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REVIEW

Gene therapies in canine models for Duchenne muscular dystrophy

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Abstract
Therapies for Duchenne muscular dystrophy (DMD) must first be tested in animal models to determine proof-of-concept, efficacy, and importantly, safety. The murine and canine models for DMD are genetically homologous and most commonly used in pre-clinical testing. Although the mouse is a strong, proof-of-concept model, affected dogs show more analogous clinical and immunological disease progression compared to boys with DMD. As such, evaluating genetic therapies in the canine models may better predict response at the genetic, phenotypic, and immunological levels. We review the use of canine models for DMD and their benefits as it pertains to genetic therapy studies, including gene replacement, exon skipping, and gene editing.

Introduction

Duchenne muscular dystrophy (DMD) is an X chromosome-linked, neuromuscular disease characterized by progressive muscle weakness and wasting (Hoffman et al. 1987). 1/3500 to 1/5000 male babies born will have a mutation in the *DMD* gene with symptoms of muscle weakness by about 5 years of age. Patients will ultimately lose the ability to walk and be wheel chair dependent between the ages of 7–16 years. Because this is an X-linked condition, females are rarely found to have DMD.

The *DMD* gene is the largest in the human genome, comprising 2.4 million base pairs (bp). The 79 exons encode the rod-like dystrophin protein weighing 427 kiloDaltons (kDa) and is encompassed by an N-terminal, actin-binding domain (exons 2–8); 4 hinge regions (exons 8 and 9; 17; 50 and 51; and 61–64); 24 spectrin-like repeats in a large rod domain (exons 9–41); an nNos-binding domain (exons 42–45); a dystroglycan-binding domain (exons 63–70); and a C-terminal domain consisting of syntrophin isoform-binding sites (exons 73–75) (Fletcher et al. 2010). Dystrophin is thought to buttress the cell membrane while linking the F-actin cytoskeleton to the sarcolemma. In its absence, muscle is prone to tearing and increased intracellular calcium perturbations, leading to bouts of necrosis and cell death, with subsequent regeneration due to activation of satellite cells (Dowling et al. 2004). Ultimately, regeneration will halt due at least partly to satellite cell senescence, leading to muscle tissue replacement with fibrofatty infiltration.

The *DMD* gene is prone to mutations compared to the rest of the genome (1×10^4 versus 1×10^5–1×10^6) (Nachman 2004). Many types of *DMD* mutations have been characterized, with deletions of one or more exons occurring most frequently, accounting for 68–72% of those reported (Aartsma-Rus et al. 2006; Bladen et al. 2015). Smaller deletions, insertions, point mutations, and duplications make up the remainder. Mutations typically occur in hotspot areas, the primary one between exons 45 and 53 and secondary between exons 2 and 20. Frame-disrupting mutations lead to minimal to no dystrophin expression and the Duchenne phenotype. A truncated dystrophin protein can be expressed when the reading frame remains intact, leading to a less severe phenotype in Becker muscular dystrophy patients (Monaco et al. 1988). There are exceptions to the reading frame rule (Aartsma-Rus et al. 2006).

Diagnosis is suspected based on characteristic symptoms, increased creatine kinase serum assays, muscle biopsies to detect dystrophin protein expression, and eventually studying the transcript. Multiplex ligation-dependent probe amplification combined with Sanger sequencing is frequently used to characterize the *DMD* mutation (Grimm et al. 2012). Corticosteroids remain the gold standard of care for DMD boys. A daily regimen is most efficacious, temporarily improving

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muscle strength and delaying the loss of ambulation by an average of 2–3 years (Matthews et al. 2016). Supportive and palliative care for respiratory and cardiac abnormalities help prolong the life span of affected boys. Recent advances in genetic therapies have been made possible by initially testing in the murine (mdx) and canine models for DMD.

The mdx mouse was first discovered as a naturally occurring mutation in a colony of research mice (Bulfield et al. 1984 and; Sicinski et al. 1989). Since then, mdx mice have been the most commonly used animal model to determine proof-of-concept for various therapies (Kornegay et al. 2014). The mouse model has certain drawbacks, including a very mild phenotype that requires treadmill exercise to worsen histology.

On the other hand, the canine model for Duchenne, specifically, the golden retriever muscular dystrophy (GRMD) dog, closely recapitulates the DMD phenotype (Kornegay 2017). Affected dogs have a mutation in the acceptor splice site of intron 6, resulting in exon 7 skipping, a stop codon in exon 8, and subsequently minimal to no dystrophin protein expressed (Schatzberg et al. 1999). The frame-shifting mutation leads to a progressive phenotype. Affected dogs show signs of muscle weakness in the neonatal period, requiring supportive care for the first 2–3 weeks of life (Kornegay et al. 1988). They have progressive weakness and joint contractures, but tend to stabilize somewhat after 6 months of age.

