Molecular Therapy
Review

Hematopoietic Stem Cell Gene Therapy for Storage Disease: Current and New Indications

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Lysosomal storage disorders (LSDs) are a broad class of monogenic diseases with an overall incidence of 1:7,000 newborns, due to the defective activity of one or more lysosomal hydrolases or related proteins resulting in storage of un-degraded substrates in the lysosomes. The over 40 different known LSDs share a life-threatening nature, but they are present with extremely variable clinical manifestations, determined by the characteristics and tissue distribution of the material accumulating due to the lysosomal dysfunction. The majority of LSDs lack a curative treatment. This is particularly true for LSDs severely affecting the CNS. Based on current preclinical and clinical evidences, among other treatment modalities, hematopoietic stem cell gene therapy could potentially result in robust therapeutic benefit for LSD patients, with particular indication for those characterized by severe brain damage. Optimization of current approaches and technology, as well as implementation of clinical trials for novel indications, and prolonged and more extensive follow-up of the already treated patients will allow translating this promise into new medicinal products.

Lysosomal Storage Disorders

Lysosomal storage disorders (LSDs) are a broad class of over 40 different inherited diseases, with an overall incidence of 1:7,000 newborns, due to the defective activity of one or more lysosomal hydrolases. The enzyme functional deficit is caused by pathogenic mutations occurring in enzyme-coding genes or in other molecules that are instrumental for lysosomal function. Defective proteins can be soluble lysosomal hydrolases, transmembrane proteins (such as membrane transporters required for the incorporation of substrates into the lysosome lumen and release of degradation products out of the lysosome), co-factors or co-activators required for lysosomal enzyme function, proteins protecting lysosomal enzymes (for instance, cathepsin A in patients with galactosialidosis), proteins involved in post-translational processing of lysosomal enzymes (as is the case in multiple sulfatase deficiency), or enzymes involved in targeting mechanisms for protein localization to the lysosome (such as one of the enzymes responsible for the acquisition of the M6P marker N-acetylglucosamine-1-phosphotransferase, which is defective in mucolipidoses types II and III). Whatever the genetic defect, it results in impairment of substrate degradation, leading to abnormal accumulation (or storage) of undegraded substrates (Figure 1).

Storage material initially accumulates in endosomes and lysosomes and eventually in other intracellular compartments and in the extracellular environment, compromising cellular function and ultimately leading to cell death and organ pathology. Secondary mechanisms, such as oxidative stress and local inflammation, are also relevant in determining organ dysfunction, particularly in the CNS. LSDs are usually classified according to the nature of the primary stored material. Broad categories include mucopolysaccharidoses (MPSs), sphingolipidoses, mucolipidoses, glycoproteinoses, oligosaccharidoses, and glycogen storage diseases.

Despite the life-threatening nature of these disorders, clinical manifestations of LSDs are extremely variable and determined by the characteristics and tissue distribution of the accumulating material. Since lysosomes are present in all eukaryotic cells (with the exception of erythrocytes) and since lysosomal substrates have key roles in many cellular functions, the effects of lysosomal impairment are in most cases widespread. Storage may affect brain, viscera, bones, and connective tissues. Affected individuals generally appear normal at birth but symptoms appear soon after. Over two-thirds of LSD patients present with an involvement of the CNS at varied severity, with neurons being particularly vulnerable, which makes LSDs the most common cause of pediatric neurodegenerative disease. Clinical manifestations may involve mental retardation, progressive regression after a period of normal development, dementia, sensory loss including blindness or deafness, motor system dysfunction, seizures, brain atrophy, demyelination, and sleep and behavioral disturbances. Neuropathology is progressive and ultimately leads to death at an early age.

