Received: 6 May 2021 | Revised: 23 June 2021 | Accepted: 25 June 2021

DOI: 10.1002/jimd.12414

REVIEW ARTICLE

JIMD SSIEM WILEY

Mucopolysaccharidoses type I gene therapy

Sarah C. Hurt[1,2] | **Patricia I. Dickson**[2,3] | **David T. Curiel**[1]

[1]Cancer Biology Division, Department of Radiation Oncology, Washington University School of Medicine, St. Louis, Missouri, USA

[2]Department of Genetics, Washington University School of Medicine, St. Louis, Missouri, USA

[3]Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA

Correspondence
David T. Curiel, Cancer Biology Division, Department of Radiation Oncology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8224, St. Louis, MO 63110.
Email: dcuriel@wustl.edu

Funding information
National Institutes of Health (US), Grant/Award Numbers: UG3 TR002851, R01 EB026468, R01 NS088766, R61 NS111079; National Science Foundation (US), Grant/Award Number: DGE-1745038

Communicating Editor: Jaak Jaeken

Abstract

Mucopolysaccharidoses type I (MPS I) is an inherited metabolic disease characterized by a malfunction of the α-L-iduronidase (IDUA) enzyme leading to the storage of glycosaminoglycans in the lysosomes. This disease has longtime been studied as a therapeutic target for those studying gene therapy and many studies have been done using various vectors to deliver the *IDUA* gene for corrective treatment. Many vectors have difficulties with efficacy and insertional mutagenesis concerns including adeno-associated viral (AAV) vectors. Studies of AAV vectors treating MPS I have seemed promising, but recent deaths in gene therapy clinical trials for other inherited diseases using AAV vectors have left questions about their safety. Additionally, the recent modifications to adenoviral vectors leading them to target the vascular endothelium minimizing the risk of hepatotoxicity could lead to them being a viable option for MPS I gene therapy when coupled with gene editing technologies like CRISPR/Cas9.

KEYWORDS

adenovirus, CRISPR, gene editing, gene therapy, mucopolysaccharidosis type I

1 | INTRODUCTION

Throughout the past few decades, gene therapy for monogenic diseases has been thoroughly explored due to their perceived ease of correction.[1] In this regard, mucopolysaccharidosis type I (MPS I) is an interesting disease to the field due to its monogenic status, devastating patient outcomes, and limited current treatment options. Attempts have been made to deliver the corrective gene via several different vector types both through ex vivo and in vivo methods with limited success. Recently, however, gene editing-based therapy has led to new investigation of genetic correction. This new technology has potential for a long term in vivo gene therapy not requiring subsequent treatments for MPS I.[1] Utilizing gene editing tools to treat this disease has opened up a new and exciting avenue for those studying gene therapy of MPS I.

1.1 | Mucopolysaccharidosis type I

MPS I is characterized by malfunctioning α-L-iduronidase (IDUA) enzyme which normally catabolizes the glycosaminoglycans (GAGs) dermatan sulfate and heparan sulfate.[2] Active IDUA deficiency causes cellular lysosomes

to accumulate GAGs as oligosaccharides terminating in iduronic acid, causing disease. *IDUA* has over 100 known mutations in patients with the disease.[3] Due to the variety of possible genotypes, a spectrum of clinical severity is found in MPS I.[4] In the most severe form of MPS I (Hurler syndrome), patients exhibit progressive developmental delay, corneal clouding, airway obstruction, cardiac disease, enlargement of organs, severe joint restrictions, and have an untreated life expectancy of 10 years.[5] Patients with MPS I require a coordinated team of specialists to address symptoms as they became apparent. In addition to the specialty care required, patients currently have intensive, noncurative, expensive treatment options.[3,6]

1.2 | Current treatments

There are two clinically available treatments for MPS I: enzyme replacement therapy (ERT; laronidase) and hematopoietic stem cell transplantation (HSCT).[3,7] Laronidase is a human recombinant IDUA enzyme intravenously administered and endocytosed into the bloodstream and endocytosed into cells to catabolize accumulated GAGs in lysosomes. However, laronidase has limitations related to the blood brain barrier (BBB) leading to continued progression of neurological disease, lifelong re-administration, and the high cost of over $200 000 per year.[3,6,7] HSCT, when successful, is an effective approach for treating MPS I.[3] However, HSCT has high morbidity and mortality related to the procedure, needs to employ preparative chemotherapy, and has potential for graft failure.[3] Due to these risks, HSCT is typically reserved for Hurler patients aged two or under.[3] In addition to the issues with these treatments individually, neither treatments fully eliminate the clinical manifestations of the disease and patients may still require intervention through surgery or symptom-specific specialists.[3]

Due to the limitations of current treatments, gene therapy has been explored as a potential therapeutic for this disease.[8] Gene therapy has shown promise in treating inherited diseases characterized by loss of function, and there is precedent for clinical use.[9] Here, we will examine different gene therapy approaches attempted in MPS I and end by presenting a novel approach.

2 | MPS I GENE THERAPY STRATEGIES

2.1 | Retroviral vectors

Gene therapy for MPS I was first attempted by groups using retroviral vectors to deliver the *IDUA* gene.[10] Retroviral vectors are RNA viruses that interact with the outer glycoprotein envelope to gain entry into a cell and convert its single-stranded genome into double-stranded DNA to be incorporated into the host's genome.[11] In a gene therapy context, this mechanism can be used to introduce a corrective gene into the genome of the patient.[11] Once retroviral vectors began being used in 1992 to deliver *IDUA* in vitro, they were then investigated for their potential use both in ex vivo and in vivo methods (Figure 1).[10]

