Degenerative Neurological and Neuromuscular Disease

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Gene Editing for Duchenne Muscular Dystrophy: From Experimental Models to Emerging Therapies

Umme Sabrina Haque[1,2], Toshifumi Yokota[2,3]

[1]Department of Neuroscience, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, T6G 2H7, Canada; [2]Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, T6G 2H7, Canada; [3]The Friends of Garrett Cumming Research & Muscular Dystrophy Canada HM Toupin Neurological Science Research Chair, Edmonton, AB, T6G 2H7, Canada

Correspondence: Toshifumi Yokota, Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, T6G 2H7, Canada, Tel +1 780 492 1102, Email toshifum@ualberta.ca

Abstract: The CRISPR system has emerged as a ground-breaking gene-editing tool, offering promising therapeutic potential for Duchenne muscular dystrophy (DMD), a severe genetic disorder affecting approximately 1 in 5000 male births globally. DMD is caused by mutations in the dystrophin gene, which encodes a critical membrane-associated protein essential for maintaining muscle structure, function and repair. Patients with DMD experience progressive muscle degeneration, loss of ambulation, respiratory insufficiency, and cardiac failure, with most succumbing to the disease by their third decade of life. Despite the well-characterized genetic basis of DMD, curative treatments- such as exon skipping therapies, micro-dystrophin, and steroids- remain elusive. Recent preclinical studies have demonstrated the promise of CRISPR-based approaches in restoring dystrophin expression across various models, including human cells, murine systems, and large animal models. These advancements highlight the potential of gene editing to fundamentally alter the trajectory of the disease. However, significant challenges persist, including immunogenicity, off-target effects, and limited editing efficiency, which hinder clinical translation. This review provides a comprehensive analysis of the latest developments in CRISPR-based therapeutic strategies for DMD. It emphasizes the need for further innovation in gene-editing technologies, delivery systems, and rigorous safety evaluations to overcome current barriers and harness the full potential of CRISPR/Cas as a durable and effective treatment for DMD.

Keywords: CRISPR, Duchenne muscular dystrophy, *DMD*, dystrophin, gene editing

Introduction

Duchenne muscular dystrophy (DMD) is one of the most severe monogenic disorders, characterized by progressive muscle degeneration and weakness.[1] Primarily affecting boys, DMD arises from mutations in the X-linked *DMD* gene, which encodes dystrophin, a protein critical for maintaining muscle fiber integrity. Due to lack of dystrophin protein production, DMD patients experience progressive muscle degeneration, leading to increasing muscle weakness and eventual loss of ambulation.[2,3] The impact extends further into adolescence, with patients, between 18 and 20 years of age, often requiring ventilatory support as the disease affects respiratory and cardiac muscles.[4,5] Tragically, most patients with DMD face premature mortality, often before the age of 30. Affecting approximately 1 in 5,000 male births worldwide,[6] DMD imposes a profound clinical and societal burden due to its early onset and lack of curative therapies.

Current management strategies, such as corticosteroid therapy can only delay the progression of disease and prolong ambulation.[4,7] However, corticosteroid treatment is linked to several side effects, including unintended weight gain, growth delay, delayed puberty, bone fragility, and adrenal insufficiency.[8] Advanced therapeutic approaches include exon skipping with oligonucleotides, stem cell-based therapies, and gene replacement to restore dystrophin expression.[9] While some have shown transient benefits, most target the symptoms rather than correcting the root genetic defect, and their long-term efficacy remains under investigation in ongoing clinical trials.

In recent years, significant advancements in molecular genetics have paved the way for innovative therapeutic approaches targeting the root cause of DMD. Gene editing technologies, particularly CRISPR/Cas9, have shown promise

Received: 23 January 2025
Accepted: 8 April 2025
Published: 12 April 2025

in correcting the mutations within the *DMD* gene.[10,11] Preclinical studies have demonstrated the potential of CRISPR-based strategies to restore dystrophin expression in animal models, offering hope for a transformative treatment that could alter the disease trajectory. It has been estimated that up to 80% of DMD patients could potentially benefit from gene editing strategies targeting common and less frequent mutations.[12] Despite these promising advances, numerous challenges remain to be addressed before gene editing can become a viable clinical treatment for DMD. These include optimizing editing efficiency, ensuring precise targeting, developing effective delivery methods to reach all affected muscles, and mitigating potential immune responses to the therapy.[13,14]

This review article aims to provide an update on the current state of gene editing approaches for DMD, discussing recent advancements, potential opportunities, and remaining obstacles in the field. By examining these facets, we seek to elucidate the potential of gene editing as a viable therapeutic avenue for individuals afflicted with Duchenne muscular dystrophy.