In many diseases, nervous system involvement is particularly prevalent with minimal peripheral involvement (for example in Krabbe disease, metachromatic leukodystrophy, and Sanfilippo disease). Presence of CNS manifestations is one of the critical aspects determining not only the severity of patients' prognosis but also treatment opportunities for the affected patients. Generally, a broad clinical spectrum is also recognized within each of the LSDs, with clinical

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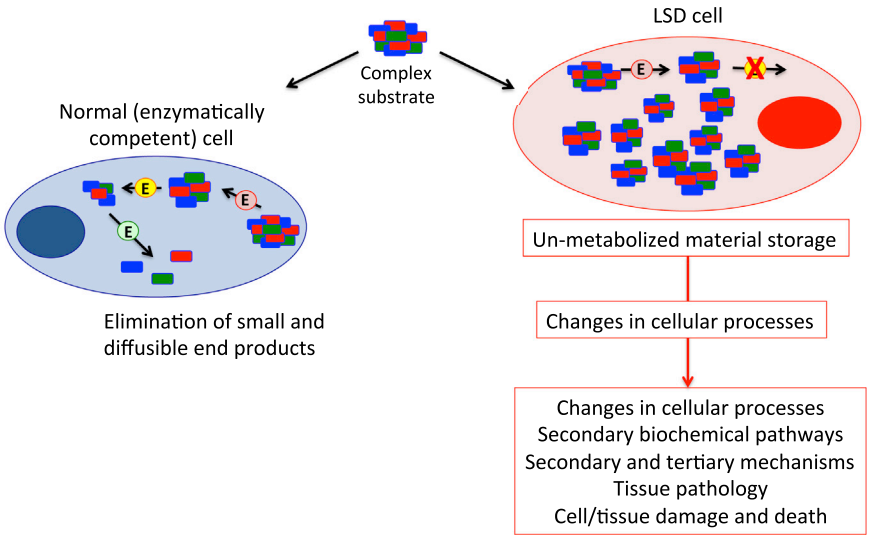

Figure 1. Lysosomal Enzyme Deficiency and Its Consequences
The defective activity of a lysosomal enzyme (E) determines the accumulation of undegraded substrates in LSD cells, leading to the activation of a pathologic cascade of events.

variants of the same disease differing from each other for the age at onset of the presenting symptoms and/or for the penetrance of the disease manifestations. A clear genotype-phenotype correlation that allows predicting actual phenotypic features of the affected patients based on their genotype exists for only a few LSDs. For most of the patients, phenotypic variability cannot be anticipated by knowledge of disease-causing mutations that are also frequently private or rare. This lack of correlation between disease manifestations and their genetic bases, together with the limited therapeutic opportunities available for LSD patients, constitutes one of the key hurdles in the application of newborn-screening programs for these diseases. Indeed, an inability to properly predict the precise disease characteristics and prognosis of many of the patients identified at newborn screening based on their genotype could affect the ability of properly directing them to available standardized or experimental treatments. Importantly, however, the majority of LSDs lack a curative treatment. This is particularly true for LSDs severely affecting the CNS. Therefore, LSDs represent an urgent curative need.

Cross-Correction and Rationale for Hematopoietic Stem Cell-Based Approaches

Most treatment opportunities for LSDs are based on the 1968 observations of E.F. Neufeld and colleagues of mutual correction of the biochemical defect in in-vitro-cultured skin fibroblasts from patients affected by Hurler and Hunter syndromes, when mixed with each other or with normal cells.[1] This effect was attributed to the release into the medium of "substances" that today we know were the lysosomal enzymes iduronate-2-sulfatase (IDS, defective in Hunter syndrome) and alpha-L-iduronidase (IDUA, defective in Hurler syndrome). It was only 12 years later that Hasilik and colleagues[2] first described the cross-correction mechanism and identified the "corrective factor" as being the functional enzyme secreted by wild-type cells, taken up and sorted to the lysosomal compartment of the deficient fibroblasts through the mannose-6-phosphate receptor (M6P-R) pathway (Figure 2).

