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REVIEW

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Canine and Feline Models of Human Genetic Diseases and Their Contributions to Advancing Clinical Therapies

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For many lethal or debilitating genetic disorders in patients there are no satisfactory therapies. Several barriers exist that hinder the developments of effective therapies including the limited availability of clinically relevant animal models that faithfully recapitulate human genetic disease. In 1974, the Referral Center for Animal Models of Human Genetic Disease (RCAM†) was established by Dr. Donald F. Patterson and continued by Dr. Mark E. Haskins at the University of Pennsylvania with the mission to discover, understand, treat, and maintain breeding colonies of naturally occurring hereditary disorders in dogs and cats that are orthologous to those found in human patients. Although non-human primates, sheep, and pig models are also available within the medical community, naturally occurring diseases are rarely identified in non-human primates, and the vast behavioral, clinicopathological, physiological, and anatomical knowledge available regarding dogs and cats far surpasses what is available in ovine and porcine species. The canine and feline models that are maintained at RCAM are presented here with a focus on preclinical therapy data. Clinical studies that have been generated from preclinical work in these models are also presented.

INTRODUCTION

For many lethal or debilitating genetic disorders in patients there are no satisfactory therapies. The critical barriers to developing effective treatments for genetic diseases in humans include limited natural history studies, an insufficient understanding of how genotype and phenotype correlate with disease, a paucity of validated surrogate markers, and the dearth of potential therapies

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†Abbreviations: RCAM, Referral Center for Animal Models of Human Genetic Disease; *MAN2B1*, lysosomal alpha-mannosidase; AMD, alpha-mannosidosis; CNS, central nervous system; AAV, adeno-associated virus; cDNA; complementary deoxyribonucleic acid; *FUCA1*, alpha-L-fucosidase; ERT, enzyme replacement therapy; HSCT, hematopoietic stem cells; PNS, peripheral nervous system; CSF, cerebrospinal fluid; GLD, globoid cell leukodystrophy; GALC, galactosylceramidase; IV, intravenous; MPS, mucopolysaccharidosis; IDU, alpha-L-iduronidase; GAGs, glycosaminoglycans; FDA, US Food and Drug Administration; EMA, European Medicines Agency; rhIDU, recombinant human iduronidase; IT, intrathecal; RV, gamma-retrovirus; NAGLU, N-acetyl-α-D-glucosaminidase; HS, heparan sulfate; ARSB, arylsulfatase; rh4S, recombinant human N-acetylgalactosamine-4-sulfatase; *GUSB*, β-glucuronidase; cGUSB, canine β-glucuronidase; NPC1, Niemann-Pick type C1; HPβCD, 2-hydroxypropyl-β-cyclodextrin; XLHED, X-linked Hypohidrotic Ectodermal Dysplasia; EDA, ectodysplasin A; Fc:EDA, recombinant ectodysplasin A; GRMD, golden retriever muscular dystrophy; IM, intramuscular; XSCID, X-Linked Severe Combined Immunodeficiency; IL-2Rγ, interleukin-2 receptor gamma chain.

Keywords: large animal models, rare disease, genetic disease, feline, canine, referral center, resource, preclinical trial

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that substantially improve disease in animal models. These barriers are partially overcome by studying naturally occurring large animal models (canine and feline) of human genetic disease for which breeding colonies are developed and natural history, clinicopathological, and histological data are collected. Animal models allow for the studies necessary to unravel the pathogenic mechanisms involved in disease progression.

In 1974, the Referral Center for Animal Models of Human Genetic Disease (RCAM) was established at the University of Pennsylvania with the overall objective to serve as a national referral and resource center to discover, characterize, maintain breeding colonies, and make available dog and cat models with hereditary diseases homologous to those found in human patients that can be used to translate preclinical trials from kennel to clinic. The naturally occurring animal models currently available represent true orthologs of their respective human disease, involving defects in homologous genes resulting in similar molecular, biochemical, pathological, and clinical phenotype as in human patients. Indeed, large animal models serve as an important intermediate for the assessment of therapeutic strategies by allowing for repeated sampling of fluids and tissue, evaluation of disease progression and safety of therapy using the same equipment and techniques used in pediatric and general human patient populations, accurate biodistribution studies due to increased brain and body size, and long-term efficacy and safety studies due to their prolonged lifespan. Although non-human primates, sheep, and pig models also share some of these attributes, naturally occurring diseases are rarely identified in non-human primates, and the vast behavioral, clinicopathological, physiological, and anatomical knowledge available regarding dogs and cats far surpasses what is available in ovine and porcine species. Finally, the tremendous expertise of veterinarians and the state of the art veterinary facilities available provide an unparalleled capability to characterize naturally occurring diseases in dogs and cats.

A comprehensive list of canine and feline models of human genetic diseases that have been identified through RCAM and are available for further study are listed in (Table 1). Below we summarize the models currently being evaluated by RCAM with attention to the genetic defect, their clinical features, and their use in preclinical studies. To varying degrees, these models have been described at the clinical, pathological, biochemical, and molecular levels confirming homology with human genetic diseases.

