HUMAN GENE THERAPY 23:808–815 (August 2012)

DOI: 10.1089/hum.2012.140

Gene Therapy Approaches for Lysosomal Storage Disease: Next-Generation Treatment

Barry J. Byrne, Darin J. Falk, Nathalie Clément, and Cathryn S. Mah

Abstract

Lysosomal storage diseases are a group of rare inborn errors of metabolism resulting from deficiency in normal lysosomal function. These diseases are characterized by progressive accumulation of storage material within the lysosomes of affected cells, ultimately leading to cellular dysfunction. Multiple tissues ranging from musculoskeletal and visceral to tissues of the central nervous system are typically involved in disease pathology. Since the advent of enzyme replacement therapy (ERT) to manage some LSDs, general clinical outcomes have significantly improved; however, treatment with infused protein is lifelong and continued disease progression is still evident in patients. Viral gene therapy may provide a viable alternative or adjunctive therapy to current management strategies for LSDs. In this review, we discuss the various viral vector systems that have been developed and some of the strategy designs for the treatment of LSDs.

Introduction

Lysosomal storage diseases (LSDs) are a class of inherited metabolic storage diseases encompassing more than 70 distinct diseases characterized by progressive storage of undigested or partially digested materials within the lysosomes of affected cells. Accumulation of substrates within lysosomes eventually leads to cellular and metabolic dysfunction, and sometimes the substrates themselves are cytotoxic. Affected tissues range from skeletal and cardiac muscle, liver, kidney, eye, and bone to tissues comprising the central nervous system. Care for patients with LSDs has been strictly palliative, but now several LSDs are managed with enzyme replacement therapy (ERT), substrate reduction therapy (SRT), or in some cases hematopoietic cell transplantation (Table 1). Although current therapies can slow disease progression and patient outcomes have significantly improved, none have been shown to be curative and complications can arise, often related to immune response to the therapeutic protein. Furthermore, current therapies have not been shown to ameliorate neurological deficits, which occurs in the majority of LSDs. As such, alternative strategies need to be developed and explored.

All LSDs are a result of two recessive mutations in a single gene locus. For most LSDs, low levels of residual protein expression resulting from missense mutations elicit significant improvement of the clinical phenotype and carriers remain largely unaffected. These attributes make LSDs an ideal fit for gene therapies. Considerations for gene therapy strategies include the target and route of delivery and the vector construct.

Cross-Correction

Most LSDs result from a deficiency of a lysosomal enzyme. Lysosomal enzymes have a unique characteristic in that, although most of the expressed enzyme is targeted to the endosomal system via binding of the enzyme to the mannose 6-phosphate receptor, a small percentage of expressed enzyme is also secreted from the cell, which can be taken up by distal cells and trafficked to the lysosome to perform its enzymatic function. Current ERT and bone marrow transplantation therapies take advantage of this process of uptake of secreted enzyme, which is also known as *cross-correction*.

In initial gene therapy studies, muscle was first targeted as the potential endogenous source of enzymes for cross-correction because of the ease of accessibility by intramuscular injection. However, these strategies have been relatively unsuccessful, due in part to the inefficiency of muscle in those secretion processes (Ellinwood *et al.*, 2004; Hodges and Cheng, 2006; Sands and Davidson, 2006). In addition, multiple gene therapy studies have indicated that muscle tissue may be more prone to the development of humoral and/or cytotoxic immune response, ultimately resulting in elimination of the therapeutic protein (Warrington and Herzog, 2006).

To date, most gene therapy-mediated cross-correction strategies have targeted the liver as the production depot of therapeutic protein, as hepatocytes normally synthesize and

Department of Pediatrics and Powell Gene Therapy Center, University of Florida, Gainesville, FL 32610.

Table 1. Lysosomal Storage Diseases Managed with Enzyme Replacement Therapy and Substrate Reduction Therapy

LSD	*Prevalence*[a]	*Genetic inheritance*	*Approved treatment*	*Gene therapy clinical trial; viral vector*
Fabry disease	1:40,000–60,000	X-linked recessive	ERT (agalsidase alfa[b] and agalsidase beta)	NCT00001234; RV-a-Galactosidase A
Gaucher disease type I	1:50,000	Autosomal recessive	ERT (imiglucerase) (velaglucerase alfa) (taliglucerase alfa); SRT (miglustat)[b]	NCT00001234 and NCT00004294; RV-glucocerebrosidase
Gaucher disease type III	1:100,000	Autosomal recessive	ERT (imiglucerase)	
Glycogen storage disease type II (Pompe disease)	1:40,000	Autosomal recessive	ERT (alglucosidase alfa)	AAV/NCT00976352; rAAV-CMV-GAA
Metachromatic leukodystrophy	1:40,000–160,000	Autosomal recessive		NCT01560182; LV-ARSA
Mucopolysaccharidosis type I	1:100,000	Autosomal recessive	ERT (laronidase)	
Mucopolysaccharidosis type II	1:100,000–170,000	X-linked recessive	ERT (idursulfase)	NCT00004454; RV-iduronate-2-sulfatase
Mucopolysaccharidosis type IIIA (Sanfilippo disease type A)	^1:70,000[c]	Autosomal recessive		NCT01474343; rAAV-SGSH and rAAV-SUMF1
Mucopolysaccharidosis type IVA	1:200,000–300,000	Autosomal recessive	ERT (galsulfase)	
Mucopolysaccharidosis type VI	1:250,000–600,000	Autosomal recessive	ERT (galsulfase)	
Neuronal ceroid lipofuscinosis (Batten disease)	1:25,000–50,000	Autosomal recessive		NCT00151216, NCY01411985, NCT01161576; rAAV-CUhCLN2
Niemann-Pick disease type C	1:150,000	Autosomal recessive	Hydroxypropyl-β-cyclodextrin; SRT (miglustat)[b]	