Epidemiologic studies have shown that the first year of a golden retriever's life is analogous to the first 20 years of a human (Patronek et al. 1997). With this in mind, the GRMD phenotype can be subdivided into quadrants, with the first 3 months of age analogous to the first 5 years of a boy with DMD. The next 3 months, from 3 to 6 months of age, correspond to 5–10 years of age for a child with Duchenne. Experimental therapies in GRMD are typically assessed over the 3- to 6-month period. Functional analyses to evaluate therapies in GRMD have been tested and perfected throughout the years (Kornegay et al. 2012). A number of tests have been assessed, including twitch and tetanic flexor and extensor force; eccentric contraction decrement (ECD); joint angle measurements of the pelvic limb; 6-min walk testing; skeletal muscle and cardiac magnetic resonance imaging (MRI); echo- and electrocardiography; respiratory function; and positron emission tomography-computed tomography (PET-CT) (Fan et al. 2014; Acosta et al. 2016; Schneider et al. 2018).

With regard to pre-clinical therapeutic trials, a review from 2009 detailed gene therapy in large animal models for DMD (Wang et al. 2009). Since this time, several exciting advancements have been achieved in the research community. In this current review, we will discuss these advancements pertaining to the three types of gene therapies and how the canine model has provided significant pre-clinical data for inclusion in human clinical trial applications. These include gene replacement, exon skipping, and gene editing (Table 1).

Gene replacement

The National Institutes of Health's definition of gene therapy is an "experimental technique" that uses genes to treat or prevent disease (https://ghr.nlm.nih.gov/primer/therapy/genetherapy). Although the term, "experimental technique" may be slightly outdated, there are now several FDA-approved genetic therapies for conditions such as retinal dystrophy, B-cell acute lymphoblastic leukemia, and refractory large B-cell lymphoma (https://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/ucm581222.htm). Both the NIH and FDA describe several approaches to gene therapy, including replacing a mutated gene (leading to disease) with a healthy gene copy; inactivating (knocking out) a mutated gene that is behaving improperly; or introducing a new gene to help fight a certain disease. Most commonly used vectors for therapeutic delivery of genetic material are recombinant viruses, including adeno-, lenti-, retro-, and adeno-associated viruses (AAV), with the latter most commonly used in diseases of skeletal muscle (Wirth et al. 2013).

Gene replacement therapy can be hypothetically administered to any boy with DMD regardless of his *DMD* mutation. Ideally, one would deliver the entire 14 kilobase (kb) *DMD*

Table 1 Genetic therapies evaluated in CXMD dogs

	Gene replacement	Exon skipping	Gene editing
Canine model	GRMD	$CXMD_J$	GRMD, ΔE50
Frequency of administration	Single	Multiple	Single
Dosage	1×10^{12}–6.94×10^{14} vg/kg BW	100–400 mg/kg (morpholino); 12 mg/kg (PPMO)	1×10^{14} vectors/kg BW
Functional studies	MRI, muscle force, ECD	ECG	Muscle force, ECD (GRMD)
Reported side effects	Neonate: delayed growth, pelvic limb atrophy, joint contractures, worsening histology	None reported	None reported

cDNA. However, due to packaging limitations (~4.5 kb) AAV, only the most functional regions of the *DMD* gene are included in the construct. That said, the sizes of the actin, nNOS, dystroglycan, and syntrophin-binding domains are approximately 800, 700, 1 kb, and 225 bp in length, respectively. The corresponding bp length of hinges 1, 2, 3, and 4 are 311, 176, 342, and 277 bp. Regarding vectors, a suitable promoter to drive dystrophin expression is required (typically creatine kinase, myosin heavy chain, others), taking up additional packaging space. Finally, inverted terminal repeats and a poly-A tail should be included for proper function. Taken together, dystrophin functional domains and other structures must be carefully selected.