LYSOSOMAL STORAGE DISEASES

Alpha-Mannosidosis (AMD) is a rare disorder characterized by a deficiency in the α-D-mannosidase enzyme resulting in the accumulation of undegraded mannose-rich oligosaccharides. Affected individuals may be homozygous or compound heterozygous for mutations in the *MAN2B1* gene, encoding for the lysosomal alpha-mannosidase. The frequency of AMD in humans is estimated to be approximately 1:500,000 worldwide with an autosomal recessive inheritance pattern. Children affected with AMD are usually born without deficits, and in childhood develop delayed developmental milestones, ataxia, facial deformities, skeletal defects, recurrent infections, and hearing deficits. Clinical signs vary from severe forms with pronounced intellectual disability, hydrocephalus, hepatosplenomegaly, and early death, while milder forms include moderate intellectual disability, mild dysostosis, and survival into adulthood. In comparison, the existing cat model presents with an early onset of clinical signs similar to the milder forms of AMD in humans with the absence of prominent skeletal deformities, hepatomegaly, and ocular abnormalities. However, cats develop cerebellar dysfunction including action tremors, loss of balance, nystagmus, and dysmetria [1,2]. There is no cure for AMD and treatments continue to be symptomatic. Promising therapies tested in the cat model include bone marrow transplantation and adeno-associated virus (AAV) mediated gene therapy [3-5]. Hematopoietic stem cell transplant (HSCT) studies in cats identified a reversal of storage vacuoles in the central nervous system (CNS) and peripheral tissues, as well as a slowed progression of cerebellar dysfunction [4]. Gene therapy studies using AAV targeted to the CNS of cats have demonstrated remarkable preliminary results. Injection of AAV carrying a functional copy of the complementary DNA (cDNA) into multiple sites of the feline brain resulted in improvements in neurological dysfunction and increased life span [5]. Alternatively, a single-AAV injection into the cisterna magna also exhibited correction of storage lesions in the CNS of AMD cats, slowed clinical progression of the disease, and extended lifespan of treated animals [3]. The major difference between these gene therapy studies was the extension of neuronal transduction and widespread expression achieved via the cisterna magna injections.

Fucosidosis is a rare disorder characterized by a deficiency in the lysosomal enzyme α-L-fucosidase. Only ~100 cases have been reported worldwide with an estimated incidence of less than 1:200,000 live births, however, the disease is believed to be underdiagnosed making its frequency difficult to determine. Fucosidosis is inherited as an autosomal recessive trait and affected individuals have mutations in the *FUCA1* gene. Children develop signs of delayed motor skills and intellectual disability within the first year of life and gradually develop coarse facial features, skeletal deformities,

Table 1. Large animal models of human genetic diseases at the Referral Center for Animal Models of Human Genetic Disease (RCAM).

Disease*	Defective gene	OMIM	Species	Mutation
		Lysosomal storage disease		
Alpha-mannosidosis*	*MAN2B1*	248500	feline	c.1748delCCAG [129]
Fucosidosis*	*FUCA1*	230000	canine	c.379_392del14bp [130]
Globoid cell leukodystrophy*	*GALC*	245200	canine	c.473A>C [131]
Mucolipidosis II	*GNPTAB*	252500	feline	*unpublished*
Mucopolysaccharidosis I*	*IDUA*	607014	canine, feline	c.155+1G>A [132] c.1107-1109 del [21]
Mucopolysaccharidosis IIIA	*SGSH*	252900	canine	c.708-709insA [133]
Mucopolysaccharidosis IIIB*	*NAGLU*	252920	canine	*unpublished*
Mucopolysaccharidosis VI*	*ARSB*	253200	canine, feline	*unpublished* c.1427T>C [134]
Mucopolysaccharidosis VII*	*GUSB*	253220	canine, feline	c.559G>A [135] c.1074G>A [136]
Niemann Pick Type C1*	*NPC1*	257220	feline	c.2864G>C [137]
		Dermatologic diseases		
Ectodermal dysplasia/Skin fragility syndrome	*PKP1*	604536	canine	c.202+1G>C [138]
X-linked ectodermal dysplasia, hypohidrotic*	*EDA*	305100	canine	c.910-1G>A [95]
Epidermolysis bullosa	*PLEC1*	601975	canine	c.3823G>A [139]
Exfoliative cutaneous lupus erythematous	*SIPA1*	602180	canine	*unpublished*
Ichthyosis	*NIPAL4*	175800	canine	g.52737379delC [140]
Lethal acrodermatitis	unknown	201100	canine	*unknown*
		Cardiovascular diseases		
Juvenile dilated cardiomyopathy*	*MTHFD1*	*n/a*	canine	*unknown*
Tricuspid valve dysplasia	unknown	224700	canine	*unknown*
		Nervous/muscular system diseases		
Epilepsy	*ANK4*	600699	canine	*unknown*
Glycogenosis type VII	*PFKM*	232800	canine	c.222G>A; c.550C>T [141]
Glycogen storage disease IV	*GBE1*	232500	feline	334bp*ins;*6.2kb*del* [142]
X-linked muscular dystrophy*	*DMD*	310200	canine	A>G intron 6 [143]
Fetal-onset neuroaxonal dystrophy	*MFN2*	608507	canine	c.1617_19delGGA [144]
Myotonia congenita	*CLCN1*	118425	canine	c.803C>T [145]
Non-syndromic neuroepithelial deafness	*CHD23*	601386	canine	*unpublished*
		Hematological/immunological system diseases		
Erythrocytic pyruvate kinase deficiency	*PKLR*	266200	canine	c.693+304G>A [146]
Factor VII deficiency	*F7*	227500	canine	c.407G>A [147]
Factor VIII deficiency	*F8*	306700	canine	c.98G>A [148]

