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A Review of Gene Therapy in Canine and Feline Models of Lysosomal Storage Disorders

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Abstract

Lysosomal storage disorders (LSDs) are inherited diseases that result from the intracellular accumulation of incompletely degraded macromolecules. The majority of LSDs affect both the peripheral and central nervous systems and are not effectively treated by enzyme replacement therapy, substrate reduction therapy, or bone marrow transplantation. Advances in adeno-associated virus and retroviral vector development over the past decade have resurged gene therapy as a promising therapeutic intervention for these monogenic diseases. Animal models of LSDs provide a necessary intermediate to optimize gene therapy protocols and assess the safety and efficacy of treatment prior to initiating human clinical trials. Numerous LSDs are naturally occurring in large animal models and closely reiterate the lesions, biochemical defect, and clinical phenotype observed in human patients, and whose lifetime is sufficiently long to assess the effect on symptoms that develop later in life. Herein, we review that gene therapy in large animal models (dogs and cats) of LSDs improved many manifestations of disease, and may be used in patients in the near future.

Introduction

Lysosomal storage disorders (LSDs) encompass over 50 individual diseases that result from defective catabolism of macromolecules and their subsequent accumulation within lysosomes. The majority of these disorders result from deficiency of a hydrolytic enzyme; however, select LDSs are attributed to defects in membrane-bound or activator proteins. All LSDs are an effect of a single gene defect, and the vast majorities are inherited in an autosomal recessive fashion. While individually they are rare, the combined prevalence of all LSDs is $\sim$1 in 5000 live births.[1–3] For many of these diseases, there are over 100 known mutations, leading to a spectrum of onset of symptoms and disease progression.

Several therapeutic approaches have been employed to treat LSDs. Most commonly, enzyme replacement therapy (ERT) delivers a recombinant form of the defective enzyme, while substrate reduction therapy reduces synthesis of the substrate that cannot be catabolized. Bone marrow or cord blood transplantation from a normal donor functions to provide a new source of cells that are capable of migrating and secreting the deficient enzyme. Repeated, systemic delivery of ERT has become the standard of care for some LSDs, such as type I Gaucher disease,[4] in which there is no central nervous system (CNS) involvement. However, the inability of large recombinant proteins to efficiently penetrate the blood–brain barrier (BBB) renders this therapy ineffective for the majority of LSDs in which neurologic disease is a prominent feature. The feasibility of infusing recombinant enzymes directly into cerebrospinal fluid (CSF) circulation is currently being assessed in numerous animal models of LSDs. However, the necessity of repeatedly injecting the CNS comes with safety, practicality, and financial concerns.

An alternative to infusion of recombinant enzyme is the use of a viral vehicle to deliver the deficient enzyme. LSDs are an ideal candidate for gene therapy because they are monogenetic and the therapy can be administered as a one-time treatment. Furthermore, gene therapy can exploit an advantageous mechanism of lysosomal enzyme uptake known as cross-correction.[5] The majority of lysosomal enzymes are secreted into the extracellular space and, once they have exited a cell, can subsequently be taken up by a mannose 6-phoshate receptor on neighboring cells' plasma

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membrane. Therefore, it is not requisite for gene therapy to target every cell, as delivery to a subpopulation of cells that produce and secrete a portion of the deficient enzyme can cross-correct cells the vector failed to transduce.

Success of gene therapy is dependent on numerous considerations, including but not limited to, vector type, dosage, injection route, and age at treatment. Many studies have relied on the ability of viral vectors to target specific organs, and although strategies to better equip retroviruses for targeted delivery have been underway, the advent of the adeno-associated viruses (AAV) has allowed for such ambition.[6–8] Systemic expression of a therapeutic gene has most commonly been obtained through liver-directed gene therapy as the liver naturally acts as an endogenous source of circulating proteins. Indeed, this approach has been demonstrated in the clinical setting for hemophilia B, although preexisting antibodies to the AAV vector in some animals have proven to be a difficult obstacle.[9–11] As passage of circulating therapeutic protein across the BBB also remains an issue, direct brain injections of viral vectors were tested. Although adenovirus and retroviruses offer therapeutic advances for cancer-based therapies, their use in CNS gene therapy is limited due to their ability to initiate an innate immune response.[12] Offering a safer alternative, studies in the CNS using AAV serotypes have been completed.[13–20] From these studies also came the important discovery identifying select serotypes that have the ability to undergo axonal transport, and transduce cells that are some distance from the injection site, redefining this area of the field.[14,16] Indeed systemic administration of AAV serotypes with a CNS transduction profile, such as AAV9 and AAVrh10, offers a new wave of therapies allowing access to the CNS from the periphery. However, old challenges have been re-identified such as peripheral antibody responses and new challenges have evolved, such as cell-specific transduction mediated by age.[21,22]

Animal models of LSDs provide an important intermediate to optimize gene therapy protocols and assess the safety and efficacy of therapy prior to initiating human clinical trials. Numerous LSDs are naturally occurring in large animal models, particularly cats and dogs, that closely recapitulate the pathological and biochemical abnormalities observed in human patients. Furthermore, in contrast to murine models, the size and complexity of a cat or dog's brain is more comparable to that of a child, providing stronger translational potential for neuropathic LSDs. Finally, the longer lifespan of large animal models compared with mice allows the long-term efficacy to be assessed. Herein we review recent advancements in gene therapy in dog and cat models of LSDs (Table 1) and demonstrate the progress toward treatment in patients.