[a]http://ghr.nlm.nih.gov/.
[b]Approved by the European Medicines Agency, but not by the U.S. Food and Drug Administration.
[c]Combined incidence of all MPSIII.

secrete a myriad of proteins. Treatment of neonatal animal models with liver-targeting vectors has been successful for several LSDs including mucopolysaccharidosis (MPS) I and MPS VII; however, treatment of more mature/adult animals has proven more challenging, primarily because of the more robust immune response in mature animals (Ponder and Haskins, 2007; McKay *et al.*, 2011). For example, in the mouse model of Pompe disease, antibodies elicited in response to gene therapy completely abrogated cross-correction of other distal tissues (Cresawn *et al.*, 2005; Warrington and Herzog, 2006; Koeberl *et al.*, 2007). Efforts have been made to develop vectors that would be less immunogenic, with the most common strategy being the use of tissue-specific promoters for the target depot organ. Again, using Pompe disease studies as an example, use of a liver-specific promoter did result in a reduced immune response and in those animals, cross-correction of skeletal and cardiac muscle could be achieved. However, in those same studies, the use of tissue-specific promoters did not completely eliminate the possibility of immune reactivity to the therapeutic enzyme, revealing the complexities of immune tolerance (Cresawn *et al.*, 2005; Sun *et al.*, 2009; Zhang *et al.*, 2012).

Despite the successes of cross-correction-based strategies, for both gene therapy and ERT, these strategies cannot overcome the blood–brain barrier hurdle and as such, can only potentially treat the somatic manifestations, leaving the neuropathological problems untreated. The inability to address the neural components of disease impedes the ability to attain substantial correction, as functional deficits would remain. While the natural history of various LSDs has shifted as a result of approved therapeutics prolonging the life span and delaying disease progression, a new paradigm of multisystem involvement has materialized and brought forth a need to address all aspects to achieve productive correction and an overall improved outcome. One additional important consideration is the relative inefficiency of the secretion–reuptake mechanism versus the primary pathway by which lysosomal proteins are processed. Immediate processing of bisphosphorylated high-mannose-containing protein from the *trans*-Golgi to the lysosome is the most efficient and natural pathway. This pathway requires cell-autonomous direct correction.

Direct Correction

Delivery of gene therapy vectors directly to affected tissue, allowing the cell to produce the therapeutic protein in an autonomous manner, is the most straightforward gene therapy strategy. Especially in the milieu of LSDs, the endogenous processing and trafficking of the enzyme within the transduced target results in greater efficiency of functional protein production, thereby providing improved correction through a native activity of the wild-type protein. Furthermore, although perhaps somewhat less efficient, the transduced target tissues themselves could also act as a depot of enzyme production and secretion for cross-correctional events.

The challenge in direct correction of target tissues lies primarily in the technical aspects of delivery, especially for those tissues/organs that are structured in a manner that is too widespread to be accessed by just a few direct injections, primary examples being the CNS and skeletal muscle. As such, studies have focused on treating specific tissues such as select muscle groups or regions of the CNS that would demonstrate the most clinical benefit. Pompe disease is an LSD with profound and progressive skeletal muscle weakness. A major complication in Pompe disease is respiratory insufficiency. Direct administration of vector to the diaphragm of Pompe mice resulted in correction in the diaphragm tissue. In particular, the targeted therapy yielded significant improvement in both the contractile strength of the diaphragm muscle as well as ventilatory function (Mah *et al.*, 2004, 2010; Rucker *et al.*, 2004).

Direct delivery of vector to the CNS may be one of the most efficient methods to effectively treat the neurological pathologies of LSDs, particularly those pathologies that occur within the brain or spinal cord (Lee *et al.*, 2011). Fortunately, the design of the CNS provides advantages to direct delivery strategies in that vectors can be transported along neuronal connections to distal sites and that secreted enzymes can be transported antero- and retrograde to cross-correct cells distal from the injection site. Animal model studies of LSDs including MPS I, MPS IIIB, MPS VII, Sandhoff diseases, Niemann-Pick A, globoid cell leukodystrophy, and metachromatic leukodystrophy showed widespread biochemical and histological correction in the brain and in some cases, even improvement in behavioral symptoms after direct administration of a gene therapy vector (Biffi and Naldini, 2005; Hodges and Cheng, 2006; Ponder and Haskins, 2007; Sands and Haskins, 2008).

Vector Delivery

As mentioned previously, direct injection of target cells/tissues for the goal of either direct correction (such as intracranial injection into target brain) or for cross-correction (portal vein injection for liver targeting) has been successfully used in preclinical animal model studies and is also being investigated in current clinical studies. However, other methods of vector delivery are also being explored.

Ex vivo bone marrow or hematopoietic stem cell gene therapy is being evaluated for the treatment of some LSDs and is the basis of several clinical trials for LSDs (Naldini, 2011). These strategies provide an advantage in which autologous bone marrow or hematopoietic stem cells are transduced with a gene therapy vector to express the therapeutic protein and are then introduced into the affected individual. Like with traditional bone marrow transplantation, the primary goal is to establish a long-term source of circulating enzyme for cross-correction of affected tissues and possibly the reconstitution of affected tissues with corrected stem cells. The potential benefit of this strategy is the reduction in graft rejection and other complications associated with the use of allogeneic or unrelated cells for transplantation.