This phenomenon is unique to lysosomal enzymes, as M6P groups are added exclusively to the N-linked oligosaccharides of these soluble enzymes as they pass through the *cis*-Golgi network. After being modified, the lysosomal enzyme binds the M6P-R in the *trans*-Golgi network, and it is sorted to the late endosomes to reach the lysosome. About 40% of the enzyme escapes this pathway and is secreted into the extracellular space; this enzyme can then bind the M6P-R on the membrane of the producer cell or surrounding cells, and it can be endocytosed and sorted to the lysosome.[3] Based on this phenomenon, treatment modalities able to make the therapeutic enzyme available at the site of tissue damage have been developed. Enzymes can be made available to patients by different approaches. The most obvious approach consists of the parenteral supply of recombinant or purified protein, the so-called enzyme replacement therapy (ERT), aimed at reducing the storage of the substrate responsible for the disease by enzyme supply to the affected tissues where it can be taken up by the defective cells.

ERT started more than 20 years ago with the large-scale isolation of β-glucocerebrosidase from human placentas for the treatment of Gaucher disease.[4] The treatment of non-neuronopathic forms of Gaucher disease then shifted from this original source of enzyme to the recombinant form that was approved in 1991 by the U.S. Food & Drug Administration (FDA) and became clinically available as imiglucerase (Cerezyme and Genzyme). Treatment has been very successful.[5] The genes for almost all of the lysosomal enzymes have been cloned, and, theoretically, their encoded proteins could be produced in large quantities. Despite this possibility, the small number of patients affects the pharmacological development of ERT. Moreover, the inability of recombinant/purified enzymes to efficiently cross the blood-brain barrier (BBB) limits the usefulness of ERT to LSDs with minor or no involvement of the CNS.[6]

Despite some experimental studies that have shown a reduction of substrate storage in the CNS of animal models of LSD in the presence of high quantities of enzyme in the plasma,[7, 8] research is currently directed at promoting transport of the administered enzyme across the BBB. Among the recently explored strategies are the conjugation of the enzyme with molecules recognized by a specific BBB carrier, such as IGF-2, the Fc fragment of antibodies, ApoB, or the TAT protein transduction domain, or the use of nanoparticles.[9–17] Different routes of enzyme administration are also being explored, including

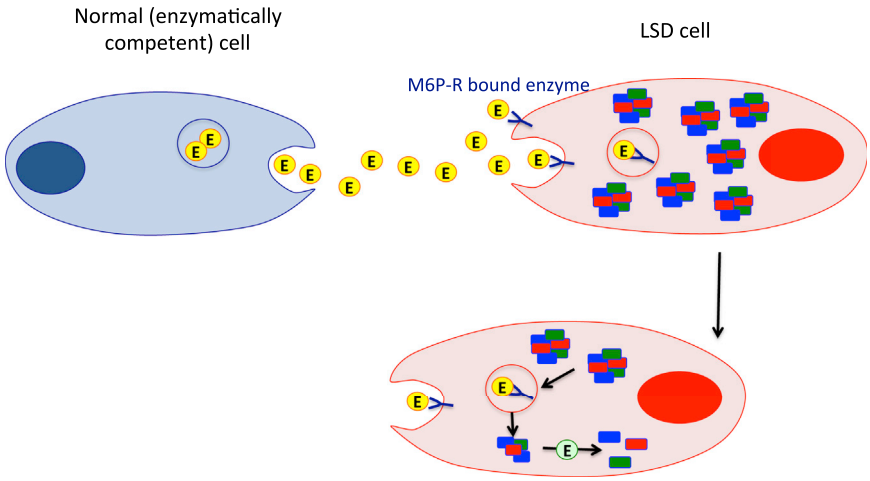

Figure 2. The Mechanisms of Cross-Correction
Functional enzyme secreted by wild-type cells is taken up and sorted to the lysosomal compartment of the LSD cells through the mannose-6-phosphate receptor (M6P-R) pathway. This leads to restoration of substrate metabolism.

intra-cerebroventricular or intra-thecal ERT agent delivery.[18–21] ERT may also be associated with the induction of immune responses against the injected protein. In LSDs characterized by a complete absence of the enzyme, parenteral administration could result in the recognition of the protein as a non-self-antigen, thus triggering a humoral response with IgG production.[22–25] Immune responses to an enzyme can lead to different adverse effects in term of severity, producing non-neutralizing or neutralizing antibodies. A direct and proportional inhibiting effect of the antibodies on efficacy has not been constantly observed, perhaps due to the complexity and heterogeneity of the disease in patients. Finally, the high cost of the recombinant enzymes, the need for lifelong treatment, and the frequency of injections are significant issues for many patients and their families.