The concept of mini-dystrophin therapies is predicated on the observation that in-frame *DMD* gene mutations that allow translation of truncated dystrophin proteins cause a less severe Becker muscular dystrophy (Ramos and Chamberlain 2015). Providing proof of concept, injection of a recombinant adenovirus carrying a 6.3 kb Becker cDNA in mdx mice led to widespread sarcolemmal expression of the dystrophin transgene (Ragot et al. 1993) and reduced ECD (Deconinck et al. 1996). Use of viral vectors to carry transgenes is complicated by the potential for an immune response to either viral capsid antigens or the transgene. In this context, another study of mdx mice treated with adenovirus-mini-dystrophin construct showed that protein expression declined by ~50% (Gilbert et al. 2003). This decline was presumed to occur due to an immune response to the transgene mediated through antigen-presenting dendritic cells (Jooss et al. 1998). Adeno-associated viral vectors failed to transduce dendritic cells in mice, which led to using this construct to introduce reporter transgenes and, ultimately, mini-dystrophins (Xiao et al. 1996). Subsequent studies of systemic AAV-mediated mini-dystrophin therapy in the mdx mouse model have demonstrated relatively long-term dystrophin expression and functional benefit (Gregorevic et al. 2008).

With regard to large animal models, Howell et al. injected an adenoviral, human mini-dystrophin gene into canine muscle, showing modest expression in 2.5% of myofibers (Howell et al. 1998a). A follow-up study by the same lab injected a replication-deficient, adenoviral, human mini-dystrophin construct into the tibialis anterior muscle of 2-day-old GRMD dogs. One group received immunosuppression while another did not. Muscle biopsies taken 10 days post-injection showed 15.8% of myofibers to be dystrophin-positive (dys+). However, in non-immunosuppressed GRMD dogs, an immune response was observed to the viral and transgene antigens, leading to a reduction in dystrophin expression after 2 months (Howell et al. 1998b).

Delivery of serotypes AAV2 and AAV6 intramuscularly to normal-genotyped dogs led to a significant T-cell-mediated immune response to the viral capsid antigens (Wang et al. 2007a). Follow-up AAV6 studies in GRMD dogs included immunosuppression (Wang et al. 2007b). A vector dosage of 1×10^{11}–1×10^{12} vector genome (vg)/kilogram (kg) of body weight (BW) was delivered intramuscularly and the authors determined that immunosuppression led to a reduced T-cell response. However, when the immunosuppression was withdrawn, the T-cell response returned followed by a reduction in AAV gene expression. The same authors improved the μ-dystrophin design and instituted a stronger immunosuppressive regimen, leading to long-term dystrophin restoration in GRMD dogs (Wang et al. 2007b).

Another GRMD study from our laboratory evaluated systemic AAV-9 μ-dystrophin therapy in three neonatal GRMD dogs (Kornegay et al. 2010). A vector dosage of 1.5×10^{14} vg/kg BW was used without immunosuppression. Variable dystrophin, ranging from 15 to 100% that of normal, was observed in skeletal muscles (Fig. 1). However, some treated dogs displayed delayed growth, pelvic limb muscle atrophy, joint contractures, and worsening