Systemic (intravenous) delivery of vectors has been explored extensively in preclinical studies for LSDs. In an ideal situation, a single peripheral injection of vector would be sufficient to treat disease, and the vector itself would be optimized to selectively infect the target cells, whether the goal is for direct correction of affected cells, providing a depot for cross-correction, or a combination of both. Factors such as the physical cell–vector interaction required for infection (such as binding to a cellular receptor, either taking advantage of the normal tropisms or engineered) and

cis-elements within the vector (such as promoter/enhancer choices) play a role in the ability of a vector to target a particular cell type. Specific examples of systemic delivery studies are discussed below.

Viral Gene Therapy Vector Systems

Gene therapy has the potential ability to correct LSDs by providing an endogenous depot for therapeutic protein production available for cross-correction and/or directly correcting affected cells and tissues, thereby tackling the disease from different fronts. Virus-based gene therapy vectors have been shown to be the most effective in preclinical studies in a variety of animal models of LSDs and are discussed below.

Retroviral Vectors

Retrovirus-based vectors (RVs) are single-stranded, RNA-containing enveloped viruses. After entry into a host cell, the retroviral genome is reverse-transcribed into DNA that can then integrate into chromosomal DNA, thus promoting stable transduction in dividing cell populations. Retroviral vectors were the first gene therapy vectors to enter clinical trials in general and also within the context of LSDs. Lentiviruses (LVs) are a class of retroviruses, with a similar basic genome structure, but with the addition of several regulatory gene expression genes and the ability to transduce nondividing cell populations at higher efficiency than RVs (Mah *et al.*, 2002).

RV-based strategies have been shown to be successful in several animal models of LSDs, and *ex vivo* RV gene transfer was the first gene therapy strategy to be tested in clinical trials for LSDs. Interestingly, within the history of RV-based preclinical studies for LSDs, there are multiple instances in which a single strategy performed in different animal models of the same disease revealed different outcomes. Systemic intravenous delivery of an RV encoding the canine α-L-iduronidase (cIDUA) gene driven by a liver-specific promoter resulted in sustained, high levels of serum cIDUA with significant storage reduction and correction of cardiac function, vision, and bone mass density in the MPS I mouse model (Liu *et al.*, 2005). However, in the MPS I dog, the same strategy resulted in 25% of the serum cIDUA levels seen in mice and only moderate clinical improvement (Traas *et al.*, 2007). And in the MPS I cat, whereas cIDUA expression was similar to that seen in dogs, expression was transient because of a robust cytotoxic T lymphocyte response against cIDUA-expressing cells (Ellinwood *et al.*, 2004; Ponder *et al.*, 2006). In another example, RV-mediated liver-directed expression of β-glucuronidase resulted in significantly increased survival and long-term expression (11 years) in both the murine and canine models of MPS VII. However, unlike in the murine model, treated MPS VII dogs still developed lumbar spinal disease, which is thought to be due to the inability of the secreted enzyme to reach and cross-correct the spine tissues (Macsai *et al.*, 2012; Smith *et al.*, 2012). Together these studies suggest that strategies may not translate in a linear fashion as we move from preclinical studies in small to large animal models of disease to clinical trials. They also highlight the benefit of testing strategies in multiple systems, when possible, as such studies could provide insight into the potential complications/hurdles that may need to be addressed in future studies (Sands and Davidson, 2006; Ponder and Haskins, 2007).

Both *in vivo* and *ex vivo* strategies have been employed for treatment of LSD by LV gene therapy. LVs have been shown to transduce neurons efficiently in both rodents and nonhuman primates and as such, have been used to target CNS pathology in LSDs (Biffi and Naldini, 2005). Ventricular infusion of an LV expressing β-glucuronidase in the MPS VII mouse resulted in widespread biochemical and histological normalization of regions within the brain with significant improvement in behavioral performance. Not surprisingly, somatic pathologies still persisted in these animals (Bielicki *et al.*, 2010). Similarly, intracerebral injection of therapeutic LVs in mouse models of Krabbe and metachromatic leukodystrophy yielded metabolic correction in the brain (Di Domenico *et al.*, 2009). These and other studies suggest that LV vectors may be useful in treating CNS pathology in LSDs; however, they also highlight the need for concomitant treatment of non-CNS pathologies, whether it is in the form of current therapies such as ERT or other gene transfer strategies.

The inherent traits of LVs to infect quiescent cells and integrate into the cellular chromosome make them a potentially ideal vector for *ex vivo* hematopoietic stem cell gene therapy. Transplantation of hematopoietic stem and progenitor cells transduced by an LV-IDUA vector improved metabolic and functional correction in MPS I mice (Wang *et al.*, 2009; Visigalli *et al.*, 2010). Furthermore, metachromatic leukodystrophy murine studies have demonstrated some reconstitution of defects in the central and peripheral nervous systems, suggesting that further refinement of an *ex vivo* gene therapy strategy could lead to simultaneous correction of both somatic and neurological pathologies.

Adenovirus

Adenoviruses are naked, double-stranded DNA viruses with a genome that is about 26–45 kb and flanked on both ends by inverted terminal repeats (ITRs). Advantages of adenoviral vectors (AdVs) are the ability to infect a broad range of cell types, both dividing and nondividing, and the capacity for large foreign DNA constructs. Furthermore, recombinant AdVs generally do not integrate into chromosomal DNA and persist as episomal DNA, thereby minimizing the risk of unwanted insertional mutagenesis (Mah *et al.*, 2002).