To overcome ERT limitations and obtain effective delivery of functional lysosomal enzymes at every disease site by a one-time intervention, alternative strategies aimed at the generation of tissue sources of enzyme have been developed. Historically, the first approach developed in this direction has been hematopoietic cell transplantation (HCT) from healthy compatible donors. The rationale to apply HCT in LSDs lies in the ability of the transplanted cells and/or their progeny to contribute to fixed-tissue macrophage populations in the affected tissues and to become local permanent sources of functional lysosomal enzymes. Moreover, metabolically active cells can improve the disease phenotype by scavenging storage material and modulating local inflammation at diseased sites. Cell turnover with the donor after HCT is supposed to affect all types of tissue-fixed myeloid populations, including myeloid cells and possibly microglia in the brain. For this reason HCT was intended as an avenue also to treat LSD patients with severe CNS involvement. Importantly, if full donor chimerism is achieved, HCT is a single intervention able to provide a durable lifelong enzyme source to the affected patient. The donor cells also re-establish a novel immune system in the patient, overcoming pre-existing and avoiding post-treatment immune responses directed at the functional enzyme. Based on this rationale, since the very first LSD patients were transplanted in the early 1980s,[26] a few thousand additional LSD patients have been treated with allogeneic HCT over the past decades.[27]

HCT: Outcomes and Implementation from Allogeneic to Autologous Transplant of Gene-Corrected Hematopoietic Stem Cells

Mucopolysaccharidosis type I (MPS I) represents the best example of LSD that can be successfully treated by HCT. The guidelines of the European Bone Marrow Transplantation (EBMT) group recommend HCT for patients affected with MPS I diagnosed before the age of 2.5 years and with clinically presumed Hurler syndrome (MPS IH) (presence of clinical signs and symptoms compatible with MPS IH, i.e., phenotypic diagnosis based on clinical expertise) and/or homozygosis or compound heterozygosis for mutations exclusively associated with the severe phenotype.[28] HCT in MPS IH has been shown to be clinically effective, increasing life expectancy and improving various clinical outcomes.[29–33] However, residual disease burden is present in transplanted patients, and the skeleton, heart valves, and CNS remain partially refractory to correction post-HCT. Age and disease state at transplant are key determinants of the long-term prognosis of the transplanted patients, and the best functional outcome is generally observed when HCT is performed in very young patients with a low disease burden at transplant. In many centers the standard practice is to deliver ERT alongside HCT to improve the condition of the patient prior to transplant and increase the feasibility, safety, and overall outcome of the procedure.[34] Despite this, low but measureable transplant-related mortality and morbidity still affect the procedure, and, in a fraction of the patients, a second transplant is needed to overcome engraftment failure due to the intrinsic defect of the bone marrow niche cause by storage accumulation.[35, 36]

Similar considerations can be applied to a few other LSDs where an HCT indication can be formulated in pre-symptomatic or very early symptomatic patients, but in none of the other LSDs is the indication to transplant as strong as in MPS IH. Indeed, a lack of benefit or limited benefit on the CNS is a common finding for the majority of the transplanted LSD patients. Multiple reasons can justify this poor outcome. First, the ability of the transplant to deliver functional enzyme to the CNS is based on a progressive cell replacement process that requires a long time to occur. For this reason, benefit consequent to enzyme delivery to the brain is expected to occur not earlier than 1 year post-HCT. Consequently, whenever HCT is administered to patients in acute or advanced disease stage, neurological disease progression will prevail over the potential for benefit in the crucial first

year post-treatment, affecting the long-term prognosis of the transplanted patients. Moreover, many of the drugs employed during and after transplant to allow the establishment of donor chimerism and prevent/control graft versus host disease (GvHD) could exert neurotoxic effects that are particularly detrimental in acutely progressing and symptomatic phases of LSD brain disease. Furthermore, the use of sibling donor carriers of a disease-causing allele could negatively impact the outcome of HCT, since an enzymatically defective source is employed.