Factor IX deficiency	*F9*	306900	canine	*unknown*
Factor XI deficiency	*FXI*	264900	canine	*unpublished*
Iron-refractory iron-deficiency anemia	*TMPRSS6*	206200	canine	*unknown*
Leukocyte adhesion deficiency	*ITGB2*	116920	feline	*unknown*
Porphyrias	*HMBS*	263700 176000	feline	c.189insT; c.842_844delGAG [149]
X-linked severe combined immunodeficiency*	*IL2RG*	300400	canine	c.582_583insC [120]
		Additional diseases		
Congenital hypothyroidism	*TPO*	274500	canine, feline	*unknown* *unknown*
Cystinuria	*SLC3A1* *SLC7A9*	220100	canine	*unknown*
Fibrodysplasia ossificans progressiva	*ACVR1*	102576	feline	*unpublished*
Multiple midline defect syndrome	*unknown*	unknown	feline	*unknown*
Primary ciliary dyskinesia	*unknown*	215518	canine	*unknown*

*Animal models that are currently supported by independent funding and actively producing proof-of-concept and/or preclinical data.

hypotonia, with additional complications such as visceromegaly, recurrent infections, and skin discolorations due to angiokeratomas. Progressive neurological deterioration occurs due to myelin loss in the brain generating seizures, severe intellectual disability, movement disorders, and decerebrate rigidity. Canine fucosidosis also presents as a progressive neurological disorder in which loss of myelin in the earliest myelinating tracts of the CNS is detectable by 8 weeks of age, followed by later maturing tracts such as the corpus callosum around 16 weeks of age, and lastly of the sensory and motor tracts around 8 to 12 months of age [6]. Loss of oligodendrocytes due to down regulation of genes responsible for oligodendrocyte and myelin maturation occur early in the disease course and follow a similar spatiotemporal pattern as seen in hypomyelination [7]. Infiltration of macrophages, widespread vacuolization, and gliosis are also hallmarks of pathology in advanced disease stages. Clinically, dogs present with anxiety, learning delays, ataxia, proprioceptive deficits, and poorly modulated postural adjustments [6].

HSCT and enzyme replacement therapy (ERT) have been evaluated in the canine model of fucosidosis. HSCT prior to the onset of signs resulted in increased levels of α-L-fucosidase, improvement of peripheral nervous system (PNS) and CNS lesions, delay in disease onset, and substantial increase in lifespan. However, transplantation after the onset of clinical signs was not effective [8]. More recently, monthly ERT via direct infusion of enzyme into the cerebrospinal fluid (CSF) via intrathecal (IT) or intracisternal infusion resulted in increased enzyme activity in the spinal cord and most areas of the brain and subsequent substrate reduction [9,10]. Reductions in vacuolation, astrocytosis, microgliosis, vacuoles per neuron, and hypomyelination were found in the cerebral cortex, cerebellum, medulla, and spinal cord; however, neuroaxonal dystrophy and Purkinje cell loss showed no response to treatment [10]. ERT resulted in partial and variable correction of enzyme activity, reduction in storage accumulation, and amelioration of neuropathology.

Globoid Cell Leukodystrophy (GLD) results from a deficiency in the lysosomal enzyme galactosylceramidase (GALC). Deficiency of this enzyme results in the accumulation of galactosylsphingosine (psychosine), which is toxic to oligodendrocytes and Schwann cells. Disease is characterized by diffuse CNS and PNS demyelination. Mutations in the *GALC* gene are inherited in an autosomal recessive pattern. In the United States the incidence is estimated at ~1:100,000, although higher incidence has been identified in smaller communities of Israel (6:1,000). Infants usually present with general irritability, weakness, anorexia, fever of unknown origin, growth abnormalities, and intellectual disability. Difficulty with ambulation, breathing, chewing, and swallowing also occur. The naturally occurring canine model of GLD closely recapitulates the clinical disease progression, neuropathological alterations, and biochemical abnormalities reported in humans [11,12]. Signs in affected dogs include ataxia, tremors, pelvic limb paralysis, and hearing and vision deficits. Diffusion tensor imaging of the canine GLD brain shows substantial

abnormalities in the internal capsule, corona radiata, and corpus callosum [13]. Consistent with imaging findings, histologic evaluation shows severe loss of myelin and globoid cell accumulation in white matter [14]. Brain biochemistry reveals decreased GALC activity and elevated psychosine levels [11].

Allogeneic HSCT to 4-week-old GLD dogs delayed onset of disease by up to 2 years of age [15]. However, all HSCT-treated dogs eventually succumbed to clinical signs of GLD and the effect on survival was variable. AAV gene therapy via a combination of intravenous (IV) and intracerebroventricular injections delayed disease progression, increased survival time, and improved CNS and PNS pathology (Bradbury et al. submitted).

Mucopolysaccharidosis type I (MPSI) is caused by loss of activity of the lysosomal enzyme α-L-iduronidase (IDU) and the disease is characterized by the accumulation of the glycosaminoglycans (GAGs) dermatan and heparan sulfate within cells. Hurler, Hurler-Scheie, and Scheie syndromes are described based on decreasing severity of clinical disease and varying phenotypes. Hurler disease is more commonly identified in patients with a frequency of ~1:100,000 newborns. The more attenuated forms, Scheie and Hurler-Scheie, are less common and estimated to occur in ~1:500,000 newborns.