Current Therapeutic Landscape of DMD

The current therapeutic landscape for DMD has evolved significantly with advancements targeting the disease's genetic and pathological basis. The *DMD* gene, one of the largest in the human genome (~2.2 Mb), is highly prone to mutations that often disrupt the open reading frame (ORF) and result in non-functional dystrophin, leading to DMD.[15–18] *DMD* gene contains 79 exons, many of which encode the central rod domain of dystrophin, consisting of 24 spectrin-like repeat domains.[12,19] Certain in-frame deletions in the central rod domain of dystrophin can preserve partially functional dystrophin, leading to the milder Becker muscular dystrophy (BMD) phenotype.[20–23] BMD patients typically exhibit less severe muscle degeneration, later disease onset, and longer survival compared to DMD patients. Therefore, therapeutic strategies aim to leverage this natural phenomenon by converting the severe DMD phenotype into the milder BMD condition.[20,21,24,25] This can be achieved through gene replacement therapies that introduce microdystrophin transgenes or through exon skipping approaches using antisense oligonucleotides (ASOs).

Exon skipping restores the open reading frame by masking specific exons during pre-mRNA splicing, allowing for the production of truncated but functional dystrophin. Since 2016, four FDA-approved ASO therapies—Exondys 51, Vyondys 53, Viltepso, and Amondys 45—have been tailored to specific exon deletions[26] (Table 1). Despite advances

Table 1 Available FDA-Approved Antisense Oligonucleotide and Gene Therapy Options for DMD

Name	Manufacturer	Chemistry & Therapeutic Target	FDA Approval Date	Eligible DMD Population	References
Exondys 51 (eteplirsen)	Sarepta Therapeutics	Antisense oligonucleotide (PMO); Targets exon 51	Sep 19, 2016	~13% of DMD patients (exon 51 mutation)	[27,28]
Vyondys 53 (golodirsen)	Sarepta Therapeutics	Antisense oligonucleotide (PMO); Targets exon 53	Dec 12, 2019	~8% of DMD patients (exon 53 mutation)	[29]
Viltepso (viltolarsen)	NS Pharma (Nippon Shinyaku)	Antisense oligonucleotide (PMO); Targets exon 53	Aug 12, 2020	~8% of DMD patients (exon 53 mutation)	[30,31]
Amondys 45 (casimersen)	Sarepta Therapeutics	Antisense oligonucleotide (PMO); Targets exon 45	Feb 25, 2021	~8% of DMD patients (exon 45 mutation)	[32–34]
Elevidys (delandistrogene moxeparvovec-rokl)	Sarepta Therapeutics	AAV vector gene transfer; delivers micro-dystrophin gene to muscle (one-time infusion)	Jun 22, 2023 (expanded Jun 20, 2024)	Theoretically benefits **100%** of DMD patients regardless of mutation type (ELEVIDYS is contraindicated in patients with any deletion in exon 8 and/or exon 9 in the DMD gene due to the increased risk for a severe immune-mediated myositis reaction). Initially approved for **ambulatory patients aged 4–5 years.**	[35–37]

in ASO technology, challenges remain, including limited applicability to specific mutations, incomplete restoration of dystrophin function, and the need for systemic delivery to all affected muscle groups specifically cardiac tissue.

Gene-replacement therapy has emerged as a promising approach to replace the dysfunction endogenous dystrophin using microdystrophin, a functional but shortened version of dystrophin. In June 2023, FDA granted accelerated approval to delandistrogene moxeparvovec (Elevidys), an AAV-mediated gene therapy for DMD patients aged four and five years who do not have deletions in exon 8 and/or 9[38] (Table 1). This single-dose therapy delivers a microdystrophin transgene via AAV74.[39] Despite its potential, microdystrophin therapy has limitations, including transgene loss due to rare AAV integration, immune responses preventing retreatment, and risks of insertional mutagenesis and off-target effects.