Many of these limitations could be overcome by a hematopoietic stem cell (HSC) gene therapy approach based on the use of autologous HSCs and gene therapy vectors intended to increase enzyme production by the transplanted cells and their progeny. Indeed, transplantation of genetically engineered autologous cells can do the following: (1) increase the potential for benefit of HCT by increasing the enzyme dose delivered to the affected tissues by the transplanted cell progeny; this can be achieved by using efficient gene transfer vectors that allow the production of above-normal enzyme quantities by individual cells; and (2) reduce morbidity and mortality associated with the transplant procedure thanks to the use of milder conditioning regimens and the lack of GvHD in the autologous setting. This rationale has been proven in the animal models of several LSDs and more recently in patients.

From Preclinical Data to Initial Clinical Evidences

Therapeutic efficacy of HSC gene therapy approaches in controlling disease manifestations was first shown in preclinical experiments on LSD models lacking CNS disease.[37, 38] In this setting, HSC gene therapy based on gamma-retroviral (γRV) vectors was proven effective in restoring the missing enzyme activity and providing therapeutic effects on visceral organs. However, the proof of the therapeutic potential of this strategy for LSD characterized by extensive CNS and peripheral nervous system (PNS) involvement came a few years later with the advent of lentiviral vectors (LVs). Metachromatic leukodystrophy (MLD), an LSD historically considered refractory to HCT, represents one of the first LSDs where HSC gene therapy efficacy and the need for highly efficient gene transfer vectors were shown. Matzner and colleagues initially showed that HSC gene therapy with bone marrow cells expressing the human arylsulfatase A (ARSA, a disease-causing gene in MLD) cDNA from a γRV resulted in the expression of high enzyme levels in various tissues.[39–41] The treatment almost completely prevented sulfatide storage in liver and kidney, while only a partial correction of the lipid metabolism was detectable in the brain. This partial correction was accompanied by very modest if no amelioration of neuropathology and behavioral tests.

As proven by subsequent studies, this limited outcome was due to the requirement of high levels of ARSA for the correction of the metabolic defect in the CNS. The use of an LV for HSC gene transfer allowed overcoming this requirement and achieving therapeutic benefit in the same animal model a few years later.[42, 43] By transplanting HSC transduced with third-generation LV carrying the therapeutic ARSA cDNA, enzyme activity was reconstituted in the hematopoietic system of MLD mice to above-normal levels. In this context, the development of major CNS and PNS disease manifestations was almost completely prevented upon pre-symptomatic treatment,[42] and early disease manifestations were corrected upon treatment administration in symptomatic stage.[43] Remarkably, gene therapy had a significantly better therapeutic impact than wild-type HSC transplantation, indicating a critical role for enzyme overexpression in the HSC progeny in determining benefit. Other investigators confirmed these initial findings in models of other LSDs characterized by severe CNS involvement, such as GM1 gangliosidosis, MPS IH, MPS IIIA, globoid cell leukodystrophy, and others.[44–49] These results highlighted the concept that therapeutic HSC transplantation actually corrects a nervous system damage-response pathway defective in LSD. Vector-mediated overexpression of the therapeutic enzyme in macrophages/microglia may serve to enhance the corrective potential of this pathway and to dampen its destructive capacity.