MPSI has been identified in both the canine and feline species [16,17]. The canine model of MPSI has clinical signs similar to the intermediate form, Hurler-Scheie disease, in humans with common symptoms and signs including stunted growth and facial dysmorphia; joint and bone disease including joint laxity and cervical vertebral disc disease; cardiac disease including thickened valves, aortic dilation, and eccentric hypertrophy, and corneal opacity [17]. CNS abnormalities include storage material in neurons, astrocytes, tissue macrophages, and leptomeninges, as well as defects in myelination [18,19]. The severity of clinical disease in the MPSI dog model varies widely among individual animals and many of the hallmarks of clinical disease in patients are not seen in this model including hepatosplenomegaly, cognitive deficits, joint stiffness, and many of the key features of dysostosis multiplex [20]. Affected MPS1 cats develop facial dysmorphia, corneal clouding, cardiac murmurs, and pelvic limb gait abnormalities. Interestingly, the skeletal malformations are limited to facial changes, hip subluxation, widening of the cervical vertebrate, and pectus excavatum [21]. Dwarfism, common in human MPSI patients, is not observed. Storage lesions in cats are comparable to those identified in the severe form of the human disease, Hurler's disease [22].

HSCT in the dog model showed reduced storage in most tissues, and overall slowed disease progression [20,23]. However, HSCT had minimal effects on storage in chondrocytes and disease progression of the cervical spine [20]. Similar findings were observed in HSCT studies in the MPSI cat [24]. In comparison, ERT studies provided important data for transition of US Food and Drug Administration (FDA)-approved products into clinical use. Initial systemic injections of recombinant human IDU (rhIDU) in both the canine and feline MPSI models revealed weight gain and overall viability improvement, as well as reduction of storage material in multiple peripheral tissues. However, heart valves, cornea, CNS tissue, and articular cartilage were left virtually untreated [25-27]. Of clinical significance, treated animals developed complement-activating antibodies to the rhIDU product. However, the use of immune tolerance protocols based on canine organ transplantation procedures allowed for improved tissue enzyme activity, including hard-to-treat tissues such as the cornea, synovium, and heart valve, decreased lysosomal storage burden, and reduced tissue and urine GAG content [28,29]. Alternatively, IT-rhIDU injections resulted in reduced storage material and histological improvement in brain and meninges, as well as prevention of spinal cord compression, especially when young affected animals were treated before the onset of clinical signs [30-33].

As with ERT, gene therapy studies in the canine model of MPSI also resulted in antibody production against the protein product. Early somatic gene therapy studies using myoblasts and retroviruses attempted tolerance using oral rhIDU, however, an immune response developed and poor to no enzyme expression resulted [34]. Similar findings were identified with trials using autologous stem cells transduced *ex vivo* with a retrovirus [35]. However, IV injections of a gamma-retrovirus (RV) in 2 to 3 day old MPSI dogs resulted in sustained circulating enzyme levels at 28-fold above normal at 1.8 years of age and decreased peripheral clinical severity [36]. Interestingly, these RV-treated MPSI dogs did not mount an immune response to the IDU enzyme. Intracerebral injections of AAV carrying the IDUA transgene and immune tolerance protocols generated therapeutic effects in the brain tissue of treated MPSI dogs with greatly reduced antibody production [37,38]. More recently, liver directed AAV gene therapy in 3 to 5-day-old MPSI dogs induced a persistent state of immune tolerance and allowed for 100-fold lower antibody levels in the CNS following subsequent IT-AAV injections [39]. This finding ultimately allowed for an induction of tolerance to rhIDU in MPSI dogs treated at 5 days of age with an AAV8-liver directed vector expressing human IDU and showcased that immaturity of the immune system was responsible for the blunted immune responses [40]. Similar studies in the cat model have generated complete resolution of cardiac abnormalities via liver-directed gene therapy with an AAV vector, and reduction of histopathological abnormalities within the

brain [41,42].

Mucopolysaccharidosis IIIB (MPSIIIB), also known as Sanfilippo syndrome, results from deficient N-acetyl-α-D-glucosaminidase (NAGLU), another enzyme along the degradation pathway for heparan sulfate (HS). MPSIIIB is estimated to occur in ~1:200,000 new births. Affected children are born without deficits yet gradually develop mildly coarse facial features, macrocephaly, hepatomegaly, skeletal abnormalities, hernias, gastrointestinal disorders, and recurrent infections. The onset of CNS signs occurs in adolescence and includes delayed speech and behavior problems. Children may become restless, destructive, aggressive, anxious, and often have difficulty with social interaction. Cognitive development usually peaks at 3 to 6 years of age but gradually declines after this time. The naturally occurring canine model was identified in Schipperke dogs [43]. Clinical findings in the dog included severe cerebellar disease, dystrophic corneas, and retinal degeneration. Biochemical analysis identified HS in urine samples and residual NAGLU activity in fibroblasts (~4 and 9 percent) [43]. Gross examination revealed cerebellar atrophy and histopathology identified cytoplasmic vacuolation in neurons and perithelial cells of the CNS.