Gene Therapy in Feline and Canine Models of LSDs

Alpha-mannosidosis

Alpha-mannosidosis (AMD) is an inherited lysosomal storage disease caused by the deficient activity of the lysosomal enzyme α-mannosidase. Deficient enzymatic activity results in the accumulation of mannose-rich oligosaccharides within lysosomes.[23,24] In humans, the disease is characterized by intellectual disability, ataxia, and progressive skeletal abnormalities.[25–28] Hepatosplenomegaly, recurrent bacterial infections, gingival hyperplasia, synovitis, hearing loss, hydrocephalus, paraplegia, and corneal and lenticular opacities can also occur.[23,24,27] Neuropathological findings include vacuolated neurons, glia, and endothelial cells throughout the brain and spinal cord; Purkinje cell loss; and myelin deficiency of the central and peripheral nervous systems.[27]

Feline alpha-mannosidosis

Spontaneously occurring AMD has been reported in cats,[28–34] cattle,[35–42] and guinea pigs,[43–45] and a knockout mouse has been created.[46] Interestingly, disease can also be induced by ingestion of the indolizidine alkaloid swainsonine, which is found in locoweed plants.[47] Hereditary AMD occurs in Persian, longhair, and shorthair cats and, in Persian cats, is caused by a 4 bp deletion in the α-mannosidase gene (*MANB*) resulting in a frameshift and premature termination.[30,33,48] Disease in cats is characterized by progressive cerebellar ataxia, skeletal abnormalities, hepatomegaly, thymic aplasia, gingival hyperplasia, corneal and lenticular opacities, and polycystic kidneys.[29,31,34] Neuropathologic findings are similar to those found in human patients.[29–32,49,50] Surrogate MRI markers of CNS disease severity have been developed in the AMD cat.[51–53]

In 2001, a comparison of the ability of three AAV serotypes (AAV1, AAV2, and AAV5) to transduce the cat brain was examined, and it was determined that AAV1 resulted in the greatest transduction of both gray and white matter.[54] Eight-week-old cats with AMD received six injections of an AAV1 vector carrying the normal feline alpha-mannosidase cDNA into each rostral cerebral hemisphere and the rostral brainstem, and two injections into the cerebellum.[51] Surprisingly, all treated cats showed improvement in signs of cerebellar dysfunction (intention tremor, truncal ataxia, and tremor) from 12 to 18 weeks of age. Treated cats were euthanized at 18 weeks of age, an age when untreated cats are euthanized because of severe cerebellar dysfunction. Histological analysis of the brains of treated cats showed complete resolution of storage in neurons, glia, and endothelial cells up to 4.5 mm from the injection track, and up to 2 mm from cells producing *MANB* mRNA. While lysosomal storage increased as the distance from the needle track increased, no regions of the treated cat brain showed lysosomal storage as severe as that found in untreated AMD cats. Even in regions of the brain distant from the injection tracks, such as the occipital cortex, cells were not as swollen as those seen in untreated AMD cats. Myelination abnormalities also improved throughout the brains of treated cats. Finally, resolution of storage also could be seen in cells of the choroid plexus, ependyma, and meninges. Although disease was not cured in the treated cats, these studies suggested that direct gene therapy to the brain could delay, ameliorate, and even improve disease in the cat. These studies led to the study of third-generation AAV vectors and intrathecal administration of AAV in this feline model (currently unpublished) and the proposal for a clinical trial in children with AMD.

GM1 gangliosidosis

GM1 gangliosidosis is an LSD that results from a deficiency of the hydrolytic lysosomal enzyme β-galactosidase (β-gal; EC 3.2.1.23). Functional β-gal is responsible for the initial step in ganglioside catabolism and cleavage of the terminal galactose residue, and in its absence GM1 ganglioside

accumulates throughout the CNS. GM1 gangliosidosis presents as three clinical forms: (1) infantile, (2) late infantile/juvenile, and (3) adult onset, with severity decreasing as the age of onset increases. Infantile and juvenile patients most commonly experience developmental regression, muscle weakness, skeletal abnormalities, visceromegaly, and neurological signs progressing to seizures, blindness, and ultimately a vegetative state.[55] Disease onset of the infantile form typically occurs in the first 6 months of life and is fatal by 5 years of age. In the juvenile form, symptoms most commonly arise between 7 months and 3 years of age, and lifespan does not frequently exceed 15 years of age. The adult onset is highly variable in the age of onset, presentation of symptoms, and lifespan.