Glucocorticoids like prednisone/prednisolone and deflazacort remain a mainstay for managing secondary symptoms of DMD, such as inflammation, fibrosis, impaired angiogenesis, altered calcium homeostasis, and mitochondrial dysfunction. However, their non-selective action leads to significant side effects, including weight gain, growth suppression, bone fragility, and adrenal insufficiency, limiting long-term use.[8,40–42] To reduce steroid toxicity, alternative dosing regimens have been explored. A study by Quattrocelli et al demonstrated that pulsed weekly steroids can confer metabolic advantages and improved muscle performance[43] while reducing corticosteroid-associated side effects compared to conventional daily dosing.[43] Translating this concept to patients, the FOR-DMD trial (a large multicenter randomized study) directly compared daily prednisone with an intermittent regimen (10 days on, 10 days off) in boys with DMD. The trial found that although intermittent prednisone was associated with a modest reduction in some side effects, it was significantly less effective than daily dosing in slowing disease progression and improving motor outcomes.[44]

In parallel with dosing modifications, Vamorolone (brand name Agamree), a first-in-class dissociative corticosteroid, was recently approved for the treatment of DMD.[42] It is specifically designed to preserve the anti-inflammatory efficacy of traditional glucocorticoids while minimizing their detrimental effects on bone health, metabolism, and growth. In the pivotal VISION-DMD trial, vamorolone demonstrated clinical efficacy comparable to prednisone along with a more favorable safety and tolerability profile. Nevertheless, some adverse events characteristic of corticosteroid therapy were still observed under vamorolone. According to trial data and subsequent post-approval analyses, the most common side effects reported with vamorolone included Cushingoid features (eg rounded face), behavioral and mood disturbances (psychiatric disorders), vomiting, increased body weight, and vitamin D deficiency.[45]

Adding to the therapeutic arsenal, the FDA recently approved givinostat, a histone deacetylase inhibitor, in March 2024 for DMD patients aged six years and older.[46] Unlike other therapies, givinostat is applicable irrespective of specific genetic mutations and has shown efficacy in reducing fibrosis and improving muscle function. Ongoing studies aim to evaluate its long-term safety and effectiveness, marking a significant step forward in non-steroidal treatment options for DMD.

While significant progress has been made, there remains a critical need for more comprehensive and effective therapies that address the underlying genetic causes of DMD. Overcoming challenges such as systemic delivery, mutation-specific limitations, and ensuring durable efficacy will require ongoing innovation to advance treatment options and improve the quality of life for all DMD patients. Gene editing can offer the potential to directly target the underlying genetic causes of DMD, providing a more effective, durable, and broadly applicable solution.

Gene Editing as a Promising Therapeutic Approach for DMD

CRISPR/Cas System

Clustered regularly interspaced short palindromic repeats (CRISPR) originally discovered as a bacterial immune mechanism, has been adapted as a powerful genome-editing tool.[47–49] In bacteria, CRISPR incorporates segments of viral DNA into the host genome, where they are transcribed into RNAs that guide Cas endonuclease to recognize and destroy invading viral DNA. The system has been repurposed for genome editing, involving two key components: a single guide RNA (sgRNA) that directs the Cas protein to a specific genomic target sequence near a PAM (protospacer adjacent motif) region and the Cas endonuclease itself, which creates double-stranded DNA breaks (DSBs).[47,49]

Gene editing is facilitated by two repair mechanisms after creating a double-stranded DNA break (DSB). In the presence of a DNA donor template, homology-directed repair (HDR) can introduce precise modifications at the target site. However, HDR is active only in proliferative cells and has limited efficiency in quiescent cells and postmitotic cells, such as muscle stem cells and myofibers and cardiomyocytes.[50,51] Nonhomologous end-joining (NHEJ), on the other hand, operates in both dividing and non-dividing cells, introducing small insertions or deletions (indels) at the site of the DSB.[52] This process is especially effective in correcting out-of-frame mutations, such as restoring the open reading frame (ORF) of the *DMD* gene by targeting splice donor or acceptor sequences.