Although a phase 1/2 safety and efficacy study of IV-ERT for MPSIIIB is being conducted (NCT02324049), studies in animal models have identified poor blood-brain-barrier penetration of enzyme after IV-ERT leading to the development of strategies that target brain delivery [44]. One such study is currently recruiting for delivery of a modified recombinant human NAGLU via a reservoir placed in the cerebral ventricles of MPSIIIB patients (NCT02754076). Studies in the MPSIIIB animal models have supported phase 1/2 clinical trials of intracerebral AAV gene therapy to treat brain disease (ISRCTN19853672). Data supporting this trial were generated in the canine model where direct brain injections of an AAV.NAGLU vector indicated a reduction in biochemical markers of disease (GM2/3 gangliosides and GAGs) and vacuolation, as well as an increase in enzyme activity in brain tissue [38]. However, regions of the brain not targeted were still diseased and pathology of the cerebellum and periphery was not alleviated.

Mucopolysaccharidosis VI (MPSVI) is an autosomal recessive disease caused by a deficiency of arylsulfatase B (ARSB) leading to the lysosomal accumulation of dermatan sulfate. Exact frequency of disease is unknown but is estimated to occur in 1:250,000 to 600,000 newborns. Referred to as Maroteaux-Lamy syndrome in human patients, the disease is somatic with no CNS dysfunction. However, abnormal neuronal metabolism and storage was identified in the animal model [45]. Infants generally do not display signs at birth, however, by early childhood they gradually develop coarse facial features, an enlarged head, hydrocephalus, organomegaly, heart disorder due to excessive storage in the valvular leaflets, hernias, chronic upper respiratory infections, sleep apnea, corneal clouding, and hearing loss. Skeletal deformities are often obvious and include shortened stature, dysostosis multiplex, and cervical spinal stenosis. Intelligence is generally not affected, however, reports of cognitive impairment are not uncommon. Life expectancy often depends on severity of the disease, yet heart disease and airway obstructions are a common cause of death. Both canine and feline models of MPSVI have been identified [46,47]. Facial dysmorphia, including a small head, broad shortened ears, and bilateral corneal clouding; cardiac abnormalities, involving valvular thickening and aortic dilation; progressive skeletal deformities, including epiphyseal dysplasia of long bones and cervical spine, bilateral hip subluxations, pectus excavatum, and odontoid hypoplasia, as well as locomotor difficulties and spinal cord compressions occur [46-49]. The majority of tissues are grossly normal and histology reveals storage material mainly in the cytoplasm of connective tissue and leukocytes [50].

The feline model has been used extensively for therapeutic studies. Early HSCT indicated improvement in systemic storage abnormalities, reduced urine GAG excretion, as well as a reduction in osteopenia and reduced long bone deformities [51-53]. Weekly infusions of high-dose recombinant human N-acetylgalactosamine-4-sulfatase (rh4S) were found to alleviate systemic storage with exceptions in corneas and cartilage. Joint changes and spinal cord compressions persisted [54,55]. However, early treatment before the onset of skeletal malformations and intra-articular injections were found to alleviate many of the pathological changes in bone and joints respectively [55,56]. Although CNS abnormalities are not a hallmark of MPSVI, IT injections of rh4S in the cat model were found to prevent pelvic limb paralysis and storage in the meninges [56,57]. The identification that high levels of circulating enzyme were necessary to gain therapeutic efficacy led to successful studies in animal models with vector mediated gene therapy. Systemic injections of RV or AAV vectors encoding for ARSB led to expression of high circulating enzyme via liver transduction, reduced GAG storage in tissues, increased long bone length, and improved spontaneous mobility [58-60].

Mucopolysaccharidosis VII (MPSVII), also known as Sly syndrome in humans, results from mutations in the gene that encodes for the lysosomal hydrolase β-glucuronidase (*GUSB*). The incidence is estimated at ~1: 250,000 newborns. The resultant storage products are chondroitin 4- and 6-sulfates, heparan sulfate, and dermatan sulfates. Patients develop facial dysmorphia;

corneal clouding due to storage in stromal keratocytes; hepatomegaly; moderate to severe skeletal disease including, growth retardation, epiphyseal dysplasia, joint swelling and laxity, bilateral hip subluxation or complete luxation, angular deformities of the ribs, luxating patellae, progressive pelvic limb weakness, and vertebral malformations; tracheal hypoplasia; and variable cardiac abnormalities including thickened aortic valves and mitral insufficiency. Intelligence is usually normal yet progressive intellectual decline has been noted. Patients with mild symptoms often live well into the second decade of life; however, life expectancy is generally reduced due to chronic upper respiratory infections, intestinal abnormalities, and neurodegenerative complications. Spontaneous models of the disease have been identified in both dogs and cats, which faithfully recapitulate many of the clinical signs seen in human patients [61,62]. Biochemical analysis indicates residual tissue activity ranging from 0.2 to 1.7 percent normal, serum activity at ~6.4 percent normal, and urinary GAGs consisting of chondroitin and dermatan sulfates [62,63]. Histological studies in both models shows storage vesicles in cells of all tissues examined including sphingolipid storage in ganglion cells of the brain [61,62]. Targeted studies in the canine model helped identify novel therapeutic targets in hard to treat tissues such as heart and bone, as well as biomarkers for therapy trials [64-66].