Feline GM1 gangliosidosis

Feline GM1 gangliosidosis results from a G-to-C substitution at position 1448 resulting in an arginine-to-proline substitution at amino acid 483, which is analogous to a mutation in humans.[56] Feline GM1 gangliosidosis was initially described in 1971[57] and is considered to be a model of late infantile/juvenile onset.[58] Disease onset occurs at 4.1 ± 0.6 months with fine tremors and progresses to muscle weakness, ataxia, overt full body tremors, and eventually the inability to stand, which defines the humane endpoint at 8.0 (± 0.6) months of age.[59] Histopathological examination reveals enlarged neurons with cytoplasmic inclusions and intense staining with periodic acid Schiff and hepatocellular vacuolation. Biochemical analysis is comparable to juvenile patients, with a deficiency of β-Gal activity ($< 10\%$ of normal) and substantially increased levels of GM1 ganglioside (~ 8 times normal).[60]

Gene therapy experiments were conducted in GM1 cats with an AAV vector expressing feline β-Gal injected bilaterally into the thalamus and deep cerebellar nuclei (DCN). Twenty-three GM1 cats were treated between 1.3 and 3.0 months of age, prior to the average age of onset of clinical signs, with either AAV1 or AAVrh8 serotypes and outcomes were assessed at 16 weeks after injection (short-term, $n = 7$) or at the humane endpoint (long-term, $n = 16$).[59] Short-term studies at 16 weeks postsurgery demonstrated that β-Gal activity throughout the brain, spinal cord, and CSF in GM1 cats exceeded that of normal animals and there was no significant difference between the AAV serotypes. Treatment with either vector serotype led to significant reduction of ganglioside storage in all CNS samples analyzed. Long-term studies in GM1 cats established statistically significant increases in survival for both serotypes to nearly 40 months of age, or 5 times that of untreated GM1 cats, at the time of publication. However, 8 of the 12 treated cats remained alive with subtle or no discernable clinical phenotype, and to date treated cats have exceeded 5 years of age (D.R. Martin, personal communication). Of the 4 deceased cats, 2 reached neurological humane endpoint and 2 cats, with mild or no signs of neurologic disease, failed to recover from anesthesia and were euthanized. MRI demonstrated normalization of white to gray matter intensities and overall brain architecture in AAV-treated GM1 cats. There was an apparent dose response, as cats treated with 1/10th of the original dose had a significantly shorter survival time (17.1 ± 3.6 months; $p = 0.0046$) than those that received the higher dose. Gene therapy has restored breeding function to the GM1 colony, allowing for litters completely comprised of kittens homozygous for the GM1 mutation.[59]

GM2 gangliosidosis

Tay–Sachs, along with Sandhoff disease (SD), comprises a category of LSDs known as monosialoganglioside 2 (GM2) gangliosidoses, arising from a deficiency of the hydrolytic lysosomal enzyme β-nacetylhexosaminidase (Hex; EC 3.2.1.52). Hex is comprised of two subunits, α and β, which dimerize to form three distinct isozymes with different physiological functions. HexA is comprised of an α/β subunit heterodimer, HexB is a β/β homodimer, and a α/α homodimer leads to an unstable isozyme, Hex S. HexA cleaves charged substrates and is the isozyme responsible the hydrolytic cleavage of GM2 ganglioside. A defect in HexA results in accumulation of GM2 ganglioside in neurons throughout the CNS leading to progressive and fatal neurodegeneration.

Three forms of GM2 gangliosidosis are defined based on age of onset and subsequent disease severity: (1) infantile (classical), (2) juvenile, and (3) adult onset, with the latter two having a more heterogeneous disease progression. Infantile GM2 has a mean age at onset of 5.0 months, age at diagnosis of 13.3 months, and life span of 47 months. The most frequent initial symptoms reported are developmental arrest, abnormal startle response, and low muscle tone, which progress to seizures, blindness, and ultimately a vegetative state.[61] Signs of neurodegeneration are visible upon gross examination of the brain at the time of autopsy. Namely, the ventricles are enlarged in accordance with macrocephaly and the gyri become broad and the sulci shortened because of cortical atrophy.[62] Upon histological examination, neurons laden with storage of glycosphingolipid become enlarged, attributable to cytoplasmic distention by accumulation of foamy, vacuolated material. This material often displaces neuronal nuclei peripherally and obscures visualization of Nissl substance. Ultrastructure examination reveals the presence of electron-dense lamellated material known as membranous cytoplasmic bodies as well as transversely stacked myelinoid membranes known as zebra bodies.[62] Many pathological signs of aberrant storage are also detectable in visceral organs, including the liver, heart, spleen, and skeletal system.