CRISPR systems are categorized into two classes based on the Cas proteins involved. Class 1 systems require multiple Cas effectors, while Class 2 systems, which include Cas9, Cas12, and Cas13, use a single Cas protein and are more commonly employed in genome editing.[53] The most commonly used Cas9 enzyme, derived from *Streptococcus pyogenes* (SpCas9), has 1368 amino acids and typically generates blunt-end cuts by recognizing PAM sequences of 5′-NGG-3′ or 5′-NAG-3′.[47,49,54] Smaller Cas9 variants, such as *Staphylococcus aureus* Cas9 (SaCas9, 1053 amino acids) and *Campylobacter jejuni* Cas9 (CjCas9, 984 amino acids), recognize more restrictive PAM sequences (eg, 5′-NNGRRT -3′ and 5′-NNNVRYM-3′, respectively) but are better suited for delivery via adeno-associated virus (AAV) vectors due to their smaller size.[55–57] Similarly, Cas12 enzymes like LbCas12a (1228 amino acids) and AsCas12a (1307 amino acids) target T-rich PAM sequences (5′-TTN-3′), expanding the editing possibilities.[58–60]

Advances in Cas protein engineering have further enhanced specificity and reduced off-target activity, leading to high-precision variants like eSpCas9, Spy-mac Cas9, HypaCas9, evoCas9, HeFSpCas9, xCas9, HiFiCas9, Sniper-Cas9, SpCas9-HF1, SpCas9-NG, FnCas9-RHA, SaCas9-KKH, AsCas12a-RVR, and enAsCas12a.[61] Additionally, modified forms such as nickase Cas9 (nCas9) and deactivated Cas9 (dCas9) expand its applications to base editing and transcriptional regulation, further increasing its utility.[62,63]

These features, combined with the range of PAM sequences and sizes available, make CRISPR/Cas the preferred choice for DMD therapeutics and other genetic disorders, where targeted, efficient, and scalable gene-editing solutions are critical. Most importantly, it outperforms older technologies like transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs). While ZFNs lack targeting specificity, increasing the risk of cytotoxic off-target effects, TALENs face challenges in delivery due to their large size.[64–67] In contrast, CRISPR/Cas9, with its smaller sgRNA-directed mechanism, offers greater flexibility and ease of delivery.

For DMD, the modularity and efficiency of CRISPR/Cas9 make it uniquely suited to correct mutations in the *DMD* gene. Its ability to restore dystrophin expression by precisely targeting and repairing disrupted exons offers hope for transforming DMD treatment, surpassing the limitations of older gene-editing approaches and providing a pathway to durable and effective therapeutics.

Gene Editing Therapeutic Strategy for DMD Using CRISPR/Cas System

The first proof-of-concept study for in vivo CRISPR-mediated gene editing in DMD was demonstrated in 2014 by Long *et al*.[68] This study utilized the *mdx* mouse model, which carries a nonsense mutation in *Dmd* exon 23. Researchers injected zygotes with SpCas9, a single guide RNA (sgRNA), and an exogenous single-stranded oligodeoxynucleotide (ssODN) DNA template to facilitate homology-directed repair (HDR).[68] A key finding of this study was that approximately 15% genetic correction was sufficient to restore dystrophin expression to normal levels in nearly all myofibers. This outcome is attributed to the syncytial nature of skeletal muscle, where corrected nuclei can distribute dystrophin protein throughout the muscle fibers, enabling therapeutic benefits even with partial correction.

Although germline editing demonstrated potential in this study, ethical considerations and regulatory policies have since directed research toward postnatal gene-editing approaches for human DMD therapy. Subsequently, postnatal editing was accomplished using recombinant adeno-associated viruses (AAVs) to deliver CRISPR/Cas9 components, enabling in vivo exon skipping or deletion to correct the dystrophin gene. These findings highlight the therapeutic potential of CRISPR-based strategies to target various DMD mutations effectively. The primary strategies for CRISPR-mediated therapeutic correction of DMD mutations include exon excision, exon skipping, exon reframing, exon knock in, base editing, and prime editing.