HSCT studies in the dog model reported minimal therapeutic efficacy, but cardiac abnormalities appeared to be reduced and secondary enzymes and GAG levels were normalized in the aorta and myocardium [67]. Initial somatic gene therapy using RV-transduced fibroblast based neo-organs identified normalized liver GAG with < 2 percent normal enzyme activity, indicating that a small amount of enzyme can have therapeutic effects in some tissues [68]. This initial finding was supported when proof of concept gene therapy studies with retrovirus vectors expressing canine GUSB (cGUSB) were IV injected into neonatal MPSVII dogs. Liver and spleen were transduced in treated dogs, however, enzyme activity was not identified in any other organs [69]. Long-term studies reported that treated animals could run, albeit with a stiff gate, had reduced corneal clouding, and no mitral valve thickening after 17 months of treatment [70]. Tissues that were more difficult to treat, such as bone and heart, indicated moderate alleviation of disease pathology and there were improvements in facial dysmorphia and decreased storage lesions in osteocytes and synovium, but not chondrocytes [70]. Partial improvements of qualitative abnormalities in long bones were reported, as well as improvements in mitral and aortic valve thickening [71-73]. Therapeutic efficacy after more than a decade of therapy in RV-treated dogs revealed amelioration of cardiac disease, a substantial increase in the overall quality of life, but only mild to moderate alleviation of bone and joint pathology [74,75]. CNS abnormalities were not addressed with the RV-studies. Exploiting the natural tendency for canine adenovirus serotype 2 (CAV-2) to transfect neuronal cultures, direct brain injections of a CAV-2 vector encoding for human GUSB were tested in the MPSVII dog model. Although GUSB levels were elevated and GAG storage was reduced within injected regions, immune responses to the CAV-vector necessitated implementation of immune suppression protocols [76]. In contrast, IT-AAV studies allowed for widespread expression of cGUSB in the brains of treated dogs, reduced primary and secondary storage material, normalized other lysosomal enzymes, and had minimal antibody responses when injected into the CSF [77]. All of these studies support the advance of gene therapy into the clinic for the systemic and CNS disease manifestations of MPSVII. Recently, a phase 3 clinical trial for ERT in MPSVII patients is ongoing testing the compound UX003, a recombinant human β-glucuronidase (NCT02230566).

Niemann-Pick Disease, Type C1 (NPC1) is a neurovisceral lysosomal storage disease caused by mutations in the *NPC1* gene, leading to an increase in unesterified cholesterol and glycosphingolipids that results in hepatic and progressive neurological disease. The disease has been estimated to affect ~1:150,000 individuals. Clinically, the feline model shares multiple features of the juvenile-onset form found in humans, including hepatomegaly with elevated liver enzymes, pulmonary complications due to surfactant abnormalities, and cerebellar and vestibular disease [78-81]. Histological analysis of the CNS of affected cats revealed substrate accumulation in neurons, gliosis, neuronal necrosis, axonal spheroids, severe loss of Purkinje cells, myelination abnormalities, accumulation of gangliosides (GM2 and GM3), meganeurite formation, and abnormal dendritogenesis [79,81-83]. Systemic malformations included enlarged livers with extensive vacuolation in hepatocytes and Kuppfer cells, foamy macrophages in the spleen, lymph nodes, and lung, and membranous inclusions in all affected tissues and organs examined [78,79]. Studies of the PNS in affected cats indicated decreased motor and sensory nerve conduction velocities with histological changes including reduction in fiber and axon diameters, decreased myelin thickness, thin myelin sheaths, and lipid storage in Schwann cells [84]. Lipid analysis of storage material revealed a complicated mixture of cholesterol, glucosylceramide, lactosylceramide, and sphingomyelin [78]. The feline model has been critical for identifying biomarkers that can be used in the clinical setting as an outcome of therapeutic studies [85-87]. Interestingly, studies in the feline model

also identified the potential for heterozygote carriers to have partial storage disease due to the identification of intermediate levels of unesterified cholesterol storage in the liver and brains of heterozygous NPC1 cats [88]. Abnormalities in the metabolism of amyloid-β, a peptide amyloid associated with Alzheimer disease in humans, were found in the NPC1 cat [89].

The only therapy currently approved for use in some countries focuses on the inhibition of glucosylceramide synthase, which is involved in the production of glycosphingolipids. N-butyl-deoxynojirimycin (or Miglustat) given daily to 3-day-old affected NPC1 cats delayed the onset of neurological disease, increased the lifespan of treated cats, reduced GM2 ganglioside storage, and increased Purkinje cell survival [90]. Although a phase 2 trial was completed in the United States for Miglustat, an approved therapy for type 1 Gaucher disease, the FDA withheld approval for use of the drug in NPC1 patients (NCT00517153). Cholesterol reduction using the small molecule 2-hydroxypropyl-β-cyclodextrin (HPβCD) is currently going through clinical testing for approval. This molecule was found to ameliorate hepatic disease when given subcutaneously to affected cats, however pulmonary toxicity was observed when doses necessary to treat CNS disease were given [91]. Conversely, direct injections of HPβCD into the CSF of pre-symptomatic NPC1 cats prevented the onset of cerebellar dysfunction for more than one year, reduced Purkinje cell loss, and generated near normal levels of cholesterol and sphingolipids in the CNS [91]. Unfortunately, a dose-dependent ototoxicity was observed in treated cats leading to the recommendation of auditory testing in patients receiving the therapy [91]. The NPC1 cat model has accelerated the translation of a promising finding in the NPC1 mouse model to children by providing critical information on route of delivery, scaling of dose, and adverse events that would not have been feasible using the mouse model [91,92]. Notably, this work led to the development of a phase 1/2a trial of HPβCD (NCT01747135) and also supported the initiation of a multinational phase 2b/3 clinical efficacy trial that has been approved by both the FDA and EMA (NCT02534844).