Feline GM2 gangliosidosis

Feline GM2 gangliosidosis results from a mutation in the *HEXB* gene (the β subunit) causing a deficiency in both HexA (α/β) and HexB (β/β) enzymes, and thus is a true model of SD. The GM2 gangliosidosis cat model was first described in 1977,[63] and has been well characterized in the intervening years.[64,65] There are four known mutations in the *HEXB* gene that result in feline SD: a 25 bp inversion at the 3′ terminus,[65] a single base deletion in exon 1,[66] a nonsense mutation in exon 7,[67] and a 15 bp deletion encompassing the splice acceptor site of intron 11.[68] Cats homozygous for the 25 bp inversion mutation have premature termination of the coding sequence that results in $< 3\%$ of normal Hex enzyme activity in the brain and peripheral tissues and progressive neurologic disease similar to infantile patients. Disease onset occurs at ~ 1.7 months of age with slight intention tremors of the head and tail. Signs gradually progress to ataxia, hind limb

weakness, overt whole-body tremors, and inability to stand (humane endpoint) by 4.5 ± 0.5 months. The pathological presentation of feline GM2 gangliosidosis is very similar to that seen in human disease and includes distended neurons and membranous cytoplasmic bodies in the CNS and storage inclusions, resulting in vacuolation of cells in the viscera.[63]

Sandhoff disease cats treated by bilateral thalamic injection with AAV1 vectors encoding human α and β Hex subunits lived to 7.0 and 8.2 months of age.[69] The limited therapeutic effect may have been because of robust humoral immune response to the AAV capsid and/or human Hex protein. Subsequently, AAV vectors encoding feline α and β Hex subunits were injected bilaterally into the thalamus of SD cats, which reduced the immune response and increased survival to 10.4 ± 3.7 months of age, or >2 times that of untreated cats. After bilateral thalamic injection, Hex activity was restored to near or above normal levels throughout the cerebrum, but mean activity was substantially below normal in the cerebellum.[69] To achieve better enzymatic distribution, the thalamic infusion was combined with direct targeting of the DCN. SD cats were treated between 4 and 6 weeks of age, prior to symptom onset, with an AAVrh8 vector encoding feline Hex. At 16 weeks postsurgery, HexA activity was >2-fold normal throughout the brain, spinal cord, and CSF. GM2 ganglioside storage was significantly reduced in all areas of the brain and spinal cord analyzed when compared with untreated SD cats. AAV therapy delayed disease onset up to 16 weeks posttreatment, the age in which untreated SD cats typically reach humane endpoint. Treated SD cats developed generalized hind limb weakness and subtle tremors, but did not progress to severe ataxia and overt whole-body tremors that are prominent in untreated SD cats.[70]

Because of added surgical risk associated with directly injecting the cerebellum, SD cats were alternatively treated by intracerebroventricular (ICV) injection via the lateral ventricle in combination with the bilateral thalamic injection. HexA activity was >4-fold normal throughout the brain and spinal cord.[71] Sixteen weeks after injection, GM2 ganglioside storage was reduced by $>90\%$ in all CNS regions analyzed. The thalamus + ICV injection route resulted in a delay in onset of signs; however, correction of the disease phenotype was not as complete as previously seen with the thalamus + DCN injection route.[71] Untreated SD cats and cats from both AAV treatment groups were analyzed for potential biomarkers of disease and therapeutic efficacy. Alterations were found in blood, CSF, and clinical evaluations that increased with disease progression. Importantly, many of these factors were normalized after intracranial AAV gene therapy and thus could serve as secondary outcome measures.[72] Ongoing long-term studies are demonstrating substantial therapeutic efficacy, with treated SD cats surviving at least four times longer than untreated cats (D.R. Martin, personal communication).

Mucopolysaccharidosis I

Mucopolysaccharidosis I (MPS I) is an LSD that is characterized by a deficiency of the lysosomal enzyme α-L-iduronidase (IDUA, EC 3.2.1.76). The subsequent accumulation of partially degraded dermatan and heparan sulfates in lysosomes is responsible for the primary manifestations. Currently, 119 different mutations have been identified leading to a variety of clinical phenotypes. The clinical disease is categorized as the severe form, or Hurler syndrome (MPS IH), and the attenuated form, which includes Hurler–Scheie (intermediate, MPS IH/S) and Scheie syndromes (mild, MPS IS). MPS IH is most commonly diagnosed and is clinically characterized by a combination of retarded physical and mental development, corneal clouding, high urine glycosaminoglycans (GAGs), organomegaly, coarse facial features, dysostosis multiplex, joint stiffness, cardiovascular involvement, respiratory problems, and early childhood death.[73,74] In contrast, Scheie syndrome is compatible with normal intelligence, stature, and lifespan with attenuated disease progression including corneal clouding, joint stiffness, and aortic valve disease. MPS IH/S is more variable but is usually characterized by normal intelligence with physical manifestations that have intermediate severity in clinical presentation. No single feature is diagnostic, while combinations suggestive of MPS supported with urine GAG analysis and enzyme activity assays are most often definitive.