Single-Cut Exon Editing

Single-cut exon editing requires only one sgRNA to create a single double-stranded DNA break (DSB) at the target intron-exon boundaries or splice signal sequences of *DMD*. Following the DSB, the endogenous non-homologous end-joining (NHEJ) repair mechanism introduces insertions or deletions (INDELs) at the break site, leading to two potential outcomes[69] (Figure 1A). One is exon skipping, where INDELs disrupt the splicing consensus sequence, causing the exon to be skipped during mRNA splicing. This restores the open reading frame (ORF) of dystrophin, enabling the production of a functional protein.[13] As almost 80% of the DMD patients could benefit from exon skipping based on the Leiden DMD mutation database, this strategy has high potential as therapeutic option for DMD.[70]

Another strategy is exon reframing where the generated indels lead to a targeted frameshift to restore ORF.[13] While theoretically, one-third of INDELs should be in-frame, studies suggest that the outcomes of NHEJ can often be predicted by analyzing the fourth nucleotide upstream of the PAM sequence.[69] This predictability enhances the precision of the single-cut editing process. In cases where INDELs inadvertently recreate the original uncorrected DNA sequence, the sgRNA can continue directing the Cas enzyme to recut the DNA. This iterative process continues until the PAM site or DNA target sequence is eliminated, ensuring the intended genetic correction. Additionally, Min et al developed another strategy where optimized sgRNA generates a single adenosine insertion at the DSB due to a strategically created single nucleotide overhang. This targeted approach can consistently restore the ORF in cases where exons are out of frame by only one nucleotide, significantly enhancing the reliability of reframing.

A study by Li et al demonstrated the very first example of single-cut exon editing in in vitro model. In the study the authors used a patient-derived iPSCs carried a deletion of exon 44 in the *Dmd* gene.[71] This mutation caused a frameshift in the open reading frame (ORF), preventing the production of functional dystrophin protein, a hallmark of DMD. To correct the mutation, the authors used specific single-guide RNAs (sgRNAs) to target the sequences flanking exon 44 either to disrupt the splicing acceptor to skip exon 45 which will connect exons 43 and 46 and would restore the reading frame. Another strategy was to introduce small indels to modulate the protein reading frame. After correction, the edited iPSCs were differentiated into skeletal muscle cells, which expressed full-length dystrophin protein, demonstrating successful restoration of gene function.[71]

In another in vitro study, Long et al used patient-derived iPSCs with exon 48–50 deletions which creates an out-of-frame exon 51 with a stop codon.[72] By designing single-guide RNAs (sgRNAs) to the splice acceptor site of exon 51, the researchers induced exon skipping and effectively restored the open reading frame of the dystrophin gene. Most importantly, the corrected iPSCs were then differentiated into cardiomyocytes, which showed restored dystrophin protein expression. Three-dimensional engineered heart muscle (EHM) derived from corrected cells demonstrated restored dystrophin expression and improved mechanical force of contraction. Notably, correcting only 30–50% of cardiomyocytes was sufficient to rescue the mutant EHM phenotype to near-normal control levels.[72]

Apart from exon 45–55 mutational hotspot, single-cut exon skipping has been used to skip exons within the exon 2–9. In a very recent study, researchers employed a single-guide RNA (sgRNA) CRISPR/Cas9 strategy to excise duplicated exons—specifically exon 2, exons 2–9, and exons 8–9 in patient-derived myogenic cells—thereby restoring the ORF of the dystrophin gene. The method showed high specificity with minimal off-target effects, demonstrating its potential as an efficient and precise therapeutic strategy for correcting DMD mutations involving exon duplications.[73]

In animal models, several studies have demonstrated the high efficiency of single-cut sgRNA strategies in restoring dystrophin expression. For example, Amoasii et al treated ΔEx50 mice[74] and dogs[75] with a single-cut exon-skipping approach targeting exon 51. This strategy successfully reframed the dystrophin transcript by skipping exon 51 and restored up to 90% of dystrophin protein expression in skeletal muscles and the heart. The treatment significantly improved muscle function and reduced histopathological markers of muscular dystrophy without adverse effects such as immune responses, off-target mutagenesis, or liver toxicity. Similarly, Min et al (2019) reported ~90% restoration of dystrophin protein expression across all muscle groups, including the heart, in ΔEx44 mice within four weeks of a single systemic dose of AAV9-encoded gene editing components.[76] A recent study by Rok et al (2024) demonstrated that targeting the splice donor site of exon 55 with a single guide enhanced editing efficiency and dystrophin recovery compared to a dual-guide exon excision approach.[77] While this method showed potential in preventing early-onset