Based on these preclinical findings, clinical testing of HSC gene therapy in LSDs has started. Pioneering was again the experience in MLD. Indeed, based on the efficacy preclinical data mentioned above, as well as a solid package of preclinical safety data and an optimized manufacturing strategy, a phase I/II clinical trial of HSC gene therapy in MLD patients was started in early 2010 and recently provided promising results.[50, 51] Early-onset MLD patients were treated in pre- or very early symptomatic stage with autologous HSC transduced with an LV encoding the human ARSA cDNA under the control of the human phosphoglycerate kinase promoter after exposure-targeted busulfan conditioning. Stable, sustained engraftment of gene-corrected HSCs was observed in the hematopoietic tissue of the treated children. A progressive reconstitution of ARSA activity at or above normal values in circulating hematopoietic cells and in the cerebrospinal fluid was documented, in association with a reduction of the storage material in peripheral nerve samples, thus providing formal evidence of seeding of the CNS and PNS by HSC-progeny cells also in humans. In the patients who were treated in the precocious stage, a marked benefit was demonstrated as prevention of disease onset and/or halted progression. Treatment resulted in protection from CNS demyelination and, in at least a fraction of patients, amelioration of PNS abnormalities, with signs of re-myelination at both sites. These patients showed continuous motor and cognitive development, in sharp contrast with the natural course of the disease. These exciting results pave the way for the clinical development of HSC gene therapy for novel indications.

Novel Indications

HSC gene therapy based on LVs was the first among tested gene therapy approaches to provide evidence of a correction to normal of the MPS IH neurologic and skeletal defects in mice.[45] Of note, also in this setting the therapeutic benefit provided by gene therapy on critical disease manifestations greatly exceeded that exerted by HCT, the standard of care treatment for Hurler patients, and therapeutic efficacy of HSC gene therapy was strictly dependent on the achievement of above-normal enzyme activity in the tissues of transplanted mice.

The amount of enzyme delivered to the CNS by this approach employing an ubiquitously expressed promoter was likely higher than what was observed with erythroid- or platelet-specific expression,[52, 53] indicating that myeloid cells overexpressing the enzyme could significantly increase IDUA delivery to the CNS (and possibly to the skeleton). In line with this observation are the results obtained by Sergijenko and colleagues[47] in the animal model of MPS IIIA, where neurologic disease correction was achieved by HSC gene therapy employing a myeloid lineage-specific promoter to drive expression of the therapeutic N-sulfoglucosamine sulfohydrolase enzyme cDNA. LV HSC gene therapy was also shown to be a promising approach for the treatment of CNS lesions in MPS II, a neuropathic LSD caused by a deficiency of IDS. Indeed, HSC gene therapy corrected neuronal manifestations by ameliorating lysosomal storage and autophagic dysfunction in the brains of MPS II mice.[54] Importantly, these data are particularly relevant since MPS IIIA and MPS II represent prototypical LSDs with severe CNS involvement for which indication to allogeneic HCT is not unanimously accepted due to poor neurologic outcome in the transplanted patients.

Similar promising results were also reported in the animal model of globoid cell leukodystrophy, where restriction of galactocerebrosidase enzyme expression to mature hematopoietic-lineage cells was needed in order to guarantee feasibility of the approach.[48] Also in this complex setting, vector-driven enzyme above-normal expression in hematopoietic progeny cells resulted in being instrumental for driving superior efficacy over more traditional transplant strategies. Additional further examples exist, confirming these evidences. Overall, these LSDs where HSC gene therapy demonstrated therapeutic efficacy in controlling neurologic disease manifestations are homogenously characterized by a severe involvement of the CNS and lack of registered efficacious treatments. Thus, they represent new relevant indications where HSC gene therapy could be offered to patients as a novel opportunity of efficacious treatment. Phase I/II trials are soon expected for many of these indications. Instrumental for this to happen is the generation of solid feasibility and safety packages,[46] as well as optimized, large-scale, and accessible manufacturing that would guarantee an early and feasible transition into clinical testing.