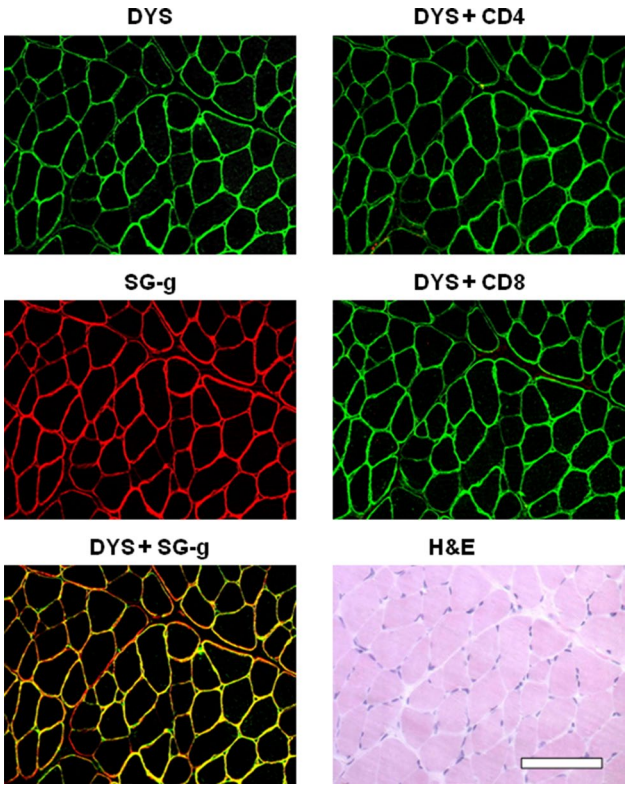

Fig. 1 Lack of immune infiltration and dystrophic lesions in muscles with uniform human mini-dystrophin expression 16 weeks after intravenous injection of AAV9-CMV-mini-dystrophin vector in a GRMD dog at 4 days of age. The long head of the triceps muscle was cryothin sectioned. Consecutive sections were stained with H&E and antibodies against mini-dystrophin (DYS, green color), sarcoglycan-γ (SG-γ, red color), CD4 (red color), and CD8 (red color) T cells. Note the lack of T-cell infiltration and dystrophic lesions in the muscle. Bar = 150 μm. *H&E* hematoxylin and eosin. Figure and legend were previously published in Kornegay et al. (2010) (Reference #32)

histopathology several weeks after therapy. We attributed the worsening of phenotype to the use of a CMV promoter, higher vector dosage, and a human-specific dystrophin cassette. A follow-up study from another group assessed three GRMD dogs that received AAV-9 canine μ-dystrophin at dosages of $1.96–6.24 \times 10^{14}$ vg/kg BW plus an immunosuppression regimen. These dogs showed variable dystrophin levels without adverse effects (Yue et al. 2015).

A long-term systemic study using a recombinant (r) AAV2/8 vector expressing a canine μ-dystrophin without immunosuppression restored and sustained dystrophin levels and improved the clinical phenotype for up to 2 years (Le Guiner et al. 2017). A dosage range of $2 \times 10^{13}–1 \times 10^{14}$ vg/kg BW was administered, resulting in 20–80% dys+ fibers and, in some muscles, up to 50% of μ-dystrophin detection with western blot compared to normal. Treated dogs had improved clinical scores and gait quality. No toxicity or adverse immune response was reported.

We recently completed a dose escalation study administering AAV9 canine μ-dystrophin to three groups of three GRMD dogs each (1×10^{13}, 1×10^{14}, and 2×10^{14} vg/kg BW) and an additional control group (Birch et al. 2017). A regimen of prednisone was instituted a week before treatment and continued for 4 weeks, for a total of 5 weeks. We observed dose-dependent increases in dystrophin expression with the strongest expression in the highest dosage group. Tetanic force increased after 45 and 90 days of treatment. There were no noticeable adverse events attributed to the AAV vector.

To date, there are three clinical trials in DMD evaluating μ-dystrophin replacement therapy (Pfizer/Bamboo, Solid Biosciences, and Sarepta Therapeutics). The GRMD dog model was utilized at the pre-clinical level for the Pfizer/Bamboo and Solid drug applications. Although human studies are ongoing, results appear encouraging thus far (Table 2). Long-term studies in GRMD dogs should be considered to determine if re-dosage of the μ-dystrophin construct will be necessary and, if so, whether it will be tolerated from an immunological standpoint.

Exon skipping

Frame-shifting *DMD* mutations that lead to minimal to no dystrophin expression and a severe clinical phenotype can potentially be managed therapeutically through exon skipping to reestablish the reading frame. This approach is sequence specific and is particularly appealing for a subset of mutations amenable to exon 51 skipping (e.g., mutations in exons 45–52). Since the majority of *DMD* mutations are deletions of one or more exons, single exon skipping can be applicable to 50–64% of DMD patients, while multi-exon skipping may be applicable to 90% of deletions, 80% of duplications, and 98% of nonsense mutations (Yokota et al. 2007; Echigoya and Yokota 2014; Aoki et al. 2012).

Exon skipping is achieved by anti-sense oligonucleotides (AOs), which are synthetic, non-protein-coding RNAs that are complementary to the mutated region of interest (Echigoya et al. 2015). These AOs are 8–30 bp in length (depending on AO type) and typically act as molecular 'pastors', blocking splicing or translation mechanisms, ultimately resulting in skipping of exons during frame reading. Several types of AOs exist, including 2′*O*-methyl phosphorothioate, locked nucleic acids, morpholinos, vivo morpholinos (containing an octaguanidine motif), and peptide-conjugated phosphorodiamidate morpholinos (PPMOs). Each has its benefits, with safety, cell penetrability, nucleic acid binding, and half-life being the primary qualities evaluated. Secondary qualities include formation of mRNA structures, GC content, and melting temperature (Shimo et al. 2018).