Feline MPS I

The feline model of MPS I is homozygous for a 3 bp deletion translating to loss of an aspartate that is highly conserved among man, dog, and mouse.[75] Clinical presentation most closely resembles the severe human counterpart, MPS IH, with high urine GAG, no enzyme activity, broad facial features, short ears, hepatosplenomegaly, corneal clouding, skeletal and joint deformities including coxofemoral subluxation, and fusion of the cervical vertebrae.[76] Behavioral and learning deficits are difficult to accurately analyze in the feline model; however, storage lesions have been identified in feline MPS I CNS tissue including increased lysosomal vesicles laden with GM3 ganglioside, cholesterol, and GAG accumulation.[77]

Knowledge of the biological component responsible for MPS I[5] has allowed for further therapeutic studies, including viral vector-mediated gene therapy studies in large animal models. Previous studies have identified that the feline model is sensitive to the species differences in the IDUA protein.[78,79] Indeed, Ponder et al. indicated a significant immune response to the canine IDUA (cIDUA) gene product; however, stable expression was achieved with additional immunosuppression given for 1 month at the time of gene therapy.[79] Until Hinderer and colleagues[77] cloned the feline *IDUA (fIDUA)* cDNA, gene therapy-based studies were limited to the canine model. Intrathecal (IT) delivery of AAV9-*fIDUA* resulted in stable CSF and serum IDUA activity at or just below normal values. Although a systemic IDUA antibody response was detected, overall results indicated global CNS transduction, normalization of secondary lysosomal enzymes, and the reduction of GAG, cholesterol, and GM3 ganglioside-associated storage lesions.[77] In addition, storage was reduced in livers of all treated animals, while those with low antibody titers exhibited cross-correction of the spleen.[77] Further studies with an intravenously delivered AAV8-*fIDUA* highlighted reversal of systemic storage lesions and complete correction of cardiovascular lesions in animals expressing sustained supraphysiological levels of IDUA in serum.[80]

Canine MPS I

MPS I in dogs is caused by a donor splice site mutation in intron 1 of the *IDUA* gene[81] and is characterized by stunted growth,[82] facial dysmorphia,[83] joint, bone, heart, and CNS

disease. Specifically, chondrocytes are affected by storage material leading to joint hypermobility and swelling,[82] erosions, and ulcerations on articular surfaces of joints along with joint effusion.[84,85] Synovial cells in joints are vacuolated and thickened with brown-red villous projections into the joints.[84,86] Degenerative osteoarthritis develops in all affected dogs over time[86] and distal limb joint laxity may be present by 9–12 months of age leading to the difficulty in ambulation.[84] The bony changes, thickened meninges, and disc degeneration in the spine may cause spinal cord compression mainly in the cervical spine and thoracic spine, which can be quite severe by over a year of age.[87] The heart is enlarged and rounded with thickened valves and intimal surfaces of the pulmonary arteries and aorta diffusely roughened.[86] Vacuolation of mesenchymal cells in arteries, including the coronary arteries,[82,86] thickening of valves and the aortic wall, and dilation of ventricles leading to thinner ventricular walls,[84] are evident in affected dogs. Aortic dilation has been shown to be most severe by a year of age in affected dogs.[88,89] Hepatocytes and Kupffer cells in affected dogs are vacuolated[86] and MPS I dogs often develop portal hypertension and nodular regenerative hyperplasia of the liver.[90] Lymph nodes are generally enlarged (3–4 times normal), and the mesenchyme in most other organs and epithelial cells in kidney and adrenal gland contain cytoplasmic vacuolation.[84,86] Corneal opacities develop by about a year of age in affected dogs[84,91] and corneal stromal cells accumulate storage and worsen with age.[89]

Storage in the nervous system seen microscopically as cytoplasmic vacuolation is mainly observed in neurons and astrocytes, but also in fibroblasts and tissue macrophages.[86] Studies in affected dogs demonstrated that leptomeninges of CNS were thickened because of mesenchymal cells packed with GAGs. Some cerebral cortical neurons also contained storage material leading to marginated nuclei, and mild to moderate axonal degeneration was noted in one dog.[84] Perivascular mononuclear cell infiltration was present in both gray and white matter.[86] On electron microscopy, inclusions (zebra bodies) were noted.[86] Increased concentrations of GM2, GM3, GD3, and β-hexosaminidase and decreased β-galactosidase concentrations were present in the CNS.[86]

Only few early gene therapy experiments have been performed to correct MPS I in the canine model.[88,92–95] Some involved transducing cells *in vitro* and then transplanting. Canine bone marrow cells transduced *in vitro* with retroviruses expressing the human gene were administered to MPS I-affected dogs between 3 and 11 months of age with low to no survival of transplanted cells, no correction of disease, and a strong immune responses against the enzyme.[94] In another experiment, *in utero* transplants between gestational day 35 and 38 were performed in heterozygous MPS I bitches mated to heterozygous males. There were no immune responses to IDUA but antibodies developed against proteins in the culture medium after antigen challenge. While the provirus was identified at low levels in hematopoietic cells, no enzyme was detected and no improvement in MPS I phenotype.[93]