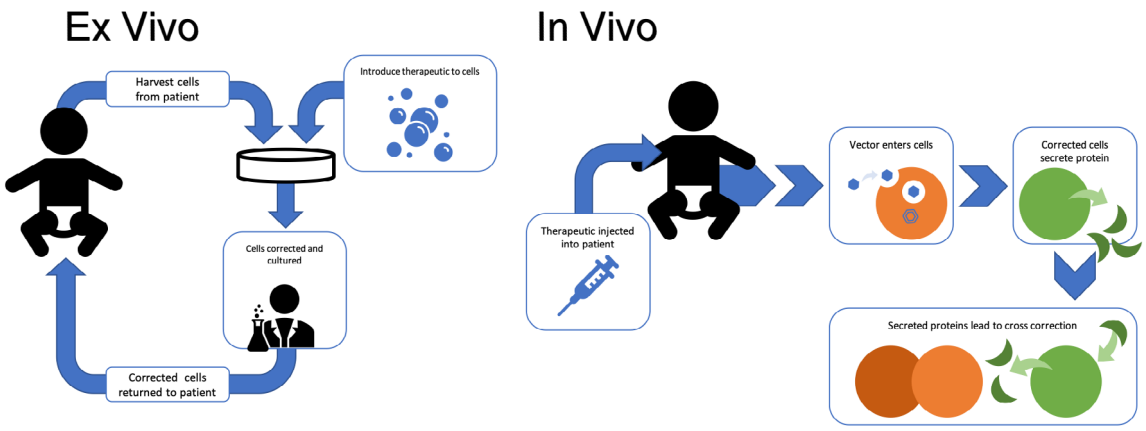

FIGURE 1 Ex vivo vs in vivo gene therapy. Ex vivo gene therapies follow a method in which one harvests cells from the patient and then makes the necessary correction with a system to deliver the gene therapy in a petri dish. Following correction, the cells are cultured in a sterile setting before ultimately being returned to the patient. Ex vivo gene therapy methods for mucopolysaccharidoses type (MPS I) have been attempted to improve upon hematopoietic stem cell transplantation (HSCT) and lessen the possibility of graft failure without worrying about dose-dependent reactions in vivo. In contrast, in vivo gene therapies are less invasive than ex vivo methods and typically involve a patient receiving a single dose with the gene therapy via injection. The gene therapy is then delivered by a vector to the target cell. The target cells then produce the corrective protein and secrete them to allow for cross correction to other unmodified cells. In MPS I, an in vivo strategy is highly desirable due to the minimally invasive process for patients and potential long-term effects

To lessen risks related to HSCT, Fairbairn et al sought to use autologous hematopoietic stem cells (HSCs) transduced with a retroviral vector carrying the *IDUA* gene in an ex vivo approach to gene therapy.[12] In vitro, they were able to show successful gene transfer to MPS I patients' CD34+ cells, but had efficiency limitations because retroviral vectors only transduce cycling cells and, when harvested, many HSCs are out of cycle.[12] To address this concern, they recommend using long-term bone marrow cultures; however, this would ultimately lead to a delay for patients whose disease may progress during the waiting period.[12] As a response to Fairbairn et al's original study, Stewart et al and Huang et al reported that IDUA-deficient cells absorb IDUA enzyme from the supernatant of fibroblasts transduced with a retrovirus expressing *IDUA* due to a mannose-6-phosphate interaction on the cells' surface.[13,14] Additionally, Stewart et al concluded that IDUA uptake was more efficient in immature cells and treatment is preferred early in development, but the exact point in developmental reimplantation which occur to lead best clinical outcomes was unclear.[13]

Retroviral in vivo approaches have also been explored for MPS I with a team of researchers focusing on a liver-directed gene therapy for neonatal MPS I mice.[15-18] In one study, they compare the use of high (10^9 transducing units/kg) and a low (10^8 transducing units/kg) dose of retroviral vector delivered via tail vein injection.[15] While both were able to achieve some stable *IDUA* expression in treated animals, 4 of 17 animals receiving the low dose were unable to establish substantial *IDUA* expression.[15] They evaluated the effectiveness of the two doses, finding the high-dose-corrected lysosomal storage in several organ systems while the lower dose failed to establish the same improvement.[15,16] These results showed that a high dose of the retroviral therapy is necessary to see improvement in MPS I symptoms in the neonatal murine model for the disease.[16] In a later study, the group evaluated the effects in more mature, 6-week old mice and attempted to increase the efficacy of their vectors in these mice by inhibiting CD28 with CTLA4-Ig to minimize the cytotoxic T lymphocyte (CTL) response against transduced cells.[17] The application of CTLA4-Ig in conjunction with their vectors led to a stable expression of *IDUA* with similar results related to lysosomal storage in these more mature mice as was seen in previous studies.[15-17]

Traas et al followed up this work in mice by delivering retroviral vectors expressing the canine *IDUA* with the liver-specific α1-antitrypsin (hAAT) promoter in the canine model for MPS I.[18] When administered to the canines, the retroviral vector therapy led to >18% normal IDUA function in treated animals with improvement in musculoskeletal and cardiovascular symptoms of MPS I.[18] The vectors in this study were also designed to implement a long-terminal repeat (LTR); DNA that flanks a retroviral sequence mediating insertion into a host's genome and includes transcription factor binding sites that can autonomously drive expression.[19] They theorize this LTR drove expression in nonhepatic cells, potentially leading to the increased retroviral RNA found in neurons 1 year after treatment.[18] Concern about the possibility of insertional mutagenesis due to the use of an LTR led to a follow-up study focused on improving the vector design for less CTL response.[18,20] Herati et al inverted the expression cassette in the gamma-retroviral vector, so hAAT drove expression of the canine IDUA rather than the LTR.[20] By doing this inversion, they were able to isolate whether their expression levels previously were a result of the LTR.[18,20] They tested this vector in the MPS I murine model and found high expression levels in the liver with stable serum IDUA levels, but had relatively low IDUA activity levels in blood.[20] Six and a half months post-treatment, they found most organs had normalized GAG levels with improvements to vision and hearing abnormalities along with musculoskeletal symptoms of MPS I, but the low overall activity levels of IDUA using this treatment led to concerns about the effectiveness of the treatment.[20]

Gamma-retroviral vectors expressing *IDUA* can be used in conjunction with a transient immune suppressant to prevent immune responses and induce long-term expression in vivo.[20,21] However, immunosuppressants are not ideal in clinical settings, and, to avoid the use of one, Metcalf et al established a self-inactivating gamma-retroviral vector using the hAAT promoter expressing canine *IDUA* and an optimized woodchuck hepatitis post-transcriptional regulatory element.[21] This vector contains a deletion in the U3 region of the 3′ viral LTR resulting in the deletion of promoting and enhancing elements after transduction and reducing the insertional mutagenesis potential.[21] By modifying the LTR, the risk of insertional mutagenesis is potentially lessened.[19,21] While they saw a reduction in insertional mutagenesis potential when compared to other gamma-retroviral vectors employing a LTR, it was significantly less effective at treating MPS I, likely due to lower transduction levels.[20,21] Retroviral vectors are known to have a generally low transduction rate when the LTR is modified to combat insertional mutagenesis.[8,11]

2.2 | Lentiviral vectors

Lentiviral vectors, originally derived from the human immunodeficiency virus type-1 (HIV-1) lentivirus, can be used to deliver genes because they are nonreplicating and can stably integrate into the genome of many mammalian cell types.[22] In the context of MPS I, lentiviral vectors were first studied by Natale et al to deliver the *IDUA* cDNA to

MPS I fibroblasts in vitro, which led to a 1.5× normal level of IDUA in transduced cells with reduced intracellular GAGs.[23] A follow-up in vivo study was conducted where the *IDUA* expressing lentivirus was delivered to the murine model for MPS I leading to an observance of increased IDUA activity in the liver and spleen and decreased GAG excretion with minimal immune response.[23,24] They also conducted a study on *IDUA* expression longevity with a single injection of the lentivirus finding, after 6 months, that the mice had no detectable IDUA levels and GAG accumulation remained consistent.[24] A major limitation is the inability to establish long-term treatment with a single injection via lentiviral vector.[24]