In general, AdV-based therapies for LSDs have been used to a lesser extent than other viral vector systems. Systemic delivery of AdVs has been explored as a therapy for LSDs. Widespread correction of tissues including liver, kidney, and skeletal muscle in animal models of LSDs including MPS VII, Fabry disease, and Pompe disease has been reported. Hurdles for this strategy include long-term persistent expression of the therapeutic transgene, interaction with the host immune system, and lack of correction of isolated tissues such as brain, eye, and bone (Kosuga *et al.*, 2000; Ziegler *et al.*, 2002; Kamata *et al.*, 2003; Kiang *et al.*, 2006). AdV studies have focused more on delivery of vector directly to affected tissues, in particular the brain; however, most studies resulted in limited expression duration (Stein *et al.*, 1999; Hsich *et al.*, 2002; Eto *et al.*, 2004). Refinement of AdV design from initial first-generation vectors to the gutless vector has

contributed to the more efficient performance of these vectors and in particular, significant reduction in vector-related immunogenicity and concomitant prolonged transgene expression. Most recently, direct intracranial injection of a therapeutic AdV into a mouse model of MPS IIIA resulted in long-term (8.5 months) expression in localized regions within the brain with slight reductions in neuropathology (Lau *et al.*, 2012).

Adeno-Associated Virus

Adeno-associated viruses are nonpathogenic, single-stranded DNA-containing parvoviruses. Their genome is flanked on both ends by ITRs, which are the sole *cis*-acting elements required for packaging and stable integration. Recombinant adeno-associated viral (rAAV) vectors have been shown to stably transduce both dividing and nondividing cells efficiently (Mah *et al.*, 2002). More than 100 novel serotypes of AAV have been isolated, some of which have been developed as gene therapy vectors and have demonstrated distinct pharmacokinetics *in vivo* (Gao *et al.*, 2005, 2011).

Recombinant AAV gene therapy has been successful in both small and large animal models of several LSDs, including mucopolysaccharidosis, Fabry disease, and Pompe disease (Warrington and Herzog, 2006; Ponder and Haskins, 2007). Because of the panoply of tissues affected in LSDs, rAAV-mediated cross-corrective therapies have been intensely investigated. Most recently, long-term biochemical and physiological correction of visceral tissues has been reported in MPS VI cats that were given liver-directed rAAV. Furthermore, some skeletal anomalies were also improved in those animals that expressed normal or greater than normal levels of serum arylsulfatase B (Cotugno *et al.*, 2011). In a different study, rAAV-mediated liver-directed production of sulfamidase at supraphysiological levels resulted in partial improvement of brain pathology in MPS IIIA mice (Ruzo *et al.*, 2012). These studies show that partial cross-correction of "difficult to access" tissues such as bone, brain, cartilage, and eye may be possible by cross-corrective therapies; however, the technology still needs to be refined as currently only a superabundance of therapeutic enzyme expression and secretion is required to produce a therapeutic effect.

The use of rAAV to directly correct affected tissues, in particular neurological manifestations, is also being investigated. To date, the majority of studies for direct CNS correction for LSDs have been done with rAAVs. In more recent studies, direct intracranial delivery of therapeutic rAAV resulted in reduction of lesions in the CNS in murine models of MPS I and Batten disease and canine models of Sanfilippo and Hurler syndromes (Cabrera-Salazar *et al.*, 2007; Ellinwood *et al.*, 2011; Wolf *et al.*, 2011). Although these studies demonstrate the profound impact of rAAV-mediated transgene expression in the CNS, the effect is limited to this tissue and does not improve non-CNS-related pathology.

Vectors based on rAAV serotype 9 (rAAV9) have been shown to have unique pharmacokinetics that may make them ideal candidates as gene therapy vectors for LSDs. Specifically, rAAV9 has demonstrated efficient transduction of most tissue types affected in LSDs including liver, heart, muscle, and CNS, both after direct administration and systemic delivery. A single intravenous injection of rAAV9 in a mouse model of Pompe disease resulted in improved respiratory muscle and cardiac function indices over those seen with chronic ERT administration (Pacak *et al.*, 2006; Falk *et al.*, 2011). Intravenous rAAV9 administration has also been shown to reduce storage pathology in the CNS and periphery in a murine model of MPS IIIB (Fu *et al.*, 2011). More interestingly, rAAV9 has been shown to transduce neural tissue via retrograde transport after intramuscular delivery or even by crossing the blood–brain barrier itself (Foust *et al.*, 2009; Bevan *et al.*, 2011; Dayton *et al.*, 2012; Dimattia *et al.*, 2012; ElMallah *et al.*, 2012). Together, the emerging data suggest that rAAV9 vector administration can achieve simultaneous autonomous expression of the therapeutic enzyme in multiple organ systems affected in LSDs, in particular both visceral and neural, thereby overcoming the primary obstacle of cross-corrective therapies.

Complications of Gene Therapy for LSDs

The innate and adaptive immune system has the ability to limit the success of viral gene transfer. Immune reactions to all LSDs have been noted in individuals receiving ERT, resulting in hypersensitivity reactions, alteration of enzyme trafficking and half-life, and/or neutralization of enzyme activity. For many, such immune responses can be controlled by the use of modified administration procedures, antihistamines, corticosteroids, and/or other immune-suppressive drugs (Brooks *et al.*, 2003; Wang *et al.*, 2008). Because gene therapy provides a protein that is either foreign (in cross-reacting immune material-negative [$CRIM^-$] patients) or in quantities/quality not normally seen (in $CRIM^+$ patients), similar immune reactions are not unexpected.