DERMATOLOGICAL DISEASES

X-linked Hypohidrotic Ectodermal Dysplasia (XLHED) is a common form of ectodermal dysplasia with a frequency of ~1:17,000 people worldwide. Abnormal development of skin, hair, nails, teeth, and sweat glands occurs *in utero* causing sparse hair, an inability to sweat, decreased lacrimation, frequent pulmonary infections, and missing and malformed teeth in affected individuals. The dog model recapitulates the clinical spectrum with the addition of keratoconjunctivitis sicca and frequent pneumonias due to a lack of respiratory serous glands [93]. A colony has been created from a German shepherd background [94]. Genotyping of the dog model indicated a splice acceptor site variant generating a truncation of both isoforms of ectodysplasin A, EDA1 and EDA2, rendering the resultant proteins inactive [95]. While clinical treatments remain symptomatic, studies in the dog model using a recombinant ectodysplasin A (Fc:EDA) have generated positive data. Intravenous injections of Fc:EDA into neonatal XLHED dogs resulted in therapeutic efficacy via weight gain, restoration of lacrimation, increased sweat gland function, improved mucociliary clearance, and reduced pulmonary disease [96,97]. Unfortunately, alopecia and hypochondrosis could not be corrected in the postnatal period. The results of these experiments were submitted to the FDA and supported phase 1/2 clinical trials in children with XLHED (NCT01564225; NCT01775462).

MUSCULAR SYSTEM DISEASES

Canine X-linked Muscular Dystrophy falls under the umbrella of muscular dystrophy disorders that ultimately lead to the weakening and breakdown of muscle fibers mainly due to defects in the dystrophin protein. Duchenne Muscular Dystrophy (DMD) is a common disorder estimated to affect 1:3,500 to 5,000 newborn males worldwide in an X-linked inheritance pattern. Muscular dystrophy was initially identified in a young golden retriever (GRMD) which is currently the most clinically relevant model of DMD in humans [98]. Similar to inheritance patterns in human patients, dystrophin-deficient dogs were also confirmed to have a X-linked inheritance pattern as well as heterogeneity within the clinical disease spectrum [99]. The model recapitulates human disease with early onset muscle weakness, selective muscle atrophy and hypertrophy, a steady progression of limb splaying, stiffness of gait, and elevated serum creatine kinase [100]. Morphological analysis of muscle fibers revealed increased hypercontraction and segmental necrosis of muscle fibers with phagocytosis and regeneration leading to fatty infiltrate and fibrosis [100]. Cardiac abnormalities including left apical systolic murmurs, dilated cardiomyopathy, myocardial degeneration and calcification, congestive heart failure in aged GRMD, and decreased cardiac contractility were commonly identified [98]. An enlarged tongue base is commonly seen in male patients and dogs alike leading to excessive drooling and dysphagia, however esophageal complications and regurgitation are unique to the dog model due to anatomical differences among species [98]. Interestingly, unlike human patients, a loss of respiratory function is not seen in the dog model and

is suspected to stem from biomechanical differences among species instead of genetics [98].

Ongoing therapeutic studies in animal models for DMD include plasmid- and vector-based gene therapy, gene repair, cell-based therapies, and pharmacologics. Direct muscle injections with a human dystrophin encoding plasmid generated few dystrophin positive cells while electrotransfer of plasmid encoding for both full length and micro-dystrophin generated limited expression and elevated cell infiltrates [101,102]. Although significantly more expression was acquired with adenovirus vectors, cellular immune responses to transgene and vector alike rendered the expression short lived [103,104]. AAV-vectors have been popular for their reduced immune responses yet initial intramuscular (IM) injections of AAV into dogs generated a primary cellular response against transgene and capsid proteins [105]. Indeed, most IM-AAV studies report using an immune tolerance regime to gain long-term expression in muscle [106-109]. To circumvent this issue, one study used systemic AAV injections in neonate GRMD dogs; although they achieved robust skeletal muscle transduction using a micro-dystrophin transgene, and no immune suppression protocol, they encountered growth delays, muscle contractions and atrophy [110]. Conversely, the use of transient or sustained immune suppression combined with systemic injections of a tyrosine-engineered AAV capsid in 2-month-old GRMD dogs allowed for widespread expression of micro-dystrophin [111]. These preclinical studies supported clinical trials using AAV vectors (NCT00428935, NTC02376816).

Multi-exon skipping using antisense oligonucleotides to restore the reading frame and produce shorter but functional dystrophin proteins has generated some of the most promising data to date. Delivery of these small nuclear RNAs in AAVs, or AAV-U7, to muscle has shown sustained correction and partial recovery of muscle strength [112]. Similar strategies led to dystrophin expression for 13 months in cardiac tissue and improved cardiac function [113]. Recently, AAV-U7 in the dog model has generated safety and efficacy data for preclinical studies via a dose-dependent response, which increased dystrophin expression and decreased pathology in treated skeletal muscle [114,115]. Similar strategies are in phase 1/2 trials for exon 45 (NTC02530905) and exon 53 skipping (NTC02310906). Various compounds, including morpholinos, have been mixed with exon-skipping antisense oligomers, termed "cocktails," and directly injected through various routes in the dog model. These studies have generated positive data including up to 50 percent normal dystrophin expression, restoration of dystrophin associated proteins, diminished muscle inflammation, and increased physical stamina [116-118]. Extension of these compounds to human patient cells have also revealed exon skipping efficacy and dystrophin protein recovery [119].