Hypothesizing loss of expression may be prevented by neonatal administration, another in vivo study focused on treating MPS I mice to compare the efficacy of a liver-directed lentiviral vector in neonatal and adult mice found that neonates were able to more effectively clear lysosomal storage after treatment than their adult counterparts.[25] Additionally, they concluded mice treated as neonates developed less skeletal abnormalities than their adult counterparts and concluded that, in a clinical setting, this treatment would be best utilized in patients who were diagnosed prenatally and longitudinal studies would need to be conducted to establish the duration of the therapeutic effects.[25] This study also looked at how the treatment affected the central nervous system (CNS) by visualizing transduced cells using green fluorescent protein (GFP) and found that, while neurons had been transduced, astroglia showed very little GFP expression indicating limited transduction to those cells.[25]

A potential ex vivo gene therapy options using autologous hematopoietic stem and progenitor cells (HSPCs) transduced with lentiviral vectors were compared to standard nonautologous HSPC transfer and found that the transduced HSPCs achieved high levels of lysosomal storage clearance in several cell types.[26] However, they note that obtaining robust IDUA activity in the brain and skeleton would require a lentivirus with more efficient transduction to HSPCs.[26] Lentiviral vectors have several known limitations when used to treat a metabolic disease like MPS I including the risk of insertional mutagenesis and immunogenicity.[27,28]

2.3 | Adeno-associated viral vectors

Much of the research on gene therapy for MPS I has been conducted using adeno-associated viral (AAV) vectors which lack viral DNA and have been engineered to deliver DNA cargo through the cell membrane.[29] Many studies have been able to show success in transducing MPS I cells both in vitro and in vivo using AAV vectors.[30-39]

2.3.1 | CNS directed

The CNS has been intensely studied for MPS I therapy with AAV vectors. In 2004, Desmaris et al showed that the neuropathology of MPS I was treatable and preventable in MPS I mice using AAV serotype 2 or 5 to transfer *IDUA* and, with a single injection of either vector, showed IDUA persistence at 26 weeks postinjection.[31] Ellinwood et al followed this work with an experiment using the canine MPS I model to test each vector serotype in different age groups of dogs and concluded that, while both serotypes delivered the gene to large portions of the brain after intracerebral injection, younger dogs showed greater reduction in pathology markers than their older counterparts.[32] Despite the improvements in disease pathology, the researchers observed major residual lesions in some of the animals and limited treatment efficacy in the cerebellum.[32] Wolf et al continued the pursuit of an AAV-mediated treatment for the CNS symptoms of MPS I by delivering *IDUA* cDNA using AAV8 to the MPS I murine model's CNS. They observed high levels of IDUA activity and lysosomal storage reduction localized to the CNS with limited effects in other organ systems.[40]

One group has done several studies delivering the *IDUA* gene to the CNS via an AAV vector to feline and canine models, and rhesus macaques.[33,35] The feline and canine models showed declining IDUA activity over time post-treatment when cerebrospinal fluid samples were analyzed with no loss of transduced cells which suggests that an immune response aimed directly at the IDUA enzyme may be responsible for the loss of efficacy.[33,35] To test this hypothesis, they performed neonatal studies in canines that demonstrated lesser immune response to the IDUA produced than their more mature counterparts.[35] To model the potential immune response from human IDUA in patients lacking the enzyme endogenously, they conducted a study in rhesus macaques that are not IDUA deficient with two test groups. One received the *IDUA* gene and the other received an irrelevant transgene at birth delivered via AAV vector.[35] All groups received AAV expressing *IDUA* at 1 month postnatal, and those who received the *IDUA* AAV at birth produce no antibodies to IDUA.[35] They attempted to tolerize the canines to human IDUA with the same method used on the rhesus macaques in the hope of developing a better clinical model with more accurate immune responses to the human IDUA, but concluded that human IDUA simply was not favorable for the canine immune system.[35,36]

Belur et al explored an intranasal administration of AAV vectors for *IDUA* expression in the CNS as a less invasive option to prevent and treat CNS pathologies of MPS I.[38] After first showing possible transduction to the nasal epithelium and olfactory bulb using AAV to deliver GFP in mice, they delivered an AAV expressing *IDUA*

and found high levels of IDUA in the olfactory bulb with normal levels in the lung and liver.[38] However, this method was considerably less efficient than injection directly to the CNS, and required a large dose to be effective.[38] Another study by Belur et al compared AAV9 and AAVrh10 CNS-directed gene therapy in MPS I mice and found both serotypes could achieve IDUA expression in all tissues tested, including the brain.[39] They hypothesized that the IDUA found in the CNS was a result of diffusion after overexpression of IDUA from the CNS vasculature due to IDUA immunofluorescence labeling showing an association with blood vessels.[39]

2.3.2 | Cornea directed

Intrastromal injection of chimeric AAV8/9 vectors containing the *IDUA* cDNA into human cornea explants was investigated for the treatment of MPS I-related corneal clouding.[37] After observing restored expression of *IDUA* in MPS I fibroblasts, they studied normal human corneas in vitro and found normal TUNEL staining suggesting no increase in apoptosis.[37] However, in vivo studies are needed before moving toward clinical trials.[37]

2.3.3 | Liver directed

The phenomenon of "cross-correction," in which a lysosomal enzyme is secreted by a cell for uptake by neighboring cells, permits the exploration of methods of limited gene correction, such as targeting single organ. In this strategy, widespread biodistribution relies on high concentrations of enzyme secreted into the bloodstream, with delivery to tissues by means of the extensive capillary network. Liver-directed gene therapy has been studied in feline and murine models of MPS I.[34,36,39] Hinderer et al led a study in the feline model where four animals were injected with an AAV vector designed to target the liver and express *IDUA*, and sustained *IDUA* expression and GAG clearance were recorded in three of those four.[34] They noted a reversal of myocardial and aortic valve lesions in treated animals with high serum levels and that these animals' heart tissue was nearly indistinguishable from WT controls.[34] However, more research is needed before moving this method further due to the small sample size.[34] However, liver-directed gene therapy carries risks related to hepatotoxicity and has potential for safety concerns.[39]