Although the aforementioned generalized drug regimens can and have been used in conjunction with gene therapy, strategies to generate more "immune-compatible" vectors or specific immune tolerance protocols are also being assessed (Arruda *et al.*, 2009; Nayak and Herzog, 2010; Mays and Wilson, 2011; Mingozzi and High, 2011). Strategies such as limiting expression to target tissues and avoiding expression in undesirable nontarget cells such as antigen-presenting cells by use of tissue-specific promoters have been shown to avoid provocation of a strong immune response. Interestingly, from studies investigating targeted gene expression, it was found that rAAV-mediated hepatic expression could lead to product-specific immune tolerance partially via induction of regulatory T cells (Ponder and Haskins, 2007; LoDuca *et al.*, 2009; Nayak *et al.*, 2009; Byrne *et al.*, 2011; Zhang *et al.*, 2012).

Another strategy to avoid immune response has been the timing of gene transfer to the neonatal or even the prenatal (*in utero*) stage of development. At these very young ages, the immune system is immature and introduction of foreign proteins at this time could allow for tolerization to the therapeutic product (Ponder, 2007; McKay *et al.*, 2011). Furthermore, another advantage to initiating treatment at this age is that disease pathology is likely less severe. As LSDs are progressive diseases, it has been shown that the longer the duration of disease, combined with the inherent reduction in tissue plasticity with age, the less chance there is to reverse pathology. Neonatal gene transfer has been successful with RV, LV, and rAAV vectors in several preclinical animal models of LSDs including MPS I, MPS VII, Fabry, Batten, and Pompe disease (Meikle and Hopwood, 2003;

Rucker *et al.*, 2004; Yoshimitsu *et al.*, 2004; Mah *et al.*, 2007; Traas *et al.*, 2007; Sondhi *et al.*, 2008). How neonatal gene therapy will translate clinically is yet unknown, because in the neonatal stage humans have a more developed or mature immune system compared with lesser animals. Although it is likely that some immune reaction is possible, it may be less severe or more easily managed than when such a reaction would occur in older patients. In addition, it has been clear from ERT studies that earlier treatment initiation results in a general improved therapeutic benefit and clinical outcome, thus lending further support toward neonatal gene transfer efforts (Meikle and Hopwood, 2003; Nakamura *et al.*, 2011).

Clinical Studies and Future Outlook

To date, several phase 1/2 clinical studies have been initiated for gene therapy-based treatments for lysosomal storage diseases (Table 1). Four studies are investigating the safety of *ex vivo* retroviral or lentiviral vector-transduced autologous cells (for the treatment of Gaucher [NCT00001234 and NCT00004294], Fabry [NCT00001234], mucopolysaccharidosis II [NCT00004454], and metachromatic leukodystrophy diseases [NCT01560182]). For the Gaucher and MPS II studies, which have been completed, low expression and no improvement in disease pathology were noted (Dunbar *et al.*, 1998; Alexander *et al.*, 2007). However, these were the first two gene transfer clinical studies for LSDs and since their initiation was greater than 10 years ago, there has been a surge in the development and improvement of vector design and delivery and thus improved results. At the time of this writing, the trial for metachromatic leukodystrophy is currently recruiting subjects and as such, results are pending.

Other clinical studies are investigating the direct delivery of recombinant adeno-associated viral vectors to affected tissues for the treatment of Pompe disease (NCT00976352), Sanfilippo type A syndrome (NCT01474343), and Batten disease (NCT00151216, NCY01411985, NCT01161576). The initial study for Batten disease involved direct injection of rAAV serotype 2-based vector into the CNS of affected children. The data resulting from the study suggested that disease progression may have been slowed; however, a true therapeutic benefit was not established (Worgall *et al.*, 2008). Like the initial retrovirus-based studies, the arena of rAAV-based gene therapy has transformed substantially since the initiation of the study and a continuing study with an alternative and potentially more robust rAAV serotype vector is now pending. The Sanfilippo study involves direct injection of a therapeutic rAAV serotype 10 into the brain. This study has been initiated; however, patients are not yet being recruited.

Our group initiated an rAAV clinical study for Pompe disease. Respiratory insufficiency is one of the primary complications in Pompe disease and long-term evaluation of patients receiving ERT has revealed progressive respiratory dysfunction that may partially be attributed to the lack of correction of the neural components of respiration (Byrne *et al.*, 2011). As mentioned previously, preclinical studies showed that direct administration of rAAV1 to diaphragms of Pompe mice could lead to improvement in diaphragm contractile strength and ventilatory function. In addition, phrenic nerve activity was increased in the treated animals, suggesting that some level of correction of CNS pathology may have also occurred (Mah *et al.*, 2004, 2010; DeRuisseau *et al.*, 2009). In light of these data and the safety profile of other rAAV1 clinical trials, we initiated an open-label, phase 1/2 study investigating the safety and efficacy of intramuscular gene transfer to the diaphragm with a therapeutic rAAV serotype 1 (rAAV1) vector encoding acid-α-glucosidase (GAA). To date, no serious adverse events or systemic toxicities related to vector administration have been detected. Furthermore, significant elevation in respiratory parameters has been noted in the first cohort of subjects (Byrne *et al.*, 2012). This study is ongoing and results are pending from the high-dose second cohort of subjects.