Similar to gene replacement, extensive experiments were first conducted in the mdx mouse to skip the point mutation in exon 23, showing proof of concept of dystrophin restoration (Lu et al. 2003). Large animal studies were performed years later in the canine X-linked muscular dystrophy model

Table 2 Comparison of dystrophin expression levels in CXMD and DMD

	CXMD	DMD
Gene replacement	Kornegay et al. (2010): AAV9 microdys in GRMD: 15–100% Dys+ fibers (IF) Yue et al. (2015): 5–60% Dys+ fibers (IF) Guiner et al. (2017): rAAV2/8; up to 67% Dys+ fibers; up to 50% normal levels (WB)	Sarepta: AAV7/4; 30–200% (WB)
Exon skipping	Yokota et al. (2009) (Morpholino): 26% of normal levels (WB) Yokota et al. (2012): (vPMO) up to 75% Dys+ fibers (IF); 20% normal levels (WB) Echigowa et al. (2017): (PPMO): 5% normal levels (heart)	Sarepta (Exon51): Morpholino; 17–70% Dys+ fibers (IF); 0–2% (WB) Komaki et al. (2018) (Exon53): up to 17% Dys+ fibers; 8.1% (WB)
Gene editing	Amoasii et al. (2018): NHEJ: 3 to up to 92% in heart HDR: 0–16% in skeletal muscle (Nghiem)	N/A

in Japan ($CXMD_J$). The $CXMD_J$ dog was derived from backcrossing GRMD dogs with Beagles (Shimatsu et al. 2003) and has the original splice site mutation in intron 6, leading to exon 7 skipping and a stop codon in exon 8. Both GRMD and $CXMD_J$ would require multi-exon skipping of exons 6–8 to restore the reading frame.

Yokota et al. (2009) evaluated a cocktail of naked morpholino AOs in $CXMD_J$ dogs. They administered the AOs at 120–200 mg/kg intravenously daily for 5–11 weeks and showed variable dystrophin restoration depending on the skeletal muscle observed, with up to 27% that of normal dystrophin levels. On the other hand, there was no detectable dystrophin restoration in the heart.

Vivo morpholinos represent an upgrade in cell penetrability and subsequent efficacy compared to naked morpholinos (Wu et al. 2009). Yokota et al. administered a three-AO cocktail of vivo morpholinos intramuscularly to a group of $CXMD_J$ dogs to skip exons 6–8 (Yokota et al. 2012). A dosage of 120–400 µg of cocktail AOs was delivered, resulting in 60% of dys+ fibers along with 20% dystrophin restoration with western blotting. A four-AO cocktail injected intramuscularly further improved dystrophin restoration.

Added improvement of the morpholino backbone led to the development of arginine-rich PPMOs (Jearawiriyapaisarn et al. 2008). Canine studies using PPMOs (given at 12 mg/kg I.V. every 2 weeks for 8 weeks) showed approximately 5% dystrophin restoration in skeletal and cardiac muscle and associated improved cardiac muscle histology and reduced cardiac conduction disturbances (Echigoya et al. 2017).

Exon 51 skipping is applicable to approximately 13% of DMD boys. Clinical trials showed dystrophin restoration, but questions of long-term benefit still remain. The Food and Drug Administration approved Eteplirsen for exon 51 skipping in DMD boys. Other exon-skipping drugs are being evaluated in clinical trials, specifically for exon 53 (Table 2). Due to the half-life of AOs, lifelong administration of morpholinos may be required.

Gene editing

Gene editing is a relatively new avenue of genetic therapy research. Clustered regularly interspaced short palindromic repeats (CRISPR) combined with a nuclease such as caspase (cas)-9 or Cpf1 have provided exciting results in DMD human cardiomyocytes and mdx mice (Zhang et al. 2017). Other gene editing platforms such as transcription activator-like effector nuclease (TALEN) were utilized at the cellular level to correct the reading frame in DMD myoblasts (Ousterout et al. 2013). Using the CRISPR platform, two gene editing methods, non-homologous end joining (NHEJ) and homology-directed repair (HDR), have been utilized in the mdx mouse model (Tabebordbar et al. 2016; Long et al. 2016; Bengtsson et al. 2017). Non-homologous end joining is the process of DNA repair without the use of a template and is prone to errors (insertions, additional deletions). Homology-directed repair typically occurs in replicating cells, is more precise, and requires the use of a donor clone. A donor clone may contain the normal sequence used to replace the excised area of the *DMD* gene. Currently, HDR-mediated gene editing is difficult to achieve in post-mitotic cells such as myofibers due to the position of the cell cycle.