2.3.4 | AAV safety

While AAV has been studied intensively and produced phenotypic correction in several models for MPS I, some safety and efficacy concerns exist for AAV including the inability to integrate recombinant episomal DNA into the genome leading to short-term expression and the potential for anti-capsid and immune responses to the vectors, often addressed with immune suppression.[29,41-45] Insertional mutagenesis-related cancers and dorsal root ganglion pathology without clinical signs have been seen in experimental animal treated with AAV vectors and require further study.[46-49] In recent years, safety concerns related to cell specificity and dose levels have also come to the forefront.[43,50-53] For example, Giles et al reported that AAV vectors unpredictably experience deamidation of amino acids on the surface of the viral capsid leading to a reduction in AAV vector cell specificity and decreased batch consistency during manufacturing.[50,51] Dose-related concerns with AAV vectors have become greater after deaths occurred due to high vector copy dose-related hepatotoxicity during a gene therapy trial for X-linked myotubular myopathy.[43,52] Additional trials have faced dose-related concerns including AveXis's trial for spinal muscular atrophy (SMA) and Solid Biosciences' and Pfizer's trials for duchenne muscular dystrophy (DMD).[52,53] However, AveXis's SMA treatment went on to receive FDA approval despite some concerns with data accuracy.[54,55] As of October 2020, both DMD gene therapies were continuing clinical trials.[56,57]

2.4 | Other methods

Nanocarriers have been investigated as a nonviral gene therapy to deliver nucleic acids.[58,59] Schuh et al used nanoemulsions, polymers that interact with biological membranes to promote internalization of the attached nucleic acids, to deliver the *IDUA* cDNA to the CNS through the nasal passages.[58] When comparing treated mice to an untreated control group, a twofold increase of IDUA activity in brain tissue was observed along with marginal IDUA activity in the spleen and kidney, likely from drainage of the nasal passages leading to DNA integration into other areas.[58] However, elevated levels of IL-6 in serum shows potential immune responses and local inflammation that would need to be better characterized before moving into clinical settings.[58]

2.5 | Gene editing

Gene editing has the potential to permanently correct IDUA deficiency in patients by using specialized nucleases that recognize and cut specific DNA sequences and reconstruct cleavage sites with nonhomologous end-joining or homologous recombination (HR).[1,60] With these technologies, a gene can be delivered to a cell,

incorporated into the genome in a targeted manner, and expressed.[1] CRISPR (Clustered Regulatory Interspaced Palindromic Repeats)/Cas9 is one of the most promising gene editing tools for gene therapy purposes due to its targeting accuracy when mediating gene integration.[60] Originally, an immune system for *Streptococcus pyogenes*, CRISPR engineers DNA by utilizing a short nucleotide sequence (called a guide RNA) found on the genome directly upstream of a three nucleotide protospacer adjacent motif.[60] Cas9 recognizes and nicks the DNA creating a double-strand break (DSB) repaired via HR to insert a gene, making a permanent change to the genome of the organism.[60] Gene editing does have the potential for on- and off-target effects that can cause a mutational process called chromothripsis, where chromosomes are extensively rearranged and can give rise to cancer or human congenital disease.[61-63] When beginning the process of gene editing, the possibility of these effects must be taken into careful consideration and the guide RNA must be fastidiously designed with these possibilities in mind.

In pursuit of treatment for MPS I, two studies using CRISPR/Cas9 to integrate the *IDUA* cDNA with liposomal complexes had varied success.[64,65] In the first study, the liposomal complexes containing vectors with CRISPR/Cas9 and the *IDUA* cDNA were injected into the superficial temporal vein for integration into the ROSA26 site on the genome and were retained in the lung, heart, and liver.[64] There was significant IDUA activity in serum and reduction of GAGs in several organ systems not including the brain.[64] The second study with these complexes reported that treated mice showed improved facial bone structure and some recovery of respiratory function when measured with plethysmography, but limited overall improvement to cardiovascular pathologies.[65] Researchers note that the low transfer efficiency, need for better systemic IDUA levels, and upscaling and storage difficulties make the transition to clinical settings with this method difficult.[65,66] Another study utilized zinc-finger nuclease mediated gene editing for the treatment of MPS I mice and found they were able to induce expression of *IDUA* to reduce lysosomal storage using liver-directed AAV vectors as their delivery system.[67] They do note that, due to the necessity of including a promoter for expression, the carrying capacity of AAV is near its limit.[67] A way to combat the concerns regarding efficiency, carrying capacity, and scalability is to change the vector systems.

An understudied vector for inherited metabolic disease gene therapy is adenovirus (Ad). This is owing in part to a treatment-related death in 1999.[68,69] This patient received a very high dose (6×10^{11} viral particles per kilogram) of an Ad vector expressing ornithine transcarbamylase to the right hepatic artery resulting in a cytokine storm, multiple organ failures, and eventual death of the patient.[68,70] As of 2013, this patient is the only out of >16 000 to have died as a result of an Ad vector-mediated gene therapy and the severe dose-dependent reaction has not been replicated in nonhuman primates or patients receiving the same dose.[68] Wold and Toth hypothesize that a genetic predisposition and strong memory response to the virus may be responsible for the severe reaction to the Ad vector.[68]

Despite this history, Ad vectors have many benefits to consider such as the ability to transduce replicating and nonreplicating cells with high expression levels and long-term episomal maintenance in nonreplicating cells.[8,71] Additionally, Ad vectors do not integrate into the host genome unless coupled with gene editing tools, such as CRISPR/Cas9, and are unconnected with germ-line mutagenesis, unlike AAV.[71,72] While no study using Ads in MPS I exist, a few studies using Ad vectors in MPS VII were conducted with limited success.[73-75] In 1995, Li and Davidson delivered the corrective gene human β-glucuronidase to eyes of MPS VII mice with an Ad vector observing a reduction in lysosomal storage in the corneal endothelial cells (ECs) 3 weeks postinjection with no improvement to the corneal keratocytes despite observing higher levels of corrective protein histochemically.[73,74] Following this work, in 1999, Ghodsi et al reported limited success in treating the CNS symptoms of MPS VII with Ad vectors.[75] Both of these studies are dated regarding Ad vectors' potential given recent advancements in gene editing and cell targeting with these vectors.[72,76] For example, with a modification to the Ad vector's fiber with a myeloid binding peptide (MBP), Ad vectors can target vascular ECs and circumvent hepatotoxicity and lead to more widespread disease correction due to the close proximity of vascular ECs with the patient's bloodstream.[72,76] When considering the cell targetability with the potential use of CRISPR/Cas9 to establish long-term correction, Ads seem like a promising avenue.[72,76] Ad vectors have been able to accomplish long-term expression in hemophilia B with a similar method using AAV vectors being successful in MPS I.[6,72] Both Ou et al and Stephens et al utilize a two-vector system that separates the corrective gene on one and the Cas9 on the other; however, the AAV method from Ou et al couples the gRNA with the Cas9 vector while the Ad method incorporates the gRNA with the corrective gene vector (Figure 2).[6,72] Both strategies saw a therapeutic effect when the gene was integrated into the genome; however, with the inclusion of MBP-mediated vascular EC targeting, potential hepatotoxicity could be circumvented and lead to an effective treatment strategy for MPS I (Figure 3).[6,72,76]