Ideally, all components of disease pathology would be corrected via systemic delivery of a single vector. However, the complex nature of LSDs and multiorgan involvement make this extremely difficult. Other factors, such as the timing of gene transfer in relation to the degree of disease progression, the differing levels of therapeutic product required to correct various tissue pathologies, and the immune consequences as they relate to administration procedures, vector/cells used, and transgene product will also influence therapeutic outcome of gene therapy. It is also important to note that although for the most part heterozygotes for LSDs do not manifest clinical symptoms of disease, exceptions have been described. In particular, greater than 70% of female carriers of Fabry disease have displayed some form of disease-related pathology. Although pathology has been attributed mostly to X chromosome inactivation, studies have also shown that enzyme uptake may be compromised in otherwise normal cells in these patients (Vanier, 2010). Abnormal intracellular mannose 6-phosphate receptor trafficking has also been described in cells from Pompe carriers (Cardone *et al.*, 2008). These findings underscore the potential importance of autonomous expression of the therapeutic protein achieved through direct correction of affected cells via gene therapy. A combination of therapies (ERT, chaperone, direct gene transfer, cross-correctional gene therapy strategies, *ex vivo* therapy, different vectors, etc.), however, may be needed to fully cure the disease long term. In the meanwhile, current developed therapies may potentially address the major complicating factors of disease, thereby facilitating improved survival and quality of life. Further development and refinement of gene therapy vectors is ongoing and the field is constantly growing. Results from the ongoing and pending clinical trials incorporating gene therapy will greatly improve our understanding of therapies and outcomes in patients with LSDs.

Acknowledgments

The authors acknowledge the University of Florida Powell Gene Therapy Center Vector Core Laboratory for production of rAAV vectors for preclinical studies and the Human Applications Laboratory at the University of Florida for production of rAAV vector for clinical trial NCT00976352. This work was supported by grants from the National Institutes of Health (NHLBI P01 HL59412-06 [B.J.B.], 5F32HL095282-03 [D.J.F.], and the NHLBI Gene Therapy Resource Program).

Author Disclosure Statement

B.J.B., Johns Hopkins University, and the University of Florida could be entitled to patent royalties for inventions described in this manuscript.

References

Alexander, B.L., Ali, R.R., Alton, E.W., *et al.* (2007). Progress and prospects: Gene therapy clinical trials (part 1). Gene Ther. 14, 1439–1447.

Arruda, V.R., Favaro, P., and Finn, J.D. (2009). Strategies to modulate immune responses: A new frontier for gene therapy. Mol. Ther. 17, 1492–1503.

Bevan, A.K., Duque, S., Foust, K.D., *et al.* (2011). Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. Mol. Ther. 19, 1971–1980.

Bielicki, J., McIntyre, C., and Anson, D.S. (2010). Comparison of ventricular and intravenous lentiviral-mediated gene therapy for murine MPS VII. Mol. Genet. Metab. 101, 370–382.

Biffi, A., and Naldini, L. (2005). Gene therapy of storage disorders by retroviral and lentiviral vectors. Hum. Gene Ther. 16, 1133–1142.

Brooks, D.A., Kakavanos, R., and Hopwood, J.J. (2003). Significance of immune response to enzyme-replacement therapy for patients with a lysosomal storage disorder. Trends Mol. Med. 9, 450–453.

Byrne, B., Smith, B., Martin, A., *et al.* (2012). Phase I/II trial of adeno-associated virus acid-α-glucosidase (AAV-GAA) diaphragm gene therapy for ventilatory failure in Pompe disease. Mol. Genet. Metab. 105, S24.

Byrne, B.J., Falk, D.J., Pacak, C.A., *et al.* (2011). Pompe disease gene therapy. Hum. Mol. Genet. 20, R61–R68.

Cabrera-Salazar, M.A., Roskelley, E.M., Bu, J., *et al.* (2007). Timing of therapeutic intervention determines functional and survival outcomes in a mouse model of late infantile Batten disease. Mol. Ther. 15, 1782–1788.

Cardone, M., Porto, C., Tarallo, A., *et al.* (2008). Abnormal mannose-6-phosphate receptor trafficking impairs recombinant α-glucosidase uptake in Pompe disease fibroblasts. Pathogenetics 1, 6.

Cotugno, G., Annunziata, P., Tessitore, A., *et al.* (2011). Long-term amelioration of feline mucopolysaccharidosis VI after AAV-mediated liver gene transfer. Mol. Ther. 19, 461–469.

Cresawn, K.O., Fraites, T.J., Wasserfall, C., *et al.* (2005). Impact of humoral immune response on distribution and efficacy of recombinant adeno-associated virus-derived acid α-glucosidase in a model of glycogen storage disease type II. Hum. Gene Ther. 16, 68–80.

Dayton, R.D., Wang, D.B., and Klein, R.L. (2012). The advent of AAV9 expands applications for brain and spinal cord gene delivery. Expert Opin. Biol. Ther. 12, 757–766.

DeRuisseau, L.R., Fuller, D.D., Qiu, K., *et al.* (2009). Neural deficits contribute to respiratory insufficiency in Pompe disease. Proc. Natl. Acad. Sci. U.S.A. 106, 9419–9424.

Di Domenico, C., Villani, G.R., Di Napoli, D., *et al.* (2009). Intracranial gene delivery of LV-NAGLU vector corrects neuropathology in murine MPS IIIB. Am. J. Med. Genet. A 149A, 1209–1218.

Dimattia, M.A., Nam, H.J., Van Vliet, K., *et al.* (2012). Structural insight into the unique properties of adeno-associated virus serotype 9. J. Virol. 86, 6947–6958.

Dunbar, C.E., Kohn, D.B., Schiffmann, R., *et al.* (1998). Retroviral transfer of the glucocerebrosidase gene into $CD34^+$ cells from patients with Gaucher disease: *In vivo* detection of transduced cells without myeloablation. Hum. Gene Ther. 9, 2629–2640.

Ellinwood, N.M., Vite, C.H., and Haskins, M.E. (2004). Gene therapy for lysosomal storage diseases: The lessons and promise of animal models. J. Gene Med. 6, 481–506.

Ellinwood, N.M., Ausseil, J., Desmaris, N., *et al.* (2011). Safe, efficient, and reproducible gene therapy of the brain in the dog models of Sanfilippo and Hurler syndromes. Mol. Ther. 19, 251–259.