A recent gene editing study in the ΔE50 (hotspot mutation) canine model for DMD used CRISPR/Cas-9 and a NHEJ technique to "snip" out exon 51, creating an internally deleted dystrophin product (Amoasii et al. 2018). An AAV9 vector was used to deliver the CRISPR and Cas-9, with one dog receiving 2×10^{13} vg/kg BW and the other 1×10^{14} vg/kg BW (plus immunosuppression). Variable dystrophin levels (3–92%) were restored 8 weeks later in skeletal muscles and the authors reported up to 92% restoration in cardiac muscle of one of the two treated dogs. No side- or off-target effects were reported (Amoasii et al. 2018).

In GRMD dogs, we have recently used an HDR technique with a CRISPR/Cas-9 plasmid to excise the splice site mutation and a donor clone (containing normal intron 6 sequence) to replace the excised region. We injected the plasmids into the muscles of the cranial tibial compartment of three, variably aged GRMD dogs. We hypothesized that utilizing an HDR-mediated technique could restore full-length dystrophin levels to approximately 10%. Dystrophin restoration was observed between 0–16% that of normal levels depending on the muscle evaluated.

Non-homologous end joining and "snipping" of one or more exons appear to be more applicable to a larger number of DMD patients, similar to multi-exon skipping. We envision a smaller subset of DMD boys that will be amenable to HDR-mediated gene editing such as those with splice site mutations. Further studies are needed to enhance HDR-mediated gene editing in post-mitotic muscle, including the use of a similar technique called homology-independent targeted integration (Suzuki et al. 2016). On a similar note, a concern with delivering CRISPR/Cas-9 or a similar gene-editing platform is the potential for off-target effects on other sequence-homologous genes, which may lead to modulation of expression. In the ΔE50 study and also our GRMD dogs, there were no detectable off-target effects (Amoasii et al. 2018). Long-term studies in canine models and more in-depth clinical evaluation for mutagenesis or other side effects are required to establish if off-target and side effects may occur.

Canine models have played an important role in the evaluation of genetic therapies across a range of severe and life-threatening diseases of children and adults (Beltran et al. 2012; Nichols et al. 2016; Bradbury et al. 2018). In line

with the basic tenet of "doctor do no harm," over and above establishing potential efficacy of the proposed treatment, the major goal of all preclinical studies should be to identify potential complications. Unfortunately, the mdx mouse has often failed to predict potentially serious side effects that were subsequently demonstrated in GRMD dogs (Kornegay 2017). Consistent with sound experimental design, animal research should be conducted in ways that follow the 3Rs of replacement, reduction and refinement (Sneddon et al. 2017). Moreover, all preclinical work should be carefully considered so as to improve translation of findings to human patients (Landis et al. 2012; Willman et al. 2015). In particular, basic tenets of experimental design should be considered, extending from power analysis, to selection of biomarkers aligned with those to be used in human trials, to blinding. Speaking as veterinarians, beyond improving the condition of human patients, these therapies should ideally eventually be applied to client-owned dogs (Callan et al. 2016).

Conclusions

In the authors' opinion, gene replacement appears to be the most promising for most, if not all, DMD boys, as administering a μ-dystrophin construct is not *DMD* mutation dependent. Exon skipping may require lifelong treatments and the long-term effects of gene editing in large animal models must be further delineated.

Canine models for DMD have contributed immensely to the evaluation of genetic therapies. Ethical implications should always be considered when using animal models for disease. In the case of DMD, the condition is a severe and life-threatening disease of children and adults. Thus, studying a large animal model that closely recapitulates the disease is of paramount importance to predict response to various therapies.

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Compliance with ethical standards

Conflict of interest Dr. Nghiem is a paid consultant for Agada Biosciences. Dr. Kornegay does not have any conflicts of interest.

References

Aartsma-Rus A et al (2006) Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle Nerve 34(2):135–144 **(review)**

Acosta AR et al (2016) Use of the six-minute walk test to characterize golden retriever muscular dystrophy. Neuromuscul Disord 26(12):865–872

Amoasii L et al (2018) Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science 362(6410):86–91

Aoki Y et al (2012) Bodywide skipping of exons 45–55 in dystrophic mdx52 mice by systemic antisense delivery. Proc Natl Acad Sci USA 109(34):13763–13768

Beltran WA et al (2012) Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. Proc Natl Acad Sci USA 109(6):2132–2137

Bengtsson NE et al (2017) Corrigendum: muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nat Commun 8:16007

Birch SM et al (2017) A blinded, placebo-controlled systemic gene therapy efficacy study in the GRMD model of Duchenne muscular dystrophy. Mol Ther 25:193

Bladen CL et al (2015) The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat 36(4):395–402