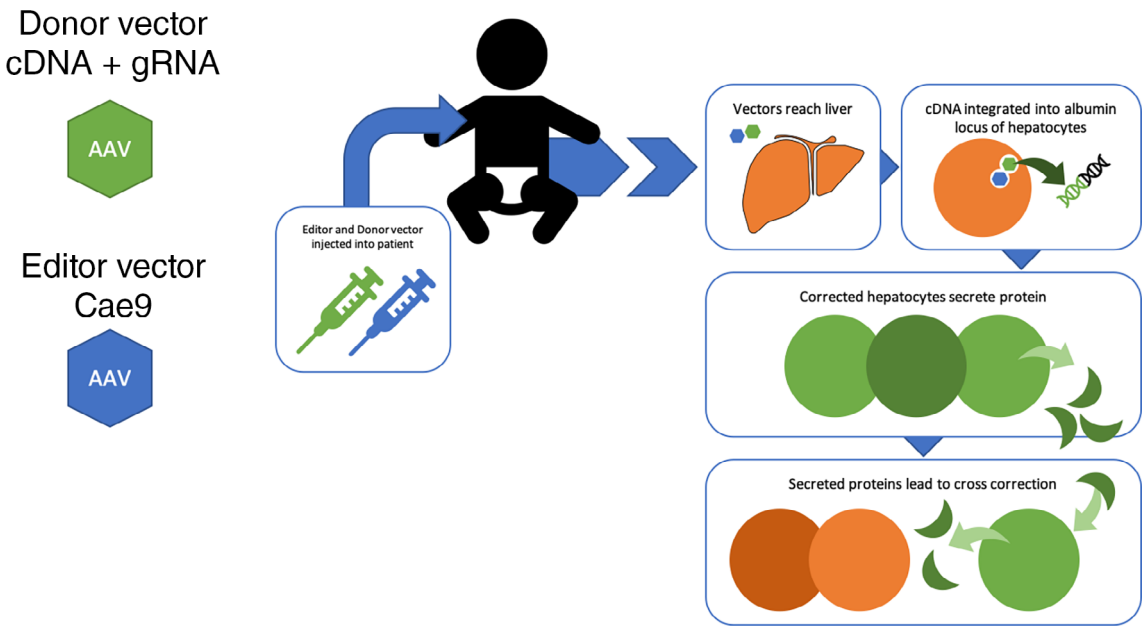

FIGURE 2 A gene editing approach to gene therapy. In the strategy pictured, based on Stephens et al's 2019 method to treat hemophilia B, two vectors are delivered to the patient via intravenous injection for a gene editing therapy strategy.[72] The donor vector includes the corrective cDNA along with the gRNA for CRISPR purposes. The editor vector includes Cas9. Placing the Cas9 and gRNA on separate vectors ensures that only cells that receive both vectors are edited. Once injected, the vectors would make their way to the liver where the viral capsid would interact with hepatocytes to release the encapsulated DNA into the cell. Once both vectors have provided DNA to the cell, the CRISPR/Cas9 system takes over to integrate the corrective gene into the albumin locus. These cells are then permanently edited to produce and secrete the corrective protein and secrete it for cross correction to surrounding cells

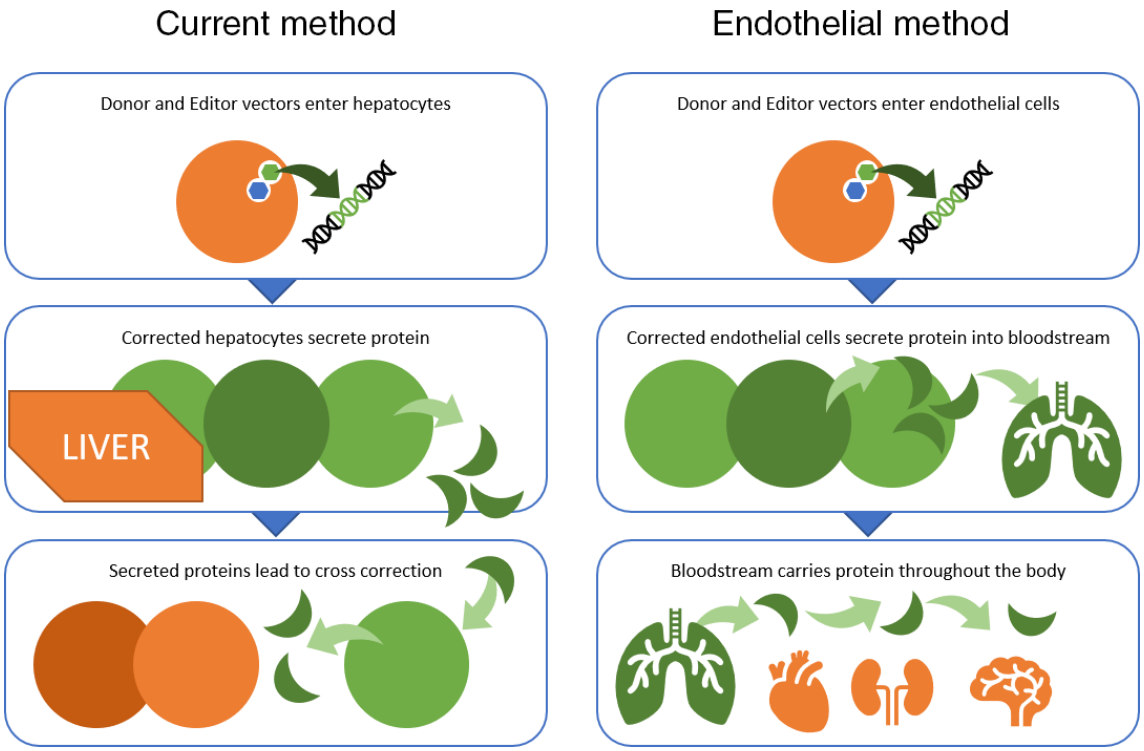

FIGURE 3 A proposed improved method to utilize the CRISPR/Cas9 system for the treatment of mucopolysaccharidoses type (MPS I). The current method of gene editing therapy focuses on targeting the liver for cellular sourcing.[6,72] However, this method could lead to concerns with hepatotoxicity due to the targeting of the liver. A method targeting the vascular endothelium rather than the liver would bypass hepatotoxicity concerns while also leading to secretion of the corrective protein directly into the blood stream for cross correction. By utilizing an adenovirus (Ad) vector modified with a myeloid binding peptide (MBP), we can achieve this and potentially establish long-term, stable correction of MPS I[76]

TABLE 1 A guide to the advantages and disadvantages of different gene therapy strategies