ElMallah, M.K., Falk, D., Lane, M., *et al.* (2012). Retrograde gene delivery to hypoglossal motoneurons using AAV9. Hum. Gene Ther. Methods (in press).

Eto, Y., Shen, J.S., Meng, X.L., and Ohashi, T. (2004). Treatment of lysosomal storage disorders: Cell therapy and gene therapy. J. Inherit. Metab. Dis. 27, 411–415.

Falk, D., Soustek, M.S., Mah, C.S., *et al.* (2011). Next generation treatment of Pompe disease using systemic gene transfer with AAV9. Mol. Genet. Metab. 102, S18.

Foust, K.D., Nurre, E., Montgomery, C.L., *et al.* (2009). Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat. Biotechnol. 27, 59–65.

Fu, H.Y., Dirosario, J., Killedar, S., *et al.* (2011). Correction of neurological disease of mucopolysaccharidosis IIIB in adult mice by rAAV9 *trans*-blood–brain barrier gene delivery. Mol. Ther. 19, 1025–1033.

Gao, G., Vandenberghe, L.H., and Wilson, J.M. (2005). New recombinant serotypes of AAV vectors. Curr. Gene Ther. 5, 285–297.

Gao, G., Zhong, L., and Danos, O. (2011). Exploiting natural diversity of AAV for the design of vectors with novel properties. Methods Mol. Biol. 807, 93–118.

Hodges, B.L., and Cheng, S.H. (2006). Cell and gene-based therapies for the lysosomal storage diseases. Curr. Gene Ther. 6, 227–241.

Hsich, G., Sena-Esteves, M., and Breakefield, X.O. (2002). Critical issues in gene therapy for neurologic disease. Hum. Gene Ther. 13, 579–604.

Kamata, Y., Tanabe, A., Kanaji, A., *et al.* (2003). Long-term normalization in the central nervous system, ocular manifestations, and skeletal deformities by a single systemic adenovirus injection into neonatal mice with mucopolysaccharidosis VII. Gene Ther. 10, 406–414.

Kiang, A., Hartman, Z.C., Liao, S., *et al.* (2006). Fully deleted adenovirus persistently expressing GAA accomplishes long-term skeletal muscle glycogen correction in tolerant and nontolerant GSD-II mice. Mol. Ther. 13, 127–134.

Koeberl, D.D., Kishnani, P.S., and Chen, Y.T. (2007). Glycogen storage disease types I and II: Treatment updates. J. Inherit. Metab. Dis. 30, 159–164.

Kosuga, M., Takahashi, S., Sasaki, K., *et al.* (2000). Adenovirus-mediated gene therapy for mucopolysaccharidosis VII: Involvement of cross-correction in wide-spread distribution of the gene products and long-term effects of CTLA-4Ig coexpression. Mol. Ther. 1, 406–413.

Lau, A.A., Rozaklis, T., Ibanes, S., *et al.* (2012). Helper-dependent canine adenovirus vector-mediated transgene expression in a neurodegenerative lysosomal storage disorder. Gene 491, 53–57.

Lee, K.Z., Qiu, K., Sandhu, M.S., *et al.* (2011). Hypoglossal neuropathology and respiratory activity in Pompe mice. Front. Physiol. 2, 31.

Liu, Y., Xu, L., Hennig, A.K., *et al.* (2005). Liver-directed neonatal gene therapy prevents cardiac, bone, ear, and eye disease in mucopolysaccharidosis I mice. Mol. Ther. 11, 35–47.

LoDuca, P.A., Hoffman, B.E., and Herzog, R.W. (2009). Hepatic gene transfer as a means of tolerance induction to transgene products. Curr. Gene Ther. 9, 104–114.

Macsai, C.E., Derrick-Roberts, A.L., Ding, X., *et al.* (2012). Skeletal response to lentiviral mediated gene therapy in a mouse model of MPS VII. Mol. Genet. Metab. 106, 202–213.

Mah, C., Byrne, B.J., and Flotte, T.R. (2002). Virus-based gene delivery systems. Clin. Pharmacokinet. 41, 901–911.

Mah, C., Fraites, T.J., Jr., Cresawn, K.O., *et al.* (2004). A new method for recombinant adeno-associated virus vector delivery to murine diaphragm. Mol. Ther. 9, 458–463.

Mah, C., Pacak, C.A., Cresawn, K.O., *et al.* (2007). Physiological correction of Pompe disease by systemic delivery of adeno-associated virus serotype 1 vectors. Mol. Ther. 15, 501–507.

Mah, C.S., Falk, D.J., Germain, S.A., *et al.* (2010). Gel-mediated delivery of AAV1 vectors corrects ventilatory function in Pompe mice with established disease. Mol. Ther. 18, 502–510.

Mays, L.E., and Wilson, J.M. (2011). The complex and evolving story of T cell activation to AAV vector-encoded transgene products. Mol. Ther. 19, 16–27.

McKay, T.R., Rahim, A.A., Buckley, S.M., *et al.* (2011). Perinatal gene transfer to the liver. Curr. Pharm. Des. 17, 2528–2541.

Meikle, P.J., and Hopwood, J.J. (2003). Lysosomal storage disorders: Emerging therapeutic options require early diagnosis. Eur. J. Pediatr. 162(Suppl. 1), S34–S37.

Mingozzi, F., and High, K.A. (2011). Immune responses to AAV in clinical trials. Curr. Gene Ther. 11, 321–330.

Nakamura, K., Hattori, K., and Endo, F. (2011). Newborn screening for lysosomal storage disorders. Am. J. Med. Genet. C Semin. Med. Genet. 157, 63–71.