Neonatal gene therapy resulted in better amelioration of disease and no side effects were noted upon administration of the vector. MPS I dogs received a retroviral vector containing human alpha1-antitrypsin promoter and the canine *IDUA* cDNA at 2–3 days of life.[88,92] All MPS I-affected, treated dogs had serum IDUA levels that were 2–68 times higher than those in the serum of normal dogs, and have been maintained for up to 8 years. All dogs survived long-term and corneal clouding was significantly decreased but not normal. There was reduction in lysosomal storage of cortical neurons and zebra bodies were absent. Clinical aspects of the disease phenotype, including facial dysmorphia, umbilical hernias, joint disease, and aortic dilation, were improved.[88] Bone disease was mild in control affected MPS I dogs making it difficult to evaluate the skeletal effects of therapy, but some of the skeletal manifestations of disease were ameliorated.[92] The results suggest that the skeletal tissues are more difficult to transduce and that treatment might need to be initiated even earlier or locally in the joint to achieve higher enzyme levels.[92]

MPS I dogs aged 3–5 months received intracerebral injections of AAV5 encoding human IDUA in conjunction with complete immunosuppression, cycolosporine (CsA) + mycophenolate mofetil (MMF), or partial immunosuppression with CsA alone.[95] Dogs with complete immunosuppression demonstrated broader dispersion of vector copies, higher IDUA activity, and greater attenuation neuropathology. Dogs with incomplete immunosuppression developed subacute encephalitis characterized by infiltration of mononuclear cells into perivascular and subarachoid spaces. Furthermore, anti-hIDUA antibodies were detectable in the brain extracts of dogs that received CsA alone, but not the brain of dogs that received CsA + MMF.[95] This study reiterates the necessity of preventing a detrimental immune reaction to the therapeutic product. Other studies have shown that the use of species-specific transgene reduces immunoreactivity of intracerebral gene therapy and may negate the need for immunosuppression.[69,96]

Mucopolysachharidosis III

Mucopolysachharidosis III (MPS III), or Sanfilippo syndrome, is characterized by mutations in the lysosomal hydrolases responsible for the catabolism of heparan sulfate (HS) oligosaccharides, and thus includes 4 types, each because of the deficiency of a different enzyme: heparan N-sulfatase (type A); alpha-N-acetylglucosaminidase (NAGLU, type B); acetyl CoA:alpha-glucosaminide acetyltransferase (type C); and N-acetylglucosamine 6-sulfatase (type D). Clinical onset is progressive in all types and generally follows a normal developmental period up to around 1–3 years of age where slowed cognitive advancement develops in the form of speech delay. Behavior issues develop around 3–4 years of age and often mirror a cognitive decline. In adolescent years, behavioral issues decline as dementia develops, along with motor function decline, a complete loss of locomotive capacity and dysphagia. Patients often develop severe hyperactivity that is unresponsive to therapy and are often misdiagnosed as attention-deficit/hyperactivity disorder or within the autism spectrum.[97] Patients may survive well into their forties depending on the phenotype, but generally succumb to death in their late twenties to mid-thirties.[98,99] Several naturally occurring animal models have been identified for types A, B, and D, but only the Schipperke dog model for MPS IIIB has been maintained for therapeutic studies.[100,101]

Canine MPSIIIB

Similar to human, the MPS IIIB dog displays normal development, until $\sim$3 years of age when evidence of

Table 1. Gene Therapy in Canine and Feline Models of Lysosomal Storage Disorders

	Disease	*Deficient enzyme*	*Model origin*	*Gene therapy*
Feline models of LSDs treated with gene therapy	α-Mannosidosis	α-D-mannosidase	Persian[28]	51
	GM1 gangliosidosis	β-Galactosidase	Siamese[57] Korat[130] Bangladeshi domestic[131] Domestic shorthair[132]	59
	GM2 gangliosidosis	β-Hexosaminidase	Domestic shorthair[63] Korat[133] Japanese domestic[134] Burmese[68]	69–71
	MPS I	α-L-iduronidase	Domestic shorthair[135]	77,79,80
	MPS VI	Arylsulfatase-B	Siamese[136]	109–114
Canine models of LSDs treated with gene therapy	MPS I	α-L-Iduronidase	Plott Hound[86]	88,92–95
	MPS IIIB	α-N-acetylglucosaminidase	Schipperke[101]	102
	MPS VII	β-Glucuronidase	German shepherd[119] Brazilian terrier[120]	92,121–126

LSDs, lysosomal storage disorders; MPS, mucopolysaccharidosis.

neurological involvement becomes obvious. Presentations generally include dullness, head tilt, and lethargy, with ataxia being a prominent feature. The animals quickly decline neurologically with clinical features involving poor condition and anorexia, cardiac involvement, tetraparesis, intention tremors, exaggerated postural reactions, and decreased menace response. Overall, the disease is considered diffuse and central with a prominent cerebellar component.[101] Behavioral studies have not been conducted to date. Animals are typically euthanized because of poor prognosis within 1–2 years from onset of clinical signs.

A single study has been published to date studying the effects of intracerebral delivery of an AAV5 vector encoding for human NAGLU. It is unclear whether this treatment delayed the onset of neurological symptoms, as this was not the ultimate goal. However, GAG and ganglioside storage was markedly reduced in treated animals compared with untreated controls.[102] Although vector genomes and NAGLU activity were found in the cerebral hemispheres surrounding the injection sites, both products were absent in the most rostral and caudal portions of the brain, especially the cerebellum.[102] Of note, immunosuppression was mandatory to prevent inflammation and allow disease correction, as animals without immunosuppressive treatment had lower (or absent) enzyme activity and inflammation in the CNS, which may have been because of the use of the human transgene.[102] Overall, this study provided evidence that AAV gene therapy delivering functional NAGLU to the brains of MPS IIIB dogs was safe and well tolerated, and could be generated as a potential clinical therapy to pursue.

Mucopolysaccharidosis type VI

Mucopolysaccharidosis type VI (MPS VI), also known as Maroteaux–Lamy syndrome, is characterized by mutations in the gene encoding for arylsulfatase B (ARSB, EC 3.1.6.12), necessary for the degradation of dermatan and chondroitin sulfates. Reduction or absence of this lysosomal enzyme leads to systemic storage of partially degraded dermatan sulfate. Disease progression can be rapid or slow with further characterization by urine GAG levels and physical presentation.[103,104] Patients with rapid onset develop coarse facial features at birth and a variety of clinical manifestations, including severe skeletal dysplasia leading to shortened stature, various bone deformities and spinal cord compression, joint stiffness and degenerative arthritis, infiltrative cardiomyopathy and respiratory dysfunction, corneal clouding, organomegaly, and communicating hydrocephalus.[103,104] Patients presenting as slow onset type develop these features at a later age with reduced severity, but eventually succumb to severe deformities and secondary complications. In comparison to many of the LSDs, cognitive decline and neurodegeneration are not major features of this disease.[105] Naturally occurring large animal models of MPS VI include both feline and canine forms.[100]

Feline MPS VI

MPS VI cats carry a homozygous *ARSB* mutation corresponding to an L476P mutation ultimately causing retention in the endoplasmic reticulum.[106] Cats predominantly present with physical deformity and orthopedic issues similar to those described in human.[99,104] They reflect the coarse facial features of MPS VI, presenting early after birth with small heads, flattened-broad faces, and short ears. The cats are often of short stature with progressive locomotor difficulty, especially in the hind limbs. Radiographic analysis reveals epiphyseal dysplasia and degenerative joint disease of long bones and osteopenia. Corneal clouding is evident as well. Animals degenerate physically over $\sim$2 years with hind limb paresis, reduced pain perception, and increased tensor tone.[107] Organomegaly and cardiac abnormalities, including mitral valve thickening, are also observed in this model.

Considering the lack of CNS involvement, ERT and systemic gene therapies are attractive options as crossing of the BBB is unnecessary. Although ERT is an approved therapy for MPS VI and has shown reduction in systemic GAG storage, it has not been shown to prevent skeletal or ocular abnormalities.[108] Current gene therapy studies in the feline model include neonatal intravenous gene therapy (see below) using retroviruses and *ex vivo* in HSCs and fibroblasts, and AAV-mediated delivery. Although the *ex vivo* studies established the feasibility of their methods in the MPS VI cat model, neither method was able to produce long-term expression or express more than 20% of normal

serum ARSB activity.[109,110] Studies using AAV delivery of feline ARSB were more successful at establishing sustained circulating enzyme levels. In an effort to specifically address retinal storage, Ho and colleagues[111] injected an AAV2 vector into the subretinal or intravitreal spaces. Subretinal injections proved to be superior to the intravitreal route and restored orientation to the layers as well as reducing vacuolization. Intravitreal injections were less dramatic, but allowed for expression in retinal ganglion cells and subsequent storage reduction. Prevention of lesions was evident in juvenile cats while alleviation of storage GAG was identified in adult cats.[111]

In preliminary studies, Tessitore et al. established that enzyme secreted from transduced hepatocytes using an AAV8 vector driven by a liver-specific promoter, thyroxine-binding globulin (TBG), was superior to an AAV1 vector driven by a muscle-specific promoter, muscle creatine kinase (MCK), for systemic expression of feline ARSB.[112] Circulating enzyme above or at normal activity levels and significant reduction in GAG storage was evident, prompting further study. A follow-up article established that high doses (6E12-E13 GC/kg) of AAV8.TBG vector were able to improve mitral valve lesions and improve long bone length and facial morphology, further resulting in improved motor activity.[113] Although high serum activity was achieved and a majority of pathologies were improved in several organs, variable circulating ARSB expression was noted. Indeed, further studies with this therapy provided evidence that preexisting antibodies to AAV8 in the cats influenced the outcomes of enzyme activity and therapy.[114] Considering the long-term expression and improvements in skeletal abnormalities and alleviation of storage material in the cats, these data support AAV8 liver-directed therapy as a potential treatment option for MPS VI.

Gamma retrovirus expressing fARSB were administered IV to neonatal cats, which resulted in normalization of body weight and facial dysmorphia, longer appendicular skeleton, improved mobility through less erosion of cartilaginous joint surfaces, and improvement of aortic valve and vessel abnormalities. There was little to no improvement of cervical vertebral length and one treated cat developed spinal cord compression at 4.6 years of age. However, the average serum and liver levels of enzyme in the treated cats were 13 and 26 times those of normal cats. Storage of GAGs in all treated cats was reduced in some organs to those found in normal cats.[115]

Mucopolysachharidosis VII

MPS VII is an LSD that results from deficient activity in β-glucuronidase (GUSB; EC 3.2.1.31), an enzyme that contributes to the degradation of heparan, dermatan, and chondroitin sulfates.[116,117] It is also known as Sly syndrome, and clinical manifestations include hepatosplenomegaly, cardiac disease, dysostosis multiplex, hernias, auditory and visual deficits, coarse facies, respiratory disease, and mental retardation. It has been treated with hematopoietic stem cell transplantation,[118] and a trial of ERT is in progress (W.S. Sly, personal communication).

Canine MPS VII

The canine model of MPS VII is because of a missense mutation that results in an arginine-to-histidine mutation at amino acid position 166 and $<1\%$ of normal enzyme activity.[119,120] Clinical manifestations in dogs include umbilical hernias, hepatosplenomegaly, portacaval shunts, an average age of death of 3 months, cardiovascular disease, degenerative joint disease, and dysostosis multiplex, with for those surviving an inability to stand or walk after 6 months of age. IV injection of a gamma retroviral vector expressing canine GUSB to newborn MPS VII dogs resulted in transduction of the liver and stable expression of mannose 6-phosphate-modified enzyme in serum for up to 11 years. This treatment increased median survival to 6.1 years, improved growth and mobility, reduced facial dysmorphia, and reduced cardiovascular disease.[92,121–125] All treated animals walked throughout their lifetime although the gait was abnormal. Cartilage was absent from articular surfaces of joints at 6 years or later.[126] The advantage of studying the effect of gene therapy in a large animal model was evident for the MPS VII dogs, where the large size made it feasible to dissect out regions such as the joints and heart valves to evaluate improvement in histopathology, and the long lifespan of dogs made it possible to determine that disease in the cartilage and intervertebral discs was not prevented long-term,[123,126] and will need to be approached with other methods.

In recent short-term proof-of-principle studies, MPS VII dogs were injected intrarthecaly (IT) with either AAV9 or AAVrh10 vectors carrying the canine GUSB cDNA. In all IT-injected dogs, enzyme activity was maintained throughout the study with supraphysiological levels circulating in the CSF and global tissue activity above or near normal levels in all CNS tissues. Storage lesions and GAG were reduced in all tissues tested and similar to the retroviral studies, all animals maintained mobility over the study with minor gait abnormalities (unpublished data).

Conclusions

Success of gene therapy experiments in animal models along with safety data collected from previous clinical trials could allow for expedited approval of human clinical trials for many of the diseases discussed above. In addition to the preclinical data described herein, human clinical trials have recently been completed or initiated for similar LSDs. A phase I/II clinical trial including four children with MPSIIIA was recently completed. Patients received 12 intracerebral injections of AAVrh10 encoding N-sulfoglycosamine sulfohydrolase at a dose of 7.2×10^{11} viral genomes/patient. All patients tolerated the neurosurgery and the safety data collected were encouraging. Efficacy data were limited by the absence of reliable outcome measures, but suggest stabilization of disease in some of the patients.[127] This study further supports the notion that early intervention is paramount to clinical trial design and obtaining therapeutic benefit. This study served as validation of direct intracranial injections of AAVrh10, which has been employed in phase I/II clinical trials for both late infantile neuronal ceroid lipofuscinosis (LINCL) and metachromatic leukodsystrophy (MLD). Lentiviral hematopoietic stem cell gene therapy has also recently shown benefit in MLD[128] and X-linked adrenoleukodystrophy.[129]

Large animal models of LSDs have been paramount to the development of effective gene therapy protocols. Data obtained from these valuable animal models continue to

support initiation of additional clinical trials. Furthermore, it is likely that this therapeutic approach may be applied to other monogenic and neurodegenerative diseases for which there is currently no effective treatment.

Author Disclosure Statement

No competing financial interests exist.

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