Gene therapy strategies	Advantages	Disadvantages
Retroviral vectors	• Incorporates gene of interest into host's genome for long-term correction.[10,11] • Use of a modified long terminal repeat can reduce the risk of insertional mutagenesis.[19-21]	• Possibility of insertional mutagenesis due to nontargeted incorporation into the genome.[21] • Ex vivo approaches may have a long waiting period in which disease progression can worsen the patient's condition.[12] • High dose of retroviral vector necessary for in vivo correction.[15] • Immune suppressant necessary for long-term expression in vivo.[20,21] • A modified LTR can reduce transduction rates to a point where their effectiveness is limited.[8,11]
Lentiviral vectors	• Nonreplicating.[22] • Stably integrate into genomes of many mammalian cell types.[22]	• Unable to establish long-term correction with single injection.[24] • Treatment of neonates may be necessary for long-term correction.[25] • Low transduction efficiency to HSPCs.[26] • Risks of insertional mutagenesis and immunogenicity.[27,28]
AAV vectors	• Lack viral DNA.[29] • Engineered to deliver DNA cargo through cell membrane.[29] • Nonreplicating.[29] • Able to target specific tissue types.[30-40]	• When targeting the liver, hepatotoxicity is a possibility.[39] • Short-term expression without gene editing.[29,41,42] • May require use of immunosuppressant.[43-45] • Possibility of inducing insertional mutagenesis related cancers.[46,48] • Possibility of inducing dorsal root ganglion pathologies.[47] • Concerns with cell specificity and necessary dose levels.[43,50-53]
Nanocariers	• Nonviral.[58,59]	• Not as well characterized as other methods.[58] • Low cell specificity.[58] • Potentially cause immune response.[58]
Liposomal complexes	• Nonviral.[64]	• Low transfer efficiency.[65,66] • Can be difficult to upscale and store.[65,66]
Adenovirus	• Able to transduce replicating and nonreplicating cells.[8,71] • Do not integrate into host genome without gene editing aids.[71,72] • Possible to target specific cell types with fiber modifications.[72,76] • Unconnected with germ-line mutagenesis.[71,72]	• Patient death in 1999 due to high dose and large immune response.[68,69] • Possibility of hepatotoxicity when liver-targeting.[72,76]
Gene editing	• Potential for permanent correction.[1,60] • The targeting ability of CRIPSR allows for carefully designed changes to the genome.[60]	• Requires a method for delivery to cells.[64-67,72,77] • On- and off-target effects.[61-63]

Notes: Each gene therapy strategy has strengths and weaknesses. Here, we show the potential pitfalls and benefits of each.

3 | CONCLUSION

Current clinical treatments for MPS I are inadequate, resulting in a residual disease burden, disability, and early death in treated individuals. Preclinical studies of gene therapy approaches to MPS I over the past few decades have demonstrated that replacement of IDUA activity is therapeutic, but have struggled with the

requirement for long-term IDUA restoration in both the systemic and CNS compartments, as well as some limitations due to safety concerns with certain vector-based approaches (Table 1). Gene editing strategies that yield high circulating concentrations of IDUA could be very effective for physical disease manifestations and could theoretically treat the CNS by penetrating the BBB in low levels. It remains to be seen how successful gene editing may be in achieving the necessary IDUA concentrations to produce a widespread therapeutic effect in the brain as well as in the harder-to-treat parts of the body such as heart valves, spinal meninges, and cartilage. However, if success is achieved, the result could be a permanent, low-risk, "cure" for MPS I.

ACKNOWLEDGMENTS

Support for this review was provided by National Science Foundation (DGE-1745038 to S.C.H.) and National Institute of Health (UG3 TR002851 and R01 EB026468 to D.T.C., R01 NS088766 and R61 NS111079 to P.I.D.).

CONFLICT OF INTEREST

Sarah C. Hurt and David T. Curiel declared no potential conflicts of interest. Patricia I. Dickson has received research support from Genzyme, Mallinckrodt, and M6P Therapeutics.

AUTHOR CONTRIBUTIONS

Sarah C. Hurt conducted literature research and wrote the manuscript. Patricia I. Dickson and David T. Curiel supervised writing and contributed to critical revisions.

ANIMAL CARE AND PATIENT CONSENT

This article does not contain any studies with human or animal subjects performed by the any of the authors.

ORCID

David T. Curiel https://orcid.org/0000-0003-3802-6014

REFERENCES

1. Prakash V, Moore M, Yáñez-Muñoz RJ. Current progress in therapeutic gene editing for monogenic diseases. *Mol Ther*. 2016;24:465-474.
2. Moore D, Connock MJ, Wraith E, Lavery C. The prevalence of and survival in mucopolysaccharidosis I: Hurler, Hurler-Scheie and Scheie syndromes in the UK. *Orphanet J Rare Dis*. 2008; 3:24.
3. D'Aco K, Underhill L, Rangachari L, et al. Diagnosis and treatment trends in mucopolysaccharidosis I: findings from the MPS I registry. *Eur J Pediatr*. 2012;171:911-919.
4. Li P, Wood T, Thompson JN. Diversity of mutations and distribution of single nucleotide polymorphic alleles in the human alpha-L-iduronidase (IDUA) gene. *Genet Med*. 2002;4:7.
5. Kakkis ED, Muenzer J, Tiller GE, et al. Enzyme-replacement therapy in mucopolysaccharidosis I. *N Engl J Med*. 2001;344: 182-188.
6. Ou L, Przybilla MJ, Ahlat O, et al. A highly efficacious PS gene editing system corrects metabolic and neurological complications of mucopolysaccharidosis type I. *Mol Ther*. 2020;28:1442-1454.
7. Sifuentes M, Doroshow R, Hoft R, et al. A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. *Mol Genet Metab*. 2007;90:171-180.
8. Ponder KP, Haskins ME. Gene therapy for mucopolysaccharidosis. *Expert Opin Biol Ther*. 2007;7:1333-1345.
9. Daley J. Gene therapy arrives. *Scientific American*; 2020. https://www.scientificamerican.com/article/gene-therapy-arrives/. Accessed April 21, 2021.
10. Anson DS, Bielicki J, Hopwood JJ. Correction of mucopolysaccharidosis type I fibroblasts by retroviral-mediated transfer of the human α-l-iduronidase gene. *Hum Gene Ther*. 1992;3:371-379.
11. Kurian KM, Watson CJ, Wyllie AH. Retroviral vectors. *Mol Pathol*. 2000;53:173-176.
12. Fairbairn LJ, Lashford LS, Spooncer E, et al. Long-term in vitro correction of alpha-L-iduronidase deficiency (Hurler syndrome) in human bone marrow. *Proc Natl Acad Sci USA*. 1996; 93:2025-2030.
13. Stewart K, Brown OA, Morelli AE, et al. Uptake of α-(L)-iduronidase produced by retrovirally transduced fibroblasts into neuronal and glial cells in vitro. *Gene Ther*. 1997;4:63-75.
14. Huang M-M, Wong A, Yu X, Kakkis E, Kohn DB. Retrovirus-mediated transfer of the human α-L-iduronidase cDNA into human hematopoietic progenitor cells leads to correction in trans of Hurler fibroblasts. *Gene Ther*. 1997;4:1150-1159.
15. Liu Y, Xu L, Hennig AK, et al. Liver-directed neonatal gene therapy prevents cardiac, bone, ear, and eye disease in mucopolysaccharidosis I mice. *Mol Ther*. 2005;11:35-47.
16. Chung S, Ma X, Liu Y, Lee D, Tittiger M, Ponder KP. Effect of neonatal administration of a retroviral vector expressing α-l-iduronidase upon lysosomal storage in brain and other organs in mucopolysaccharidosis I mice. *Mol Genet Metab*. 2007;90:181-192.
17. Ma X, Liu Y, Tittiger M, et al. Improvements in mucopolysaccharidosis I mice after adult retroviral vector–mediated gene therapy with immunomodulation. *Mol Ther*. 2007;15:889-902.
18. Traas AM, Wang P, Ma X, et al. Correction of clinical manifestations of canine mucopolysaccharidosis I with neonatal retroviral vector gene therapy. *Mol Ther*. 2007;15:1423-1431.
19. Thompson PJ, Macfarlan TS, Lorincz MC. Long terminal repeats: from parasitic elements to building blocks of the transcriptional regulatory repertoire. *Mol Cell*. 2016;62:766-776.
20. Herati RS, Ma X, Tittiger M, Ohlemiller KK, Kovacs A, Ponder KP. Improved retroviral vector design results in sustained expression after adult gene therapy in mucopolysaccharidosis I mice. *J Gene Med*. 2008;10:972-982.
21. Metcalf JA, Ma X, Linders B, et al. A self-inactivating γ-retroviral vector reduces manifestations of mucopolysaccharidosis I in mice. *Mol Ther*. 2010;18:334-342.
22. Franz K, Singh A, Weinberger LS. Chapter twenty-six - lentiviral vectors to study stochastic noise in gene expression. In: Voigt C, ed. *Methods in Enzymology*. Vol 497. Oxford, UK: Academic Press; 2011:603-622.