Factors Critical for Benefit

Preclinical and clinical experiences have identified factors that are to be critically met by HSC gene therapy in order to strengthen its potential for benefit in the unique setting of LSDs. Among these is *enzyme above-normal expression*. The value of appropriate levels of enzyme being produced by the transplanted cells and their progeny has been clearly proven in the preclinical setting as well as in clinics. Preclinical data clearly showed that the unique efficacy exerted by HSC gene therapy over healthy donor HCT is highly dependent on the higher enzyme dose associated with the use of vectors.[42, 45] Moreover, dose-effect correlations were shown in multiple animal models where the more of the enzyme that was produced in hematopoietic cells and/or tissues of reconstituted animals the more the benefit exerted at key disease sites.[42, 45, 48] Importantly, clinical experience in LSD patients is supportive of this concept. When patients with LSD treated with HCT were compared to subjects with the same disease treated with pharmacological ERT, substrate reduction was significantly greater in the former.[55] Similarly, transplant from healthy versus carrier donors resulted in a differential substrate reduction. Moreover, enzyme above-normal expression induced by HSC gene therapy in clinical testing proved to be effective in patients who are generally poorly benefited by allogeneic HCT.[51] Importantly, a benefit was also observed on disease features, such as peripheral neuropathy, on which allogeneic HCT is not effective, with a clear dependency on overall vector load and enzyme levels.

Another key component contributing to the successful implementation of HSC gene therapy for LSDs is *pre-transplant conditioning*. To achieve stable engraftment of HSCs, patients undergoing transplantation of allogeneic or autologous HSCs receive a chemotherapy- and/or radiotherapy-based preparation. When HSC gene therapy for inherited genetic disorders is concerned, the ideal conditioning regimen aims to contain toxicity by reducing the dosage and/or the number of chemotherapeutic agents administered, in comparison to fully myeloablative preparations employed in conventional allogeneic HCT. While low-dose conditioning is sufficient to create a mixed chimerism when gene-corrected cells are endowed with a natural selective advantage, such as in the case of immune deficiencies, myeloablative doses are necessary when high levels of engraftment are required in diseases such as LSDs. Importantly, conditioning in LSDs with CNS involvement has also to ensure the most effective possible reconstitution of brain myeloid cells and microglia by the gene-corrected cell progeny. In this sense, a regimen based on the alkylating agent busulfan is the preferred choice due to existing evidences of its favoring role for brain myeloid cell turnover.[56, 57]

Conditioning and possibly cell dose have also to be optimized in order to properly address the complexity of the disease in terms of overcoming or controlling (1) the detrimental effect of the storage on the bone marrow stem cell niches[35, 36] and (2) the pre-existing immunity directed toward the therapeutic enzyme in patients who would be receiving ERT during transplant preparatory phases. Indeed, as mentioned above, many LSDs are associated with niche defects due to the storage that frequently determine high-frequency engraftment failure in recipients of allogeneic HCT and the need for secondary transplant. Moreover, also to address this aspect, current practice is to pre-treat HCT candidate patients with ERT to detoxify them from storage. Since the majority of HCT candidate patients are affected by severe LSD variants and carry null mutations, immune responses to ERT are more likely to occur in them. Pre-existing immunity is not relevant and is abated by conditioning and immune suppression in the allogeneic transplant setting, but it may be more critically affecting outcome in gene therapy. Therefore, specific protocols for conditioning and controlling the immune response are to be developed and tested in patients in the context of phase I/II gene therapy clinical trials. The actual value of novel conditioning strategies based on monoclonal antibodies selectively depleting blood cells and associated with limited extra-medullary toxicity has still to be extensively evaluated in the context of HSC gene therapy for LSDs.[58]

Finally, the outcome of allogeneic HCT and conceivably of HSC gene therapy is highly influenced by the *timing of treatment administration*. Selection of patients in whom the disease has not yet caused massive degeneration of neurons and neuronal connections and functional losses was demonstrated to be critical for ensuring benefit in the treated children. Ongoing efforts directed toward the implementation of newborn-screening programs for many LSDs along with an increased understanding of genotype-phenotype correlations and phenotypic determinants would allow properly directing newly diagnosed patients to early treatment.