Bradbury AM et al (2018) AAVrh10 gene therapy ameliorates central and peripheral nervous system disease in canine globoid cell leukodystrophy (Krabbe disease). Hum Gene Ther 29(7):785–801

Bulfield G et al (1984) X chromosome-linked muscular dystrophy (mdx) in the mouse. Proc Natl Acad Sci USA 81(4):1189–1192

Callan MB et al (2016) Successful phenotype improvement following gene therapy for severe hemophilia A in privately owned dogs. PLoS One 11(3):e0151800

Deconinck N et al (1996) Functional protection of dystrophic mouse (mdx) muscles after adenovirus-mediated transfer of a dystrophin minigene. Proc Natl Acad Sci USA 93:3570–3574

Dowling P et al (2004) Drastic reduction of sarcalumenin in Dp427 (dystrophin of 427 kDa)-deficient fibres indicates that abnormal calcium handling plays a key role in muscular dystrophy. Biochem J 379(Pt 2):479–488

Echigoya Y, Yokota T (2014) Skipping multiple exons of dystrophin transcripts using cocktail antisense oligonucleotides. Nucleic Acid Ther 24(1):57–68

Echigoya Y et al (2015) In silico screening based on predictive algorithms as a design tool for exon skipping oligonucleotides in Duchenne muscular dystrophy. PLoS One 10(3):e0120058

Echigoya Y et al (2017) Effects of systemic multiexon skipping with peptide-conjugated morpholinos in the heart of a dog model of Duchenne muscular dystrophy. Proc Natl Acad Sci USA 114(16):4213–4218

Fan Z et al (2014) Characteristics of magnetic resonance imaging biomarkers in a natural history study of golden retriever muscular dystrophy. Neuromuscul Disord 24(2):178–191

Fletcher S et al (2010) Dystrophin isoform induction in vivo by antisense-mediated alternative splicing. Mol Ther 18(6):1218–1223

Gilbert R et al (2003) Prolonged dystrophin expression and functional correction of mdx mouse muscle following gene transfer with a helper-dependent (gutted) adenovirus-encoding murine dystrophin. Hum Mol Genet 12:1287–1299

Gregorevic P et al (2008) Systemic microdystrophin gene delivery improves skeletal muscle structure and function in old dystrophic mdx mice. Mol Ther 16:657–664

Grimm T et al (2012) Risk assessment and genetic counseling in families with Duchenne muscular dystrophy. Acta Myol 31(3):179–183

Hoffman EP et al (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51(6):919–928

Howell JM et al (1998a) Direct dystrophin and reporter gene transfer into dog muscle in vivo. Muscle Nerve 21(2):159–165