23. di Natale P, di Domenico C, Villani GR, Lombardo A, Follenzi A, Naldini L. In vitro gene therapy of mucopolysaccharidosis type I by lentiviral vectors. *Eur J Biochem*. 2002;269:2764-2771.
24. di Domenico C, Villani GR, di Napoli D, et al. Gene therapy for a mucopolysaccharidosis type I murine model with lentiviral-IDUA vector. *Hum Gene Ther*. 2005;16:81-90.
25. Kobayashi H, Carbonaro D, Pepper K, et al. Neonatal gene therapy of MPS I mice by intravenous injection of a lentiviral vector. *Mol Ther*. 2005;11:776-789.
26. Visigalli I, Delai S, Politi LS, et al. Gene therapy augments the efficacy of hematopoietic cell transplantation and fully corrects mucopolysaccharidosis type I phenotype in the mouse model. *Blood*. 2010;116:5130-5139.
27. Yáñez-Muñoz RJ, Balaggan KS, MacNeil A, et al. Effective gene therapy with nonintegrating lentiviral vectors. *Nat Med*. 2006; 12:348-353.
28. Escors D, Breckpot K. Lentiviral vectors in gene therapy: their current status and future potential. *Arch Immunol Ther Exp (Warsz)*. 2010;58:107-119.
29. Naso MF, Tomkowicz B, Perry WL, Strohl WR. Adeno-associated virus (AAV) as a vector for gene therapy. *BioDrugs*. 2017;31:317-334.
30. Hartung SD, Reddy RG, Whitley CB, Mcivor RS. Enzymatic correction and cross-correction of mucopolysaccharidosis type I fibroblasts by adeno-associated virus-mediated transduction of the alpha-L-iduronidase gene. *Hum Gene Ther*. 1999;10:2163-2172.
31. Desmaris N, Verot L, Puech JP, Caillaud C, Vanier MT, Heard JM. Prevention of neuropathology in the mouse model of Hurler syndrome. *Ann Neurol*. 2004;56:68-76.
32. Ellinwood NM, Ausseil J, Desmaris N, et al. Safe, efficient, and reproducible gene therapy of the brain in the dog models of Sanfilippo and Hurler syndromes. *Mol Ther*. 2011;19:251-259.
33. Hinderer C, Bell P, Gurda BL, et al. Intrathecal gene therapy corrects CNS pathology in a feline model of mucopolysaccharidosis I. *Mol Ther*. 2014;22:2018-2027.
34. Hinderer C, Bell P, Gurda BL, et al. Liver-directed gene therapy corrects cardiovascular lesions in feline mucopolysaccharidosis type I. *Proc Natl Acad Sci USA*. 2014;111:14894-14899.
35. Hinderer C, Bell P, Louboutin JP, et al. Neonatal systemic AAV induces tolerance to CNS gene therapy in MPS I dogs and non-human primates. *Mol Ther*. 2015;23:1298-1307.
36. Hinderer C, Bell P, Louboutin JP, et al. Neonatal tolerance induction enables accurate evaluation of gene therapy for MPS I in a canine model. *Mol Genet Metab*. 2016;119:124-130.
37. Vance M, Llanga T, Bennett W, et al. AAV gene therapy for MPS1-associated corneal blindness. *Sci Rep*. 2016;6:22131.
38. Belur LR, Temme A, Podetz-Pedersen KM, et al. Intranasal adeno-associated virus mediated gene delivery and expression of human Iduronidase in the central nervous system: a noninvasive and effective approach for prevention of neurologic disease in mucopolysaccharidosis type I. *Hum Gene Ther*. 2017;28: 576-587.
39. Belur LR, Podetz-Pedersen KM, Tran TA, et al. Intravenous delivery for treatment of mucopolysaccharidosis type I: a comparison of AAV serotypes 9 and rh10. *Mol Genet Metab Rep*. 2020;24:100604.
40. Wolf DA, Lenander AW, Nan Z, et al. Direct gene transfer to the CNS prevents emergence of neurologic disease in a murine model of mucopolysaccharidosis type I. *Neurobiol Dis*. 2011;43: 123-133.
41. Choi VW, McCarty DM, Samulski RJ. Host cell DNA repair pathways in adeno-associated viral genome processing. *J Virol*. 2006;80:10346-10356.
42. Duan D, Sharma P, Yang J, et al. Circular intermediates of recombinant adeno-associated virus have defined structural characteristics responsible for long-term episomal persistence in muscle tissue. *J Virol*. 1998;72:8568-8577.
43. George LA, Sullivan SK, Giermasz A, et al. Hemophilia B gene therapy with a high-specific-activity factor IX variant. *N Engl J Med*. 2017;377:2215-2227.
44. Colella P, Ronzitti G, Mingozzi F. Emerging issues in AAV-mediated in vivo gene therapy. *Mol Ther Methods Clin Dev*. 2017;8:87-104.
45. Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. *Nat Med*. 2006;12: 342-347.
46. Nguyen GN, Everett JK, Kafle S, et al. A long-term study of AAV gene therapy in hemophilia A dogs identifies clonal expansions of transduced liver cells. *Nat Biotechnol*. 2021;39:47-55.
47. Hordeaux J, Buza EL, Dyer C, et al. Adeno-associated virus-induced dorsal root ganglion pathology. *Hum Gene Ther*. 2020; 31:808-818.
48. Donsante A, Miller DG, Li Y, et al. AAV vector integration sites in mouse hepatocellular carcinoma. *Science*. 2007;317:477-477.
49. Chandler RJ, LaFave M, Varshney GK, et al. Vector design influences hepatic genotoxicity after adeno-associated virus gene therapy. *J Clin Invest*. 2015;125:870-880.
50. Giles AR, Sims JJ, Turner KB, et al. Deamidation of amino acids on the surface of adeno-associated virus capsids leads to charge heterogeneity and altered vector function. *Mol Ther*. 2018;26:2848-2862.
51. Sheridan C. To win at gene therapy, companies pick viruses with production credentials. *Nat Biotechnol*. 2019;37:5-6.
52. High-dose AAV gene therapy deaths. *Nat Biotechnol*. 2020;38: 910-910.
53. Duan D. Systemic AAV micro-dystrophin gene therapy for duchenne muscular dystrophy. *Mol Ther*. 2018;26:2337-2356.
54. Food and Drug Administration. FDA approves innovative gene therapy to treat pediatric patients with spinal muscular atrophy, a rare disease and leading genetic cause of infant mortality; 2020. https://www.fda.gov/news-events/press-announcements/fda-approves-innovative-gene-therapy-treat-pediatric-patients-spinal-muscular-atrophy-rare-disease. Accessed March 30, 2021.
55. Food and Drug Administration. Statement on data accuracy issues with recently approved gene therapy; 2020. https://www.fda.gov/news-events/press-announcements/statement-data-accuracy-issues-recently-approved-gene-therapy. Accessed March 30, 2021.
56. BioSpace. FDA lifts clinical hold on solid biosciences' DMD Gene therapy trial; October 2020. https://www.biospace.com/article/fda-lifts-clinical-hold-on-solid-biosciences-dmd-gene-therapy-trial/. Accessed April 2021.
57. Pfizer. Pfizer receives FDA fast track designation for duchenne muscular dystrophy investigational gene therapy; October 2020. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-receives-fda-fast-track-designation-duchenne. Accessed April 2021.