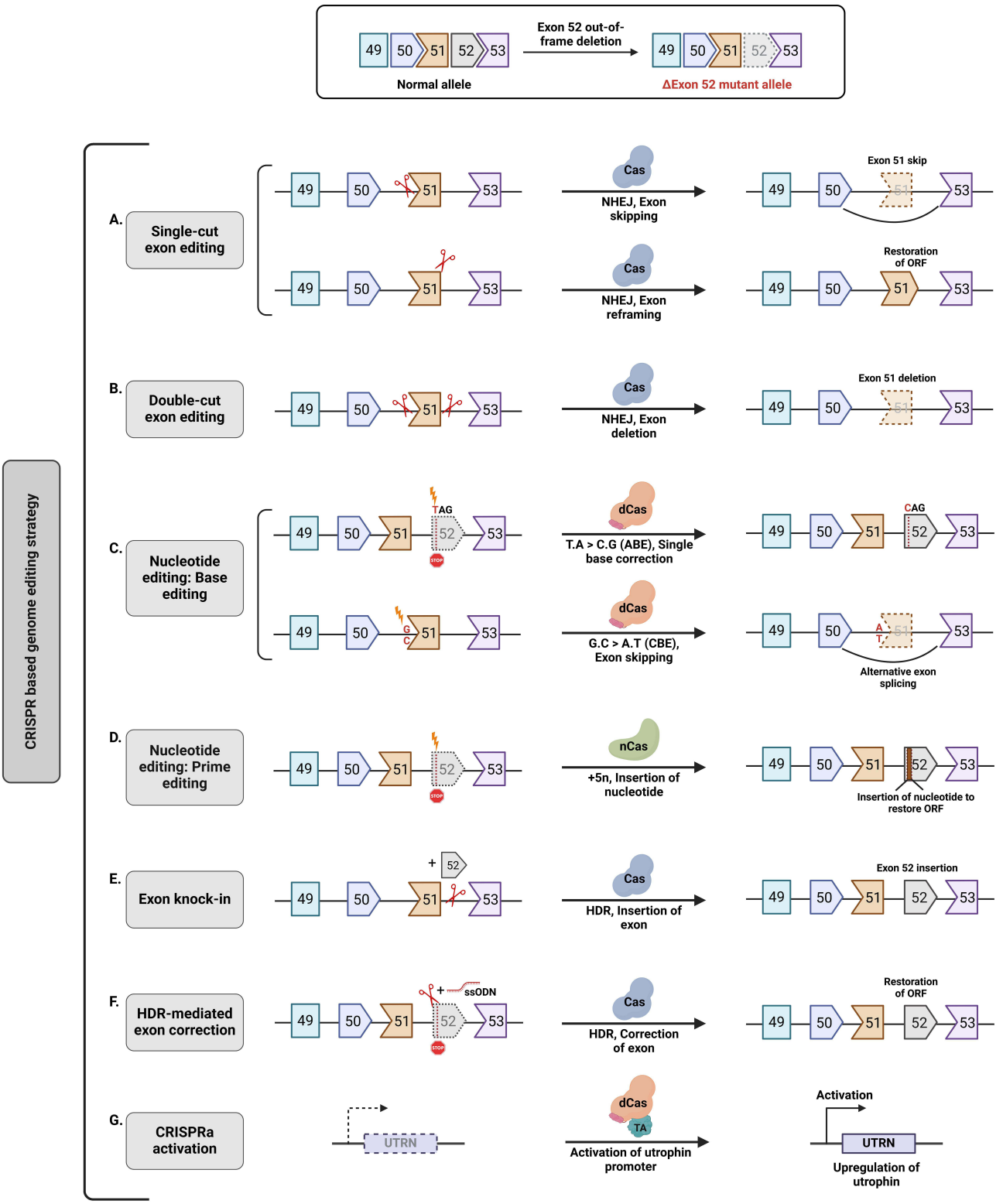

Figure 1 A schematic of CRISPR mediated therapeutic strategies for DMD including single-cut exon editing (**A**), double-cut exon editing (**B**), Nucleotide editing (**C** and **D**), Exon knock-in (**E**), HDR (Homology dependent repair) mediated exon correction (**F**), CRISPR/dCas mediated utrophin activation (**G**).

https://doi.org/10.2147/DNND.S495536

cardiac dysfunction in Δ52–54 DMD mice, dystrophin recovery in skeletal muscle was insufficient to improve motor function, highlighting the need for better AAV delivery strategies to peripheral muscles. Additionally, the efficacy of single-cut editing can vary significantly depending on the specific mutation. For instance, a study reported SpCas9 with an sgRNA targeting the splice donor site of exon 44 restored dystrophin in ~60% of myofibers in DMD ΔEx45 mice but achieved only ~36% in a DMD ΔEx43 model.[78] These findings emphasize the need to optimize sgRNA design and delivery strategies for single-cut exon editing to maximize its therapeutic potential in DMD treatment.