Naldini, L. (2011). *Ex vivo* gene transfer and correction for cell-based therapies. Nat. Rev. Genet. 12, 301–315.

Nayak, S., and Herzog, R.W. (2010). Progress and prospects: Immune responses to viral vectors. Gene Ther. 17, 295–304.

Nayak, S., Cao, O., Hoffman, B.E., *et al.* (2009). Prophylactic immune tolerance induced by changing the ratio of antigen-specific effector to regulatory T cells. J. Thromb. Haemost. 7, 1523–1532.

Pacak, C.A., Mah, C.S., Thattaliyath, B.D., *et al.* (2006). Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction *in vivo*. Circ. Res. 99, e3–e9.

Ponder, K.P. (2007). Immunology of neonatal gene transfer. Curr. Gene Ther. 7, 403–410.

Ponder, K.P., and Haskins, M.E. (2007). Gene therapy for mucopolysaccharidosis. Expert Opin. Biol. Ther. 7, 1333–1345.

Ponder, K.P., Wang, B., Wang, P., *et al.* (2006). Mucopolysaccharidosis I cats mount a cytotoxic T lymphocyte response after neonatal gene therapy that can be blocked with CTLA4-Ig. Mol. Ther. 14, 5–13.

Rucker, M., Fraites, T.J., Jr., Porvasnik, S.L., *et al.* (2004). Rescue of enzyme deficiency in embryonic diaphragm in a mouse model of metabolic myopathy: Pompe disease. Development 131, 3007–3019.

Ruzo, A., Garcia, M., Ribera, A., *et al.* (2012). Liver production of sulfamidase reverses peripheral and ameliorates CNS pathology in mucopolysaccharidosis IIIA mice. Mol. Ther. 20, 254–266.

Sands, M.S., and Davidson, B.L. (2006). Gene therapy for lysosomal storage diseases. Mol. Ther. 13, 839–849.

Sands, M.S., and Haskins, M.E. (2008). CNS-directed gene therapy for lysosomal storage diseases. Acta Paediatr. Suppl. 97, 22–27.

Smith, L.J., Martin, J.T., O'Donnell P, *et al.* (2012). Effect of neonatal gene therapy on lumbar spine disease in mucopolysaccharidosis VII dogs. Mol. Genet. Metab. (in press).

Sondhi, D., Peterson, D.A., Edelstein, A.M., *et al.* (2008). Survival advantage of neonatal CNS gene transfer for late infantile neuronal ceroid lipofuscinosis. Exp. Neurol. 213, 18–27.

Stein, C.S., Ghodsi, A., Derksen, T., and Davidson, B.L. (1999). Systemic and central nervous system correction of lysosomal storage in mucopolysaccharidosis type VII mice. J. Virol. 73, 3424–3429.

Sun, B., Zhang, H., Bird, A., *et al.* (2009). Impaired clearance of accumulated lysosomal glycogen in advanced Pompe disease despite high-level vector-mediated transgene expression. J. Gene Med. 11, 913–920.

Traas, A.M., Wang, P., Ma, X., *et al.* (2007). Correction of clinical manifestations of canine mucopolysaccharidosis I with neonatal retroviral vector gene therapy. Mol. Ther. 15, 1423–1431.

Vanier, M.T. (2010). Niemann-Pick disease type C. Orphanet J. Rare Dis. 5, 16.

Visigalli, I., Delai, S., Politi, L.S., *et al.* (2010). Gene therapy augments the efficacy of hematopoietic cell transplantation and fully corrects mucopolysaccharidosis type I phenotype in the mouse model. Blood 116, 5130–5139.

Wang, D., Zhang, W., Kalfa, T.A., *et al.* (2009). Reprogramming erythroid cells for lysosomal enzyme production leads to visceral and CNS cross-correction in mice with Hurler syndrome. Proc. Natl. Acad. Sci. U.S.A. 106, 19958–19963.

Wang, J., Lozier, J., Johnson, G., *et al.* (2008). Neutralizing antibodies to therapeutic enzymes: Considerations for testing, prevention and treatment. Nat. Biotechnol. 26, 901–908.

Warrington, K.H., Jr., and Herzog, R.W. (2006). Treatment of human disease by adeno-associated viral gene transfer. Hum. Genet. 119, 571–603.

Wolf, D.A., Lenander, A.W., Nan, Z., *et al.* (2011). Direct gene transfer to the CNS prevents emergence of neurologic disease in a murine model of mucopolysaccharidosis type I. Neurobiol. Dis. 43, 123–133.

Worgall, S., Sondhi, D., Hackett, N.R., *et al.* (2008). Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. Hum. Gene Ther. 19, 463–474.

Yoshimitsu, M., Sato, T., Tao, K., *et al.* (2004). Bioluminescent imaging of a marking transgene and correction of Fabry mice by neonatal injection of recombinant lentiviral vectors. Proc. Natl. Acad. Sci. U.S.A. 101, 16909–16914.

Zhang, P., Sun, B., Osada, T., *et al.* (2012). Immunodominant liver-specific expression suppresses transgene-directed immune responses in murine pompe disease. Hum. Gene Ther. 23, 460–472.

Ziegler, R.J., Li, C., Cherry, M., *et al.* (2002). Correction of the nonlinear dose response improves the viability of adenoviral vectors for gene therapy of Fabry disease. Hum. Gene Ther. 13, 935–945.

Address correspondence to:
Dr. Barry J. Byrne

or

Dr. Cathryn S. Mah
University of Florida
Powell Gene Therapy Center
RG-148, 1200 Newell Drive
Gainesville, FL 32610

E-mail: bbyrne@ufl.edu

Received for publication July 9, 2012;
accepted July 13, 2012.

Published online: July 13, 2012.