58. Schuh RS, Bidone J, Poletto E, et al. Nasal administration of cationic nanoemulsions as nucleic acids delivery systems aiming at mucopolysaccharidosis type I gene therapy. *Pharm Res*. 2018;35:221.
59. Teixeira HF, Bruxel F, Fraga M, et al. Cationic nanoemulsions as nucleic acids delivery systems. *Int J Pharm*. 2017;534:356-367.
60. Pineda M, Moghadam F, Ebrahimkhani MR, Kiani S. Engineered CRISPR systems for next generation gene therapies. *ACS Synth Biol*. 2017;6:1614-1626.
61. Leibowitz ML, Papathanasiou S, Doerfler PA, et al. Chromothripsis as an on-target consequence of CRISPR–Cas9 genome editing. *Nat Genet*. 2021;53:895-905.
62. Harbottle JA. Immunotherapy to get on point with base editing. *Drug Discov Today*. 2021. https://doi.org/10.1016/j.drudis.2021.04.003. published online ahead of print.
63. Kratzer K, Getz LJ, Peterlini T, Masson J-Y, Dellaire G. Addressing the dark matter of gene therapy: technical and ethical barriers to clinical application. *Hum Genet*. 2021. https://doi.org/10.1007/s00439-021-02272-5. published online ahead of print.
64. Schuh RS, Poletto É, Pasqualim G, et al. In vivo genome editing of mucopolysaccharidosis I mice using the CRISPR/Cas9 system. *J Control Release*. 2018;288:23-33.
65. Schuh RS, Gonzalez EA, Tavares AMV, et al. Neonatal non-viral gene editing with the CRISPR/Cas9 system improves some cardiovascular, respiratory, and bone disease features of the mucopolysaccharidosis I phenotype in mice. *Gene Ther*. 2020;27:74-84.
66. Gascón AR, del Pozo-Rodríguez A, Solinís MÁ. Non-viral delivery systems in gene therapy. In: Molina FM, ed. *Gene Therapy - Tools and Potential Applications*. London, UK: InTechOpen; 2013. https://doi.org/10.5772/52704
67. Ou L, DeKelver R, Rohde M, et al. ZFN-mediated in vivo genome editing corrects murine hurler syndrome. *Mol Ther*. 2019;27:178-187.
68. Wold WSM, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Curr Gene Ther*. 2013;13:421-433.
69. Wilson JM. Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. *Mol Genet Metab*. 2009;96:151-157.
70. Sibbald B. Death but one unintended consequence of gene-therapy trial. *CMAJ Can Med Assoc J*. 2001;164:1612.
71. Sawamoto K, Chen H-H, Alméciga-Díaz CJ, Mason RW, Tomatsu S. Gene therapy for Mucopolysaccharidoses. *Mol Genet Metab*. 2018;123:59-68.
72. Stephens CJ, Lauron EJ, Kashentseva E, Lu ZH, Yokoyama WM, Curiel DT. Long-term correction of hemophilia B using adenoviral delivery of CRISPR/Cas9. *J Control Release*. 2019;298:128-141.
73. Li T, Davidson BL. Phenotype correction in retinal pigment epithelium in murine mucopolysaccharidosis VII by adenovirus-mediated gene transfer. *Proc Natl Acad Sci USA*. 1995;92:7700-7704.
74. Sands MS, Wolfe JH, Birkenmeier EH, et al. Gene therapy for murine mucopolysaccharidosis type VII. *Neuromuscul Disord*. 1997;7:352-360.
75. Ghodsi A, Stein C, Derksen T, Martins I, Anderson RD, Davidson BL. Systemic hyperosmolality improves β-glucuronidase distribution and pathology in murine MPS VII brain following intraventricular gene transfer. *Exp Neurol*. 1999;160:109-116.
76. Lu ZH, Kaliberov S, Zhang J, et al. The myeloid-binding peptide adenoviral vector enables multi-organ vascular endothelial gene targeting. *Lab Investig*. 2014;94:881-892.
77. Ou L, Przybilla MJ, Ahlat O, et al. A highly efficacious PS gene editing system corrects metabolic and neurological complications of mucopolysaccharidosis type I. *Mol Ther*. 2020;28:1442-1454.

How to cite this article: Hurt SC, Dickson PI, Curiel DT. Mucopolysaccharidoses type I gene therapy. *J Inherit Metab Dis*. 2021;44(5):1088-1098. https://doi.org/10.1002/jimd.12414