Double-Cut Exon Editing

Approximately 65–72% of DMD patients carry out-of-frame mutations caused by the deletion of one or more exons.[15] Restoring the reading frame through single or multiple exon deletions has the potential to produce a shorter yet functional dystrophin protein. The double-cut exon deletion approach, which utilizes two guide RNAs (sgRNAs) targeting regions flanking the mutated exons, effectively removes the target exon(s)[79–81] (Figure 1B). This method is particularly advantageous due to its mutation-independent nature, making it applicable to exon duplications, deletions, and point mutations. Consequently, it could benefit up to 85% of DMD patients, addressing a broad range of mutational profiles.[61]

One prominent target for CRISPR/Cas9-mediated exon excision is the region spanning exons 3–9 of the *DMD* gene, a hotspot frequently implicated in N-terminal mutations.[82] However, this domain plays a critical functional role, and large multi-exon deletions within this region may yield unstable or non-functional dystrophin proteins. Clinical observations suggest that individuals with a deletion encompassing exons 3–9 (Δ3–9) often exhibit asymptomatic or mild Becker muscular dystrophy (BMD) phenotypes due to the retention of the actin-binding site ABS1 which is essential for dystrophin's role in stabilizing the muscle membrane.[17] A study by Kyrychenko et al (2017) demonstrated the efficacy of three distinct multi-exon deletions (exons 3–9, 6–9, or 7–11) in human induced pluripotent stem cells (iPSCs) for restoring the dystrophin reading frame.[82] Among these, exon 3–9 excision was the most effective, restoring contractility and calcium transients in iPSC-derived cardiomyocytes. Conversely, exon 7–11 deletion resulted in minimal recovery of cardiomyocyte function due to the production of an unstable dystrophin protein.

Single exon deletion strategies have also been explored in animal models. The deletion of exon 23 using either AAV-SaCas9 or AAV-SpCas9 systems has been validated in neonatal and adult *mdx* mice. For instance, Nelson CE et al (2019) demonstrated that genome editing in *mdx* mice was sustained for one year following a single intravenous administration of AAV-CRISPR.[83] However, the study also highlighted an unintended increase in AAV integrations at CRISPR-induced double-strand breaks than the intended exon deletion.

Expanding on these findings, Xu L. et al (2019) investigated the long-term safety and efficacy of CRISPR/Cas9-mediated multi-exon deletions in *mdx* mice.[84] Using the AAVrh.74 vector to deliver the CRISPR/SaCas9 system targeting introns 20 and 23, exon 21–23 was successfully deleted in 3-day-old *mdx* mouse pups. This approach restored dystrophin expression in cardiac muscle, which remained detectable even 19 months post-treatment. The intervention significantly improved cardiac function, with no evidence of tumor formation or other adverse effects in major vital organs, underscoring the therapeutic potential and safety of multi-exon deletion strategies in DMD.

The exon 45–55 mutational hotspot also presents a promising target for CRISPR/Cas9 mediated multi-exon deletion strategy, as small in-frame deletions within this region could serve as a therapeutic approach for over 60% of DMD patients, regardless of their mutation type.[80] For instance, Ousterout et al (2015) demonstrated that CRISPR/Cas9-mediated deletion of exon 51 or exon 45–55 successfully restored the dystrophin reading frame in patient-derived myoblasts.[80] However, the efficiency of exon 45–55 deletion was notably lower than that of single-exon deletions due to the size-dependent decline in nuclease-mediated genomic deletion efficiency. Further studies by Young et al (2017) showed that Δexon 45–55-corrected hiPSC-derived cardiomyocytes and skeletal muscle myotubes exhibited restored dystrophin expression and improved muscle stability.[79] A significant finding was the reduction in microRNA 31 (miR31) levels post-correction, aligning with patterns observed in BMD patients, suggesting a shift toward a milder phenotype. Recent advancements, such as the study by Poyatos-García (2024), provided additional insights into CRISPR/Cas9-mediated exon 45–55 excision.[85] Edited Δ45–55 clones not only restored the DMD reading frame and dystrophin expression in patient-derived myoblasts but also corrected terminal myogenic differentiation defects observed in unedited DMD myotubes.