HEMATOLOGICAL AND IMMUNOLOGICAL SYSTEM DISEASES

X-Linked Severe Combined Immunodeficiency (XSCID) in humans is associated with mutations in the gene for the gamma chain of the interleukin-2 receptor (IL-2Rγ). Affected children lack both humoral and cell-mediated immunity, and without treatment the disease is lethal within the first few years of life. Two different spontaneous mutations have been identified in the dog that result in true clinical, pathological, and immunological models of XSCID in human patients [120,121].

HSCT without pre-transplant conditioning was established as a standard of care in human XSCID patients. While HSCT resulted in engraftment of donor T cells and reconstitution of T cell function, there was insignificant engraftment of donor B cells and poor reconstitution of humoral immune function. HSCT was evaluated in XSCID dogs that underwent transplantation without pre-transplant conditioning between 2 and 3 weeks of age using a normal littermate as the bone marrow donor. Recipient dogs received untreated nucleated bone marrow cells administered intravenously at a dose of 1.0 to 1.5 × 10^8 nucleated cells/kg. In contrast to human XSCID patients, XSCID dogs demonstrated 100 percent donor derived circulating T cells, 20 to 50 percent donor derived circulating B cells, and attained full reconstitution of immunologic function [122,123]. While there was a decline in the complexity of T cell diversity seen with time post-transplant, XSCID dogs were capable of achieving long-term survival to > 10 years of age [124].

Use of genetically enhanced autologous hematopoietic stem cells can serve as an alternative to traditional HSCT when a donor match is not available and to alleviate risks associated with graft-versus-host disease. In order to evaluate this approach, twelve XSCID dogs were treated by *ex vivo* γ-retroviral gene therapy. Eight of 12 XSCID dogs successfully engrafted showing a steady increase in the proportion of gene-corrected T cells between weeks 2 and 8 post-treatment, while four of the treated dogs failed to engraft. Gene-corrected naïve T cells were initially normal at first, but gradually declined and none of the dogs survived beyond 11 months post-treatment [125].

In vivo retroviral gene therapy by direct intravenous delivery was also evaluated in XSCID dogs. Intravenous injection of a RD114-pseudotyped retrovirus vector demonstrated viral expression in peripheral blood lymphocytes 3 weeks after injection, which continued to

increase up to 85 percent gene-corrected T lymphocytes 8 weeks after treatment. Two of the four treated dogs survived long-term and at 16 and 18 months showed sustained T cell correction, up to 26 percent gene-corrected B cells, vector presence in myeloid lineages, and normalized immune function [126]. In contrast to the *ex vivo* gene therapy-treated dogs, the *in vivo* gene therapy-treated dogs showed sustained gene marking in T cells, B cells, and myeloid cells for four years following treatment [127]. Studies using a RD114-pseudotyped simian immunodeficiency viral vector generated similar results as these dogs exhibited B cell chimerism and prolonged gene-correction of myeloid cells. The gene-correction of T cells in lentiviral treated dogs was sustained for up to 4½ years [127]. Most recently, *in vivo* gene therapy utilizing a foamy virus vector was evaluated in XSCID dogs. All treated dogs exhibited gene-corrected lymphocytes as early as 2 weeks after injection and continued to expand for 12 weeks. Four out of five dogs showed normal level of gene-corrected lymphocytes and functional immune reconstitution; however, infectious complications limited the duration of follow-up. Survival in XSCID dogs treated with intravenous delivery of foamy virus was highly variable ranging from 3 to 10.5 months of age [128]. *Ex vivo* gene therapy trials using retrovirus-transduced autologous CD34+ hematopoietic stem cells in XSCID patients were evaluated in multiple clinical trials (NCT00028236, NCT01410019, NCT01410019, NCT01175239, NCT01129544). All of these trials reported increased immune function and reduced infections among treated XSCID patients. In addition to the aforementioned clinical trials, two clinical trials evaluating *ex vivo* lentiviral gene therapy are also underway and actively recruiting (NCT01512888, NCT01306019).

CONCLUSIONS

For many lethal or debilitating genetic disorders in patients there are no satisfactory therapies due to a lack of clinically relevant animal models that faithfully recapitulate human genetic disease, the relatively low incidence and high heterogeneity of these diseases that limit natural history studies, an insufficient understanding of how the genetic defect results in the phenotypic abnormalities, the paucity of validated surrogate markers that can be monitored as secondary clinical endpoints, and the dearth of potential therapies which substantially improve disease in animal models. Spontaneous mutations that give rise to genetic disease are often found to occur in the pet population and veterinary services identify these diseases as animals are brought into the clinical setting for evaluation. Collaborative efforts among veterinarians, scientists, and breeders have allowed for the discovery and understanding of disease in naturally occurring hereditary disorders in dogs and cats that are orthologous to those found in human patients. Dissemination of these models to the scientific community through breeding colonies has allowed for the discovery of disease mechanisms, generation of non-invasive biomarkers for clinical evaluation during therapeutic trials, development of drug delivery and intervention protocols, safety, efficacy, and dosing studies of novel and off-label therapies, and the eventual approval of clinical therapies for many rare and devastating diseases.

For more information on the resources available through RCAM, please visit www.vet.upenn.edu/rcam.

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