV vectors, earlier treatment, particularly in mice with aggressive neurological disease, provided a more favorable outcome than later interventions. In part, this finding may be due to the slow rate by which resident tissue microglial populations were replaced by the transplanted hematopoietic cell progeny. Finally, some measure of preconditioning of the host was necessary for optimal engraftment.

Given the encouraging clinical results using transplantation of gene-modified hematopoietic stem cells for SCID-XI, ADA-SCID, Wiskott-Aldrich syndrome and @-thalassemia (156–159), together with the supporting preclinical results in the mouse model of metachromatic leukodystrophy, a phase 1/2 clinical trial was recently initiated in patients with the late-infantile form of the disease (NCT01560182). Treatment of three presymptomatic patients who exhibited biochemical and neurophysiological evidence of the disease resulted in the reconstitution of high levels of arylsulfatase A in the cells of hematopoietic lineage and the CSF (160). Importantly, functional analysis at 18–24 months posttreatment indicated that the disease did not manifest or progress suggesting that the levels of engraftment and expressed enzyme were in the therapeutic range. However, longer-term follow-up of these patients is required to adequately assess the potential of this therapeutic strategy.

CONCLUSIONS AND PERSPECTIVES

Significant progress has been made with regards to gene therapy as a potential modality for the treatment of the CNS disease of LSDs. The promise of this therapeutic strategy is initially supported by the positive, albeit early, indications of safety in a small cohort of patients treated with either the AAV or lentiviral vector-based systems. Some studies suggested that the interventions delayed the onset or progression of the disease, although studies using larger numbers of patients will be required to confirm these observations. If these initial intriguing indications of efficacy are confirmed in longer-term studies with more patients, this would indeed represent a transformative therapy for a group of inherited diseases with high unmet medical need. Although many challenges remain (e.g., host immunity to the viral capsids or insertional mutagenesis), progress continues to be made toward this goal and there is optimism that safe and effective gene transfer vectors will be developed to treat these disorders. This optimism is also reflected, in part, by the increasing number of new start-up gene therapy companies and an increased interest by the investment community in commercializing this therapy for different disorders, including LSDs (161, 162).

If gene therapy alone proves insufficient to fully correct the CNS manifestations associated with LSDs, a combination of emerging adjuvant therapies, such as substrate reduction or chaperone therapy, may be considered (14). The concept of combination therapies that potentially provide an additive or synergistic therapeutic effect for several LSDs has already been elegantly illustrated in several nonclinical studies (163). Groupings frequently employed the use of bone marrow transplantation as one of the treating arms, which may now be extended to include gene-modified hematopoietic stem cells. Moreover, both the in vivo approach using AAV vectors and the ex vivo strategy using lentivirus-modified stem cells may also be used as a combination pair. Although more complex, such groupings may result in improved efficacy for the treatment of the CNS disease, as well as the diverse pathology noted in the visceral organs of patients with LSDs.

The development of therapies for neuropathic LSDs may also benefit from the growing realization of an association between this group of disorders and other unrelated neurodegenerative diseases, such as Parkinson’s disease and dementia with Lewy bodies (164). Indeed, there is a growing consensus that lysosomal dysfunction may represent the underlying basis for many synucleinopathies (165). If this is correct, there is the potential to repurpose drugs that were initially developed for these more common neurodegenerative diseases for LSDs and vice versa (166). Moreover, studies of apparently disparate diseases that are presumably linked through shared metabolic pathways are likely to provide greater insights into the biology of the diseases. This and other opportunities for exchange will hopefully foster an acceleration in the development of new and innovative therapies for these devastating diseases.

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