Several studies have extended this double-cut editing strategy to other multi-exon deletions, such as Δ44–54, Δ46–54, and Δ.48–57[86–88] Notably, the deletion of exon 48–57 restored a normal reading frame and produced dystrophin with normally phased spectrin-like repeats (SLRs), a structural feature often disrupted by exon skipping or complete exon deletions.[88] Furthermore, single exon deletions, such as exon 51 or 53, using SpCas9 have also proven effective in repairing the dystrophin reading frame in human DMD ΔEx48–50 or ΔEx45–52 myoblasts, respectively.[61]

In vivo studies further validated these approaches. For example, Young et al reported that electroporation of plasmids containing guide RNAs (gRNAs) targeting introns 44 and 55 successfully generated an exon 45–55-deleted in-frame dystrophin transcript in hDMD del45 *mdx* mice.[89] Similarly, targeting introns flanking exons 52–53 in *mdx*^{4cv} mice with a nonsense mutation in exon 53 restored the dystrophin reading frame, with dystrophin expression observed in cardiac, diaphragmatic, and skeletal muscles.[90] Interestingly, dystrophin expression in the heart was more robust than in skeletal muscle, likely due to the high turnover rate of skeletal muscle fibers and the persistence of AAV-mediated editing in cardiac tissue. A recent study by Rok et al (2024) explored a double-cut strategy to excise exon 55 in Δ52–54 mice using SaCas9 and intronic sgRNAs.[77] While this approach successfully removed exon 55, the overall editing efficiency and dystrophin recovery were limited and found to be restricted to cardiac tissue, underscoring the need for more efficient editing strategies.

Beyond mouse models, systemic administration of SpCas9 components via AAV9 vectors in DMD ΔEx52 pigs restored dystrophin expression across muscle tissues, ranging from 12% to 54% of normal levels. This led to improvements in muscle pathology, enhanced skeletal and cardiac function, and extended lifespan.[91]

Despite encouraging outcomes, double-cut editing strategies face notable limitations. This approach is primarily effective for excising exons that encode regions of dystrophin capable of tolerating deletions without compromising functionality. However, its efficiency is generally lower than that of single-cut strategies. The reduced efficiency is likely attributable to the requirement for precise cleavage by both sgRNAs across large genomic distances and the accurate joining of DNA ends.[83] Furthermore, the use of two distinct sgRNAs increases the risk of off-target effects and raises concerns about the potential for unintended mutations or chromosomal rearrangements caused by the simultaneous generation of DSBs.[83] These challenges emphasize the need for further refinement and optimization to maximize the therapeutic potential of multi-exon deletion strategies in DMD treatment.

Nucleotide Editing

To address the limitations of traditional genome editing methods, nucleotide editing technologies such as base editing and prime editing have emerged as promising alternatives, offering precise and permanent genome modifications without generating double-stranded DNA breaks.[14,15] Base editing, which includes cytosine base editors (CBEs) that convert DNA C•G base pairs to T•A base pairs and adenine base editors (ABEs) that convert DNA A•T base pairs to G•C base pairs, has shown potential in correcting point mutations in the dystrophin gene and inducing beneficial NHEJ-mediated exon skipping by modifying splice sites[92,93] (Figure 1C). These approaches are particularly significant for DMD, where approximately 25–35% of patients carry point mutations.[12,15] Unlike conventional CRISPR approaches, nickase Cas9 (nCas9) and deactivated Cas9 (dCas9), which are used for BEs and PEs, do not induce double-strand breaks (DSBs), thereby reducing the risks of AAV vector integration and undesired insertions or deletions (INDELs).[94]

A range of studies highlights the potential of base editing in DMD. Chemello et al (2017) employed an ABE-triggered exon skipping approach using the nSpCas9-ABEmax system to correct mutations in human DMD ΔEx51 iPSCs and neonatal DMD ΔEx51 mice, successfully inducing