The Space of Editing

Enzyme above-normal expression is a key element for benefit in HSC gene therapy, and it can be obtained upon high-frequency transduction of HSCs and integration of an expression cassette allowing sustained expression of the enzyme encoding cDNA in the presence of a reasonable number of integrant copies. Obviously this implies the use of integrating vectors and of gene addition strategies. Platforms for gene editing of mutations or, most importantly for LSDs, of targeted gene addition are becoming increasingly available and efficient, also at the level of human long-term repopulating HSCs. However, these strategies require some degree of optimization and adaptation in order to allow proper application in LSDs. As was done in the case of successful liver gene editing at the album locus,[59] also in the context of the HSC approach, loci characterized by high-level expression into the HSC-myeloid progeny should be identified and properly targeted in order to drive high-level expression of the integrant/lysosomal enzyme. Moreover, to guarantee an adequate level of success in the LSD setting, the efficiency of targeted addition should be optimized so as to guarantee that the large majority of the transplanted HSCs would carry the targeted gene addition or, alternatively, selection strategies for the successfully edited cells should be developed.

The Case of X-Linked Adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is an X-linked monogenic neurodegenerative disease due to inherited mutations in the ABCD1 gene. Mutations cause a loss of function of the peroxisomal membrane half-transporter ALD protein (ALDP) that results in impaired transport and subsequent breakdown of very long-chain fatty acids (VLCFAs) into peroxisomes, causing their accumulation in the body. The accumulation of VLCFAs may be toxic by triggering an inflammatory response in the brain, which could lead to the breakdown of myelin. It is thus classified as a peroxisomal leukodystrophy with storage accumulation. Different variants of ALD exist. The cerebral form of the disease (cALD) is the most severe variant and is characterized by inflammatory demyelination and neurodegeneration, with onset usually in childhood. Disease progression, which leads to the loss of neurologic functions and death within a few years of symptom onset in most cases, can only be halted with healthy donor HCT, provided that it can be performed at an early stage of brain disease.[60–62]

Good functional survival is observed in affected boys transplanted with pre-symptomatic disease, with early radiologic findings. However, beyond a certain stage, demyelination cannot be arrested, necessitating rapid identification of potential highly matched donors. HCT benefit in cALD is likely dependent on reconstituting myeloid brain cells and microglia, which play a key role in triggering myelin damage, with a metabolically competent and not activated donor-derived population. Being that ALDP is a transmembrane protein, correction of the metabolic defect in other non-myeloid brain cells is not expected to occur. Due to similarity in the pathogenesis and the curative effect historically exerted by HCT, HSC gene therapy has also been tested with success in cALD patients. In the initial proof-of-principle study, four boys with cALD received autologous HSCs transduced ex vivo with an LV containing an ABCD1 complementary cDNA. Results were reported on two of those patients, who demonstrated functional human ALDP expression and disease stabilization.[63] A larger multicenter study is currently ongoing that is confirming initial positive results (Williams et al., 2007, Mol Ther., conference). The goal of the procedure in the autologous setting is to restore the functional ALDP expression in reconstituted brain myeloid cells and microglia and halt disease progression, while reducing transplant-related morbidity and mortality due to the use of milder conditioning and autologous cells. Despite the nature of the defective protein, above-normal expression is probably not key to benefit. However, achievement of high rates of transplanted HSC transduction is likely critical for benefit also in cALD and points to the need for effective gene transfer vectors, such as LVs, for autologous HSC manipulation, or for optimized gene-editing strategies. Also in the autologous transplant setting, the time of treatment administration retains a key role in determining outcome of the treatment.

Conclusions

The preclinical and clinical evidences reported here indicate that HSC gene therapy has the potential to result in therapeutic benefit in patients affected by LSD and other storage disorders, with particular indication for those diseases characterized by severe CNS damage. Optimization of current approaches and technology, as well as implementation of new clinical trials in novel indications, and prolonged and more extensive follow-up of the already-treated patients will allow translating this promise into medicine development plans.

CONFLICTS OF INTEREST

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