Howell JM et al (1998b) High-level dystrophin expression after adenovirus-mediated dystrophin minigene transfer to skeletal muscle of dystrophic dogs: prolongation of expression with immunosuppression. Hum Gene Ther 9(5):629–634
Jearawiriyapaisarn N et al (2008) Sustained dystrophin expression induced by peptide-conjugated morpholino oligomers in the muscles of mdx mice. Mol Ther 16(9):1624–1629
Jooss K et al (1998) Transduction of dendritic cells by DNA viral vectors directs the immune response to transgene products in muscle fibers. J Virol 72:4212–4223
Komaki H et al (2018) Systemic administration of the antisense oligonucleotide NS-065/NCNP-01 for skipping of exon 53 in patients with Duchenne muscular dystrophy. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aan0713
Kornegay JN (2017) The golden retriever model of Duchenne muscular dystrophy. Skelet Muscle 7(1):9. **(review)**
Kornegay JN et al (1988) Muscular dystrophy in a litter of golden retriever dogs. Muscle Nerve 11(10):1056–1064
Kornegay JN et al (2010) Widespread muscle expression of an AAV9 human mini-dystrophin vector after intravenous injection in neonatal dystrophin-deficient dogs. Mol Ther 18(8):1501–1508
Kornegay JN et al (2012) Canine models of Duchenne muscular dystrophy and their use in therapeutic strategies. Mamm Genome 23(1–2):85–108 **(review)**
Kornegay JN et al (2014) Pharmacologic management of Duchenne muscular dystrophy: target identification and preclinical trials. ILAR J 55(1):119–149 **(review)**
Landis SC et al (2012) A call for transparent reporting to optimize the predictive value of preclinical research. Nature 490(7491):187–191
Le Guiner C et al (2017) Long-term microdystrophin gene therapy is effective in a canine model of Duchenne muscular dystrophy. Nat Commun 8:16105
Long C et al (2016) Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 351(6271):400–403
Lu QL et al (2003) Functional amounts of dystrophin produced by skipping the mutated exon in the mdx dystrophic mouse. Nat Med 9(8):1009–1014
Matthews E et al (2016) Corticosteroids for the treatment of Duchenne muscular dystrophy. Cochrane Database Syst Rev 5:CD003725
Monaco AP et al (1988) An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics 2(1):90–95
Nachman MW (2004) Haldane and the first estimates of the human mutation rate. J Genet 83(3):231–233 **(Erratum in: J Genet. 2008 Dec;87(3):317)**
Nichols TC et al (2016) Canine models of inherited bleeding disorders in the development of coagulation assays, novel protein replacement and gene therapies. J Thromb Haemost 14(5):894–905
Ousterout DG et al (2013) Reading frame correction by targeted genome editing restores dystrophin expression in cells from Duchenne muscular dystrophy patients. Mol Ther 21(9):1718–1726 **(Erratum in Mol Ther. 21(11):2130)**
Patronek GJ et al (1997) Comparative longevity of pet dogs and humans: implications for gerontology research. J Gerontol A Biol Sci Med Sci 52(3):B171–B178
Ragot T et al (1993) Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of mdx mice. Nature 361:647–650
Ramos J, Chamberlain JS (2015) Gene therapy for Duchenne muscular dystrophy. Expert Opin Orphan Drugs 3:1255–1266
Schatzberg SJ et al (1999) Molecular analysis of a spontaneous dystrophin 'knockout' dog. Neuromuscul Disord 9(5):289–295
Schneider SM et al (2018) Glucose metabolism as a pre-clinical biomarker for the golden retriever model of Duchenne muscular dystrophy. Mol Imaging Biol 20(5):780–788
Shimatsu Y et al (2003) Canine X-linked muscular dystrophy in Japan (CXMDJ). Exp Anim 52:93–97
Shimo T et al (2018) Designing effective antisense oligonucleotides for exon skipping. Methods Mol Biol 1687:143–155
Sicinski P et al (1989) The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. Science 244(4912):1578–1580
Sneddon LU et al (2017) Considering aspects of the 3Rs principles within experimental animal biology. J Exp Biol 220(Pt 17):3007–3016
Suzuki K et al (2016) In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature 540(7631):144–149
Tabebordbar M et al (2016) In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 351(6271):407–411
Wang Z et al (2007a) Immunity to adeno-associated virus-mediated gene transfer in a random-bred canine model of Duchenne muscular dystrophy. Hum Gene Ther 18(1):18–26
Wang Z et al (2007b) Sustained AAV-mediated dystrophin expression in a canine model of Duchenne muscular dystrophy with a brief course of immunosuppression. Mol Ther 15(6):1160–1166
Wang Z et al Chamberlain JS, Tapscott SJ, Storb R (2009) Gene therapy in large animal models of muscular dystrophy. ILAR J 50(2):187–198. Review
Willmann R et al (2015) Best practices and standard protocols as a tool to enhance translation for neuromuscular disorders. J Neuromuscul Dis 2(2):113–117
Wirth T et al (2013) History of gene therapy. Gene 525(2):162–169
Wu B et al (2009) Octa-guanidine morpholino restores dystrophin expression in cardiac and skeletal muscles and ameliorates pathology in dystrophic mdx mice. Mol Ther 17(5):864–871
Xiao X et al (1996) Efficient long-term gene transfer into muscle tissue of immunocompetent mice by adeno-associated virus vector. J Virol 70:8098–8108
Yokota T et al (2007) Optimizing exon skipping therapies for DMD. Acta Myol 26(3):179–184. Review
Yokota T et al (2009) Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. Ann Neurol 65(6):667–676
Yokota T et al (2012) Extensive and prolonged restoration of dystrophin expression with vivo-morpholino-mediated multiple exon skipping in dystrophic dogs. Nucleic Acid Ther 22(5):306–315
Yue Y et al (2015) Safe and bodywide muscle transduction in young adult Duchenne muscular dystrophy dogs with adeno-associated virus. Hum Mol Genet 24(20):5880–5890
Zhang Y et al (2017) CRISPR-Cpf1 correction of muscular dystrophy mutations in human cardiomyocytes and mice. Sci Adv 3(4):e1602814
https://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/ucm581222.htm. Accessed Jan 2019
https://ghr.nlm.nih.gov/primer/therapy/genetherapy. Accessed Jan 2019