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REVIEW

Recent advances in gene therapy for lysosomal storage disorders

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Abstract: Lysosomal storage disorders (LSDs) are a group of genetic diseases that result in metabolic derangements of the lysosome. Most LSDs are due to the genetic absence of a single catabolic enzyme, causing accumulation of the enzyme's substrate within the lysosome. Over time, tissue-specific substrate accumulations result in a spectrum of symptoms and disabilities that vary by LSD. LSDs are promising targets for gene therapy because delivery of a single gene into a small percentage of the appropriate target cells may be sufficient to impact the clinical course of the disease. Recently, there have been several significant advancements in the potential for gene therapy of these disorders, including the first human trials. Future clinical trials will build upon these initial attempts, with an improved understanding of immune system responses to gene therapy, the obstacle that the blood–brain barrier poses for neuropathic LSDs, as well other biological barriers that, when overcome, may facilitate gene therapy for LSDs. In this manuscript, we will highlight the recent innovations in gene therapy for LSDs and discuss the clinical limitations that remain to be overcome, with the goal of fostering an understanding and further development of this important field.

Keywords: human trials, clinical trials, gene therapy, lysosomal storage disease, blood-brain barrier, adeno-associated virus, lentivirus, adenovirus

Introduction

Lysosomal storage disorders (LSDs) are a group of over 40 distinct inherited diseases that result in metabolic derangements of the lysosome.[1] Clinical features of LSDs vary from disease to disease, but can include cardiomegaly, hepatosplenomegaly, skeletal deformity, cognitive disability, and premature death.[2] Most LSDs result from a loss-of-function mutation in a single gene responsible for producing a catalytic lysosomal enzyme.[3] As a result of insufficient enzymatic activity, the enzyme's substrate accumulates over variable amounts of time within the lysosomes of specific tissues, causing the pathophysiology of the respective LSD.[1,4]

Compared to many other genetic diseases, LSDs are practical targets for gene therapy because of a unique physiologic trait referred to as "cross-correction". Cross-correction allows specific extracellular LSD enzymes to be taken up and targeted to the lysosomes of otherwise enzyme-deficient cells.[5] This phenomenon was exploited to develop the first United States Food and Drug Administration-approved therapies for these diseases, known as enzyme replacement therapies (ERT).[6,7] These therapies typically involve repeated (life-long) intravenous administrations of a recombinant LSD enzyme into affected patients. From the bloodstream, the recombinant LSD enzyme can travel to affected tissues throughout the body, enter enzyme-deficient cells, and partially restore deficient lysosomal enzymatic functions. Unfortunately, recombinant

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enzymes administered intravenously do not easily cross the blood–brain barrier (BBB), so they are unable to resolve the severe neurological manifestations of many LSDs.[8,9] Additionally, in some patients, repeated administration of recombinant LSD enzymes can trigger immune reactions to the enzyme that limit the effectiveness of the therapy.[10,11]

Despite these limitations, the success of ERTs for LSDs demonstrates that improved treatment of LSDs may be achieved by development of gene therapy approaches. This article will focus on recent developments in the field of gene therapy for the treatment of LSDs, including recent successes, as well limitations that remain to be overcome.

Suitability of LSDs as targets for gene therapy

Gene therapy is based upon a simple concept – namely, a working copy of an appropriate gene is provided to a patient to either stabilize or reverse a clinical disease state. There are several caveats that must be considered when evaluating a gene therapy for a LSD, including: 1) can the existing pathology be reversed or merely slowed in its rate of progression?; 2) can the appropriate tissues or cells affected by the respective LSD be targeted by the respective gene therapy approach?; and 3) will the patient's immune system perceive the protein produced by the gene therapy treatment as foreign? Despite these caveats, LSDs provide three advantages that allow them to be practical targets for gene therapy. Firstly, each LSD is a single-gene recessive disorder and the pathophysiology of the diseases is relatively well understood. The development of several animal models that mimic the pathophysiology of various LSDs furthers accurate preclinical evaluations of various forms of gene therapy.[12,13] Secondly, clinical studies of residual LSD enzyme activities in more mildly affected LSD patients have shown that even small improvements in enzyme activity can be associated with significant impacts on the clinical course of the disease. Studies in both Pompe disease (glycogen storage disease type II [GSDII] knockout [KO])[14] and metachromatic leukodystrophy (MLD)[15,16] have demonstrated that the most severe infantile-onset forms of the disease correlate with $<1\%$ enzyme activity, and that adult onset forms correlate with $<10\%$–15% of normal enzyme activity. A similar pattern has been observed in Fabry disease, Niemann–Pick disease, and Gaucher's disease.[17,18] These results suggest that if gene therapy is able to produce even a relatively small amount of a respective LSD enzyme, it may have a large clinical impact upon the course of the disease. Finally, the most promising feature of LSDs as targets for the successful implementation of gene therapy is the "cross-correction" phenomenon. Lysosomal enzymes are secreted in small amounts to the extracellular space where they can be taken up and targeted to lysosomes of neighboring cells.[5] If supraphysiologic amounts of a respective LSD enzyme can be provided via gene therapy, secreted enzymes may enter the bloodstream and be taken up by distant organs, providing clinical benefits analogous or superior to ERT approaches. The most successful gene therapy approaches can exploit this pathway by expressing high concentrations of enzyme from the liver or other target organs and allowing that organ to secrete enzyme for "systemic cross-correction" (Figure 1).

Gene therapy vectors

There have been many different gene transfer "vectors" produced for the purpose of gene therapy; however, the vast majority of animal studies focus on the use of recombinant, virus-based vectors due to their high probability for allowing robust gene transfer and expression into a variety of cells and tissue types (Figure 2). Under the right circumstances, each of these vectors have demonstrated long-term efficacy in animal models of LSDs, and each are viable candidates for human gene therapy of various LSDs.[19–24,31]

Adenovirus

Generally speaking, adenovirus-based gene therapy vectors have been the most widely used vector in human clinical trials to date, with 23.3% of all gene therapy clinical trials utilizing adenoviruses.[25] Adenoviruses are double-stranded DNA viruses that do not integrate into the host genome, can infect nonreplicating cells, and can transfer up to 37 kb of genetic information.

Early generation vectors based on adenoviruses were potent activators of the innate immune system, activating TLR9,[26] TLR2,[26] complement,[27] and the NLRP3 inflammasome.[28] New strategies have been developed to limit immunity with adenoviruses, including the preemptive administration of medications to prevent innate immune responses to the vector,[29] the use of liver targeting veno-occlusive systems diminishing systemic exposure to high doses of recombinant adenovirus vectors,[30,31] and the development of advanced generation viruses that lack viral genes, such as helper-dependent adenoviruses (HD-Ads) that lack all viral genes.[30–33] HD-Ads have decreased immunity and sustained expression of transgenes in mice and nonhuman primates. Although HD-Ads are still capable of activating innate immunity, as would any virus-based vector,[34] the mechanism and degree of innate immune activation can be

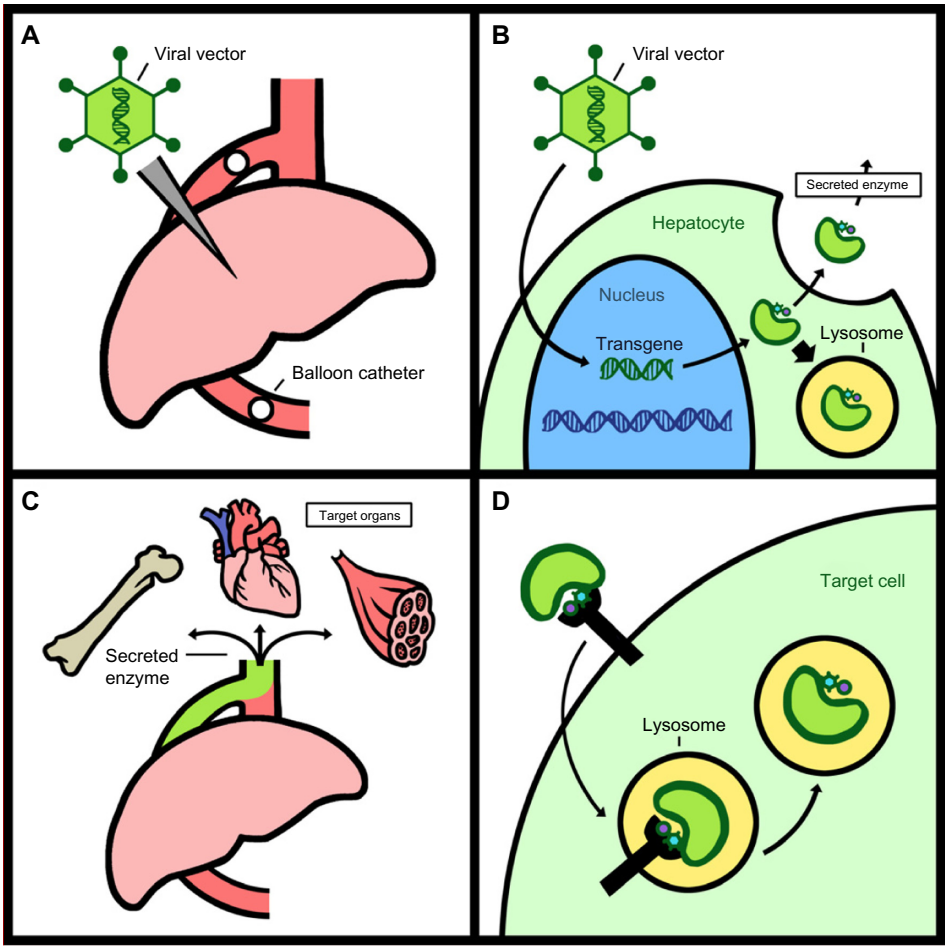

Figure 1 Systemic cross-correction.
Notes: LSDs are practical targets for gene therapy because cells that are corrected by gene therapy can secrete the transfected lysosomal enzyme, which can then be taken up by neighboring cells. Gene therapy can express superphysiologic amounts of enzyme in a target organ, such as the liver, that can then be excreted and travel to effected tissues through the blood. (**A**) The liver is infected with the viral vector. (**B**) The virus introduces its genetic cargo into the nucleus of hepatocytes. The hepatocytes produce superphysiologic amounts of enzyme, some of which are secreted. (**C**) Enzyme (green) enters the bloodstream and travels throughout the circulation, reaching the affected tissues. (**D**) Within these tissues, the enzyme binds receptors (black bar) and is trafficked to the lysosome.
Abbreviation: LSDs, lysosomal storage disorders.

comparable to nonviral methods for gene therapy.[35] Recently, HD-Ads have been demonstrated to persist in nonhuman primates for up to 7 years, demonstrating their capacity for durable expression in immune-tolerant animals.[31]

Additional advances in adenovirus-based gene transfer vectors include the development of a variety of alternative serotype-based vectors that evade pre-existing immunity, and have other interesting properties. For instance, canine adenovirus serotype 2 has been observed to be less immunogenic than classic adenoviral vectors,[36] and to have high tropism for neurons,[37] allowing it to be used for the improved treatment of neonatal mucopolysaccharisosis mice.[38]

Retroviruses and lentivirus

Retroviruses (RVs) and lentiviruses (LVs) are enveloped single-stranded RNA viruses. RVs and LVs integrate directly into the host's genome. This ability to integrate into the host's DNA is both a great advantage and a liability. On the one hand, integration allows the genes delivered by such vectors to potentially be permanent, persisting indefinitely within the host cell despite repeated cell division, such as in hematopoietic stem cells (HSCs).[39] On the other hand, multiple integrations can also promote insertional mutagenesis. For instance, in gene therapy trials using γ-RVs to treat SCID-X1 immunodeficiency, multiple patients developed T-cell acute lymphoblastic leukemia that was directly attributable to the RV randomly integrating next to, and activating the *LMO-2* oncogene.[40,41] Recent work has been aimed at improving the safety profile of RVs and LVs by targeting or limiting their ability to randomly integrate, or to include, "transcriptional insulators" that decrease the chance to transcriptionally activate bystander genes.[42,43] In gene therapy for LSDs, RVs

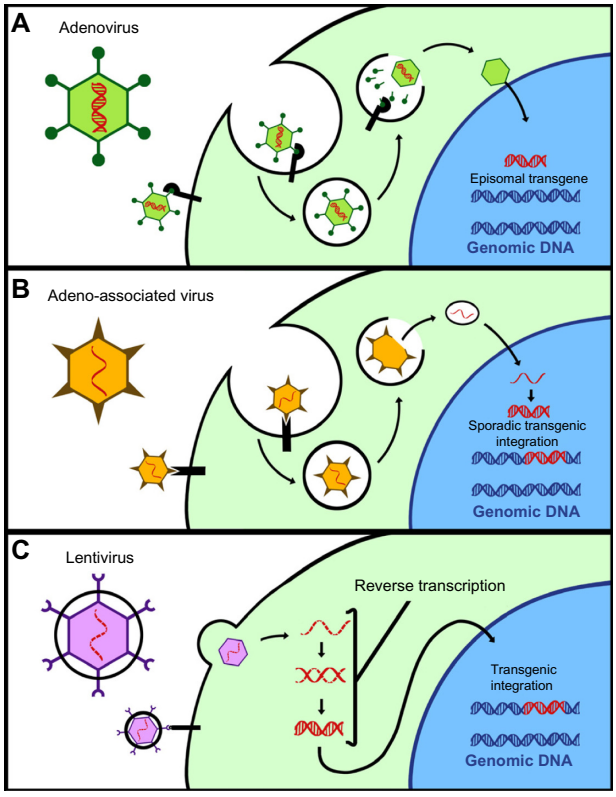

Figure 2 Commonly used viral vectors.
Notes: (**A**) Adenovirus is a nonenveloped double-stranded DNA virus. It enters the cell by binding adenovirus receptors (black bar) such as the coxsackievirus and adenovirus receptor and translocates its genome into the nuclease of the cell. Viral DNA (red helix) does not integrate into the host genome and persists as an episome. (**B**) Adeno-associated virus is a nonenveloped single-stranded DNA virus. It enters the cell using a variety of receptors, which vary by serotype (represented by the black bar). Inside the cell, it translocates its genome (red helix) into the nucleus where it is made into double-stranded DNA. This DNA may persist as an episome or sporadically be integrated into the host genome (blue helix). (**C**) Lentivirus (purple hexagon) is an enveloped single-stranded RNA virus. It enters the cell using a variety of receptors, which are different for each virus (represented by the black bar). Within the cell, it reverse transcribes its genome. The single strand of viral RNA (dashed red line) is made into a double-stranded DNA (red helix) before entering the nucleus. Retroviral DNA enters the nucleus during mitosis, while lentiviral DNA can enter the nucleus of nondividing cells via nuclear pores. Within the nuclease, the DNA integrates into the host genome (blue helix) at a random site.

and LVs are frequently used to transfect cells ex vivo. These cells can then be reinfused into the same patient that they were harvested from, and theoretically supply enzyme to neighboring cells in the body through cross-correction.

Adeno-associated virus

Adeno-associated virus (AAV) is a 4.7 kb nonenveloped single-stranded DNA parvovirus. AAVs infect cells by interacting with specific receptors that differ between serotypes. For this reason, AAV tropism is serotype-dependent.[44] Gene transfer vectors based on AAVs are capable of attaining sustained transgene expression in a wide variety of cells. Like adenoviruses and LVs, AAVs can also trigger innate or adaptive immune responses against the vector and the transgenes they express in a variety of settings.[45–52] Because they are capable of integration, another risk of AAV-based vectors is insertional mutagenesis and genotoxicity.[53,54] A unique feature of AAV vectors is the potential ability of certain serotypes to cross the BBB and enter the central nervous system (CNS), making them attractive vectors for the therapy of neuropathic LSDs.[55] For this reason, AAV9 has been used in a variety of attempts to achieve CNS-detected gene therapy for neuropathic LSDs.[56,57] The greatest limitation of AAV-based gene transfer vectors may be their potential for scalable production. Recently, a practical alternative method using a cell suspension system for AAV production has been developed.[58] This system has allowed for AAV to be produced cost effectively in current good manufacturing practices-compliance and has translated into the first approved gene therapy drug available in Europe, Glybera® (alipogene tiparvovec).[59]

Advances in gene therapy for LSDs

Over the past few years, many advances have been made in the construction and administration of gene therapy vectors. These advances either improved the safety or increased the potential efficacy of the vectors by overcoming limitations that were experienced in earlier gene therapy attempts.[62,66]

Overcoming adaptive immune responses

Overcoming the adaptive immune responses (cellular or humoral) to gene transfer is a critical issue for the successful treatment of LSDs in humans. Adaptive immune responses are a major limitation in ERT,[10,11] and adaptive immune responses can develop against the viral vector or against the transgene, and they have been reported with adenovirus,[60] AAV,[45,47,48,50] and LV.[61,62] Bypassing adaptive immunity was the main limitation in early attempts to treat LSDs with gene therapy; however, recent advances have allowed for the evasion of immune responses, and even the induction of tolerance, demonstrating an interesting potential advantage of gene therapy for the treatment of LSDs.[66,69]

These concepts are illustrated well in Pompe disease (GSDII). Pompe disease is caused by mutations in the *GAA* gene that result in insufficient acid α-glucosidase (GAA) production and the accumulation of glycogen within the lysosomes of affected tissues.[1] Infantile-onset Pompe disease presents at <1 year of age with musculoskeletal weakness, cardiomegaly, and progressive respiratory insufficiency. Without treatment, patients can succumb to cardiac or respiratory complications by 9–24 months of age. The current

treatment for GSDII is ERT, in which recombinant GAA is administered intravenously once every 2 weeks. For most patients, this therapy is quite efficacious; however, some patients develop potent immune reactions against the enzyme that can significantly limit the long-term efficacy of the therapy.[10,11]

A similar problem was encountered in the first attempts to treat GSDII with gene therapy in animal models. Early attempts to introduce a functional *GAA* gene into GSDII KO mice[63] and GSDII KO quails[64] confirmed that robust levels of enzyme expression could be achieved in vivo, but these levels of expression gradually tapered over time. This problem was ameliorated when similar attempts were made in immune-deficient GSDII KO animals, demonstrating that adaptive immunity was leading to the loss of GAA activities over time.[65] Two potentially synergistic strategies have been developed for reducing immunity induced by gene therapy for Pompe disease: one is the development of liver-targeted therapies; and the other is immunosuppression/immunomodulation.

Liver-directed gene therapy

One strategy to avoid both innate and adaptive immune responses is liver-directed gene therapy. To target transgene expression to the liver, and to avoid expression in immune cells, liver-specific promoters have been employed. This has proved effective at minimizing adaptive immunity in GSDII KO mice. Specifically, GSDII KO mice were injected with AAV2/8 vectors expressing *GAA* driven by either a liver-specific promoter or a ubiquitously expressed promoter derived from the cytomegalovirus early region enhancer and promoter elements.[66] GSDII KO mice receiving the vector with the ubiquitously expressed promoter developed antibody and T-cell responses against the transgene that limited the expression of *GAA*, and ultimately failed to correct the phenotype. In contrast, use of the liver-specific promoter prevented the formation of neutralizing antibodies and cytotoxic T-cells against the *GAA* transgene in the same animals, and it allowed for sustained plasma secretion of GAA, ultimately correcting glycogen storage and supplying GAA to muscle via systemic cross-correction.[66] Some have suggested that liver-specific promoters avoid immunity by preventing transgene expression within antigen-presenting cells.[67] While this may be the case, it appears that liver-directed gene therapy is capable of actively inducing tolerance as well.[68] This was demonstrated in the same murine model of Pompe disease. In GSDII KO mice, AAV2/8 expressing *GAA* with a liver-specific promoter avoided anti-*GAA* immunity when injected on its own; it also allowed for protection from the immune responses induced by the ubiquitously expressed vector, suggesting the induction of tolerance.[69] As immune responses are the major limiting factor for ERT, induction of tolerance suggests a role for gene therapy in patients where immune responses to the transgene are predicted to be limiting.[8,9] Similarly, liver-directed gene therapy approaches have been successfully employed to treat mucopolysaccharidosis (MPS) type I (MPSI) cats,[70] MPSIIA mice,[71] GSDII KO mice,[72] Von Gierke disease mice,[73] and Fabry mice.[74,75]

Early attempts at liver-directed gene therapy introduced the vector into the hepatic bloodstream, but they required high doses of virus to achieve significant levels of hepatocyte transduction.[76] Unfortunately, the doses required also induced dose-related toxicity.[77,78] To address these issues, a procedure was developed that utilized minimally invasive, hydrodynamic occlusion of the liver and allowed for substantially higher levels of hepatocyte transduction with substantially lower viral doses to be utilized.[79] For LSDs that can be treated with liver-directed therapy, this technique is a major breakthrough and will likely benefit future trials in large animals and humans.

Transient immunosuppression/immunomodulation

A second method for avoiding immunity is the use of transient immunosuppression. This method has been explored in a GSDII KO mouse model.[80] In that model, AAV expressing *GAA* with a ubiquitous promoter generated potent humoral immunity that limited the efficacy of the gene therapy.[80] However, pretreatment of the animals with nondepleting anti-CD4 antibodies transiently inhibited the function of CD4+ T-cells and prevented the formation of anti-*GAA* antibodies. This greatly improved the efficacy of the gene therapy attempt, resulting in decreased glycogen storage in the heart and skeletal muscles of the treated animals.[80]

The importance of transient immunosuppression has also been observed in the gene therapy of MPSI cats. MPSI is a neuropathic LSD that presents with musculoskeletal malformation, severe cognitive declines, joint pain, and hepatosplenomegaly. When a retroviral vector was used in a feline model of MPSI, potent cytotoxic T-lymphocyte (CTL) responses eliminated transfected cells and prevented long-term efficacy of the therapy.[62] In this same model, CTL responses could be avoided by transiently suppressing the animal's immune system by treatments with the use of the T-cell coreceptor-binding drug, CTLA4-Ig. This resulted in the sustained efficacy and subsequent normalization of substrate accumulations within

the liver.[62] Transient immunosuppression has also been shown to block downstream antibody responses to adenoviral vectors, allowing for their repeated administration.[81,82] This approach has also shown success in a nonhuman primate model with AAV.[83] Importantly, transient immunosuppression has been demonstrated to be safe in a human clinical trial for MPSIIIA, suggesting a future for this practice in human gene therapy approaches for many LSDs.[84]

Gene therapy for neuropathic LSDs

There is mounting evidence for the potential use of gene therapy to treat neuropathic LSDs. Over 50% of LSDs have neurologic involvement,[1] and gene therapy is particularly important for this class of LSDs, as currently no therapies exist for many of these diseases. The principal obstacle in treating these diseases is the BBB. ERT is ineffective at crossing the BBB, and most viral vectors (adenoviruses, LVs, and most AAVs) will not cross the fully matured BBB after intravenous administration.[8,9,85,86] This obstacle has led to a number of promising innovations that are each at different stages of translation.

Intracranial injections

Several strategies have been developed to bypass the BBB for the treatment of LSDs (Figure 3). The most direct and developed strategy has been to anatomically bypass the BBB with intracranial injections of the respective gene transfer vector. This approach has been utilized extensively in models of infantile neuronal ceroid lipofuscinoses (INCL) and MPSIIIA.[84,92] Both are fatal neuropathic LSDs that develop in early childhood. INCL is caused by mutations in the *PPT1* (also called *CLN2*) gene, resulting in deficiency of palmitoyl protein thioesterase-1 (PPT1). INCL presents at around 18 months with symptoms of visual defects, cognitive defects, seizures, and results in premature death.[87] MPSIIIA is caused by mutations in the *SGSH* gene that result in the deficiency of *N*-sulfoglucosamine sulfohydrolase (SGSH) and the accumulation of heparin sulfate glycosaminoglycans within the CNS. MSP IIIA patients present with cognitive delay at age 3 years, lose the ability to walk independently by age 10 years, and die by age 15 years.[88] There are currently no therapies approved for either of these fatal disorders.

Preclinical studies for INCL suggested that intracranial injections of AAV2 expressing *PPT1* cleared accumulated storage material from *PPT1* KO mice.[89] The same vector was shown to broadly transduce the brain of nonhuman primates.[91] The vector successfully produced tripetidyl peptidase (TTP)-1 within the neurons of the injected regions,

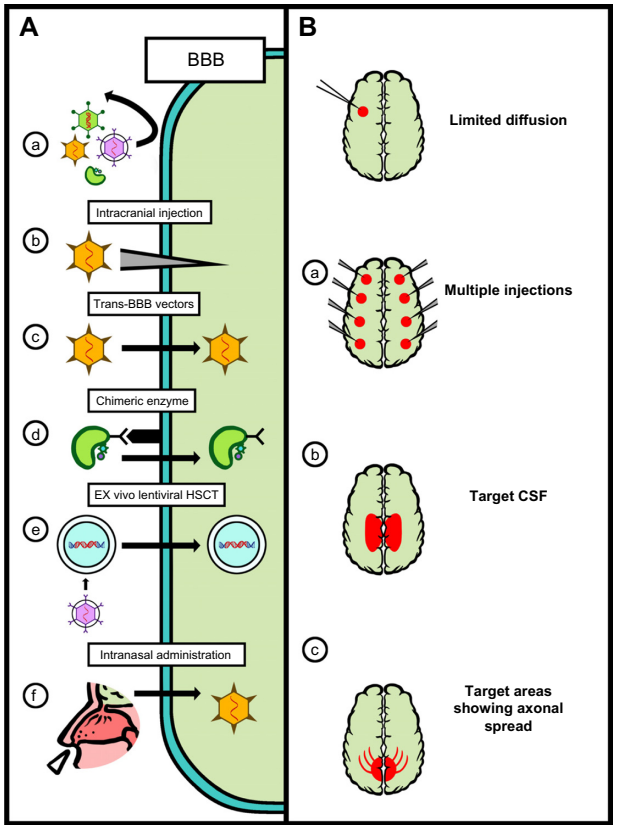

Figure 3 Strategies for CNS-directed gene therapy.
Notes: (**A**) (**a**) The BBB prevents most viruses and enzymes from entry into the CNS. (**b**) Intracranial injections anatomically bypass the BBB and have been used in human trials for infantile neuronal ceroid lipofuscinoses[92] and MPSIIIA.[84] (**c**) Certain serotypes of AAV have been reported to cross the BBB and have been used in MPSIIIB mice,[56] MPSVII mice,[99] and nonhuman primates.[96] (**d**) The enzyme can be modified to have affinity for receptors that traffic proteins across the BBB and has been used in MPSIIIA mice.[71] (**e**) Hematopoietic stem cells can be transfected ex vivo, then reintroduced to the patient. They can cross the BBB, smuggling the transfected gene into the CNS. This approach has been used in human trials for metachromatic leukodystrophy.[103] (**f**) Intranasal delivery of adenovirus[112] and AAV[113] has also been shown to bypass the BBB in rats. (**B**) There is limited spread of virus within the CNS. (**a**) Multiple injections allow for multiple areas of the brain to be targeted even with limited diffusion.[84] (**b**) Certain studies have tried to target the CSF to distribute the virus throughout the CNS.[132,133] (**c**) Certain studies have tried to target areas of the brain that have axons extending widely to allow axonal transport of the virus or enzyme throughout the brain.[134]
Abbreviations: BBB, blood–brain barrier; HSCTs, hematopoietic stem cell transplantation; CNS, central nervous system; MPS, mucopolysaccharidosis; AAV, adeno-associated virus; CSF, cerebrospinal fluid.

and although the injections themselves caused minor physical damage, no histological damage could be attributed to the vector.[90,91] This work was translated into the first gene therapy trial for a LSD.[92] Ten children with INCL received 12 intracranial injections of an AAV2 vector expressing TTP-1. A modified Hamburg neurologic rating scale demonstrated that the subjects that received gene therapy exhibited a slower cognitive decline compared to untreated controls and historical data. Unfortunately, it was also demonstrated that humoral immune responses developed in four of the ten subjects, which may have limited the overall effectiveness

of the therapy. Similar humoral immunity developed in a clinical study of Canavan disease following intracranial administration of AAV2 vectors expressing aspartoacylase.[51] These early studies demonstrated that gene therapy could be potentially effective for neurological disorders, and they also highlighted the importance of the immune system, even within immune-privileged sites like the CNS.

Lessons learned from this trial were incorporated into a clinical trial in MPSIIIA that employed transient immunosuppression alongside intracranial injections.[84] Four MPSIIIA patients aged 2–6 years were injected intracranially with an AAV2/10 vector expressing SGSH and sulfatase modifying factor 1. They received 12 simultaneous intracranial injections into the white matter of the basal ganglia. Importantly, patients in this study received immunosuppressive agents (mycophenolate, mofetil, and tacrolimus) beginning 15 days prior to surgery and extending for 8 weeks after the procedure. No adverse outcomes were associated with immunosuppression or administration of the viral vector, demonstrating a markedly improved safety profile over the INCL trial.[92] Though the study design limits conclusions about efficacy, the patients may have had a decreased decline in cognitive abilities, and the youngest patient showed improvement in several cognitive scores, including motor skills and independent thought. The behavioral data correlated with magnetic resonance imaging data that showed decreased brain atrophy in two patients and no atrophy in the other two patients. These promising results suggest that intracranial gene therapy may be a viable option for certain neuropathic LSDs.

Trans-BBB vectors for neuropathic LSDs

Another strategy to bypass the BBB is to use alternative serotypes of viruses that may be capable of enhanced entry into the CNS directly from the bloodstream. Early work demonstrated that AAV9[55] and other AAV serotypes[93] crossed the BBB, as performed in neonatal mice. However, the large injection volumes that were used in these studies potentially could have mechanically disrupted the delicate intracerebral vasculature of the neonatal mice, complicating interpretation of these results.[94] Subsequent work demonstrated that AAV9 was capable of crossing the BBB in adult nonhuman primates,[95] and this approach has been explored as an alternative to intracranial injections in models of MPS. In MPSIIIB mice, intravenous administration of AAV9 was able to correct the pathology in the CNS and the periphery.[56] As a preclinical study, the same vector was administered intravenously into adult cynomolgus monkeys; the use of AAV9 was able to drive the expression of α-*N*-acetylglucosaminidase in both the CNS and the periphery, demonstrating that this approach may be viable for a clinical trial.[96]

In addition to AAV9, other AAV vectors have been investigated for their ability to cross the BBB. Serotypes AAVrh8 and AAVrh10 were shown to cross the BBB in adult mice, with AAVrh10 also shown to cross the BBB in adult marmosets, although with variable results.[97] Another approach has involved identifying new xenobiotic AAVs that may be capable of crossing the BBB. Two recently isolated porcine AAVs were demonstrated to have some tropism for the CNS.[98] A third approach has been to artificially manipulate the AAV capsid to produce novel AAV vectors that cross the BBB more efficiently than AAV9.[99] This approach was demonstrated in a murine model of MPSVII. Importantly, MPSVII is a difficult target for AAV9-based gene therapy because the disease results in an accumulation of sialic acid within the CNS; sialic acid generally inhibits the AAV9 transduction of cells. In this model, the capsid-modified AAV9 vectors were able to overcome these issues, enter the CNS, and correct the cognitive deficits and storage lesions.[99]

Ex vivo gene therapy for neuropathic LSDs

Another strategy to bypass the BBB is the use of HSCs or other cells naturally capable of crossing the BBB on their own. These cells can be harvested from the patient, modified with RV or LV ex vivo, and then returned to the patient, where they can theoretically cross the BBB and provide enzymes to neighboring cells within the CNS via cross-correction. This approach has been explored in MLD models.[103] MLD is a neuropathic LSD caused by a deficiency in arylsulfatase A (ARSA). Patients with <1% ARSA activity present at 1–2 years of age with progressive muscle weakness, vision loss, convulsions, and dementia, and they typically die by the age of 5 years.[15,16] There is currently no therapy approved for this fatal disorder.

Experiments in a MLD mouse model have demonstrated that HSC gene therapy can succeed in correcting lysosomal storage and neurologic damage in affected mice.[100] Interestingly, this model also highlighted the importance of gene therapy for LSDs, as only HSCs that received gene therapy were able to prevent neurologic deficits.[101] This is likely because genetically modified HSCs produce up 15 times the normal amount of the required LSD protein, and therefore were able to secrete more enzyme per cell for cross-correction.[102] This work has been translated into a Phase I/II clinical trial for MLD where three presymptomatic MLD patients were treated with HSC gene therapy.[103] Autologous

HSCs were harvested from the patients, transduced ex vivo with a LV expressing ARSA, and the transduced cells were infused into the patients that had also undergone preconditioning of their bone marrow via myeloablation with busulfan. The reinfused cells engrafted within the patients, maintaining a high-level of detectable ARSA activity within a wide variety of cells and the cerebrospinal fluid. The therapy forestalled the onset of cognitive deficits and lessened the motor deficits in all three patients, as compared to untreated older siblings with MLD and historical controls.[103] Importantly, no adverse outcomes were associated with the administration of the vector, and no antibodies developed against the vector or the transgene. The safety and efficacy of this trial demonstrated the potential of ex vivo LV gene therapy for the treatment of LSDs.

In addition to LVs, nonviral methods for modifying cells ex vivo are in development, and the use of these methods for the treatment of LSDs has recently been patented.[104] These methods include the potential use of zinc-finger nucleases,[105] transcriptional activator-like effector nucleases,[106] and systems using clustered regularly interspaced short palindromic repeat-CRISPR-associated protein 9 (CRISPR-Cas9)[107] in attempts to insert potentially therapeutic transgenes into a locus of choice. These can also potentially be used in combination with integrase-deficient LVs to allow LVs to achieve targeted transgene insertion.[108] Their potential applications in LSDs has been recently reviewed, and readers are directed there for more details.[109]

Trans-BBB enzyme for neuropathic LSDs

Another approach to supply the brain with the deficient enzyme is to modify the gene coding the enzyme so that the enzyme produced by gene therapy in the periphery is able to cross the BBB itself. For example the lysosomal enzyme ARSA was fused to several of these targeting motifs, including the Tat domain from HIV, the angiopep peptide, and the receptor-binding domains from human apolipoproteins B and E. The biodistribution of these fusion proteins was altered by the presence of the additional ligand motifs, demonstrating the potential for trans-BBB enzyme therapy.[110] Recent work has also shown that lysosomal enzymes modified with ligand motifs that allow them to bind the human insulin receptor are better able to cross the BBB and distribute throughout the brain in rhesus monkeys.[111] This approach has also been utilized in gene therapy for MPSIIIA mice.[71] A liver-directed AAV8-based gene therapy vector produced a LSD protein that had been modified to include the secretion signal from iduronate-2-sulfatase to enhance secretion and the BBB-binding domain (BBBBD) from apolipoprotein B to cross the BBB. The BBBBD from apolipoprotein B is recognized by low-density lipoprotein receptors and it allows the enzyme to be trafficked into the brain. A single intravenous injection of the AAV2/8 carrying the modified *SGSH* gene was better able to foster the detection of *SGSH* activity throughout the periphery and the CNS of the animals treated with this version of the *SGSH* gene.[71] MPSIIIA mice that received this treatment also showed decreased brain pathology and recovered normal behavior. This novel approach combines the advantages of liver-directed gene therapy with the ability to improve targeting of LSD enzymes into the CNS.

Intranasal delivery

There have also been attempts to introduce viral vectors to the brain via intranasal delivery. Early work in rats demonstrated that adenovirus could enter the CNS and transduce the *β-galactosidase* gene into the rat brain following intranasal delivery.[112] This delivery system was also used with AAV8 to introduce a physiologically relevant levels of α-l-iduronidase (IDUA) into a mouse model of MPSI (IDUA–/– mice).[113] Due to the substantial anatomical differences between the nasal cavity of rodents and humans,[114,115] this approach likely needs to be evaluated in primates to confirm its potential for high levels of efficacy in human populations.

Current limitations in gene therapy for LSDs

Despite the many recent achievements in the gene therapy for LSDs, there remain several obstacles to overcome. A major obstacle that many gene therapy vectors face is pre-existing immunity against the vector itself, which can be a major limitation when translating these approaches to the actual human population, especially with use of AAV.[116,117] Although great promise exists for the use of AAV-based therapies, most of the human population also have pre-existing humoral immunity to AAV serotypes, with 72% of the population having antibodies against AAV2, 67% against AAV1, 47% against AAV9, 46% against AAV6, 40% against AAV5, and 38% against AAV8.[116] Furthermore, under certain conditions, even low titers of anti-AAV antibodies have been shown to completely block AAV gene therapy attempts.[42,118,119] Additionally, anti-AAV capsid antibodies have been shown to cross-react unpredictably across AAV serotypes, so antibodies against one AAV serotype may prevent any other AAV from functioning as a vector.[116,120] Finally, in certain circumstances, preformed neutralizing AAV antibodies have been shown to partially decrease transgene expression,

even when injected into immune-privileged sites, such as the brain[121] or eye.[122] Current clinical trials circumvent this issue by carefully excluding research participants harboring pre-existing anti-AAV antibodies; however, this can exclude 72%–33% of the patient population from treatment.[116,123] Groups have proposed using empty capsids as decoys for antibodies (thereby decreasing the ratio of antibody to target) with variable success.[124,125]

Another limitation in the development and translation of gene therapy for LSDs is the animal models. While the animal models for LSDs are among some of the best for any disease (being true homologues), interspecies differences have still proven relevant when translating from one species to another. In addition to the limitations mentioned earlier, differences in the immune responses between animal models have demonstrated that immune responses to gene therapy vectors can vary between species. For instance, when neonatal MPSI dogs received retroviral gene therapy, they did not mount an immune response to the protein expressed by the transduced transgene (α-l-iduronidase),[126] despite the ability of adult MPSI dogs to develop a potent antibody response to the same protein directly.[127,128] However, when a very similar vector was administered to neonatal MPSI cats, the cats developed a very strong T-cell-mediated response against the protein encoded by the vector that rapidly eliminated transfected cells.[62] The critical importance of these differences was illustrated by the clinical Phase I/II study of an AAV2-based vector expressing factor IX. In preclinical trials, hemophilia B dogs showed no evidence of potent immune responses to the vector;[129,130] however, human trial participants experienced potent T-cell responses that eliminated AAV-transfected cells.[45,50] The differences in the immune responses between species are an inherent limitation as to the use of animal models; this is a caveat that must be remembered when translating any therapy from an animal model to human subjects.

Expert opinion

Of the numerous human genetic diseases, LSDs likely provide the highest chance for the clinical success of gene therapy strategies given contemporary understandings of the limitations of gene therapy approaches. Currently, gene therapy clinical trials are underway for Fabry disease, Gaucher disease, MLD, MPSII, MPSIIIA, MPSIIIB, INCL, and Pompe disease.[131] In addition, preclinical research has demonstrated special advantages that gene therapy might have for use in the treatment of LSDs. The ability of liver-directed gene therapy to produce robust amounts of LSD enzymes systemically, as well as to potentially induce tolerance, is highly promising, especially for the subset of patients who develop potent immune responses to ERT.[8] In addition, many of the neuropathic LSDs currently have no treatments, and the recent clinical trials demonstrate that gene therapy may provide a safe and potentially therapeutic option for these diseases. Taken together, these exciting advances show that gene therapy for LSDs is overcoming previous limitations and moving forward on several fronts. As these therapies are translated into clinical practice, the role of administering clinical gene therapy may reside firmly in the venue of the metabolic geneticist or other clinician that specializes in the treatment of LSDs.

Disclosure

The authors report no conflicts of interest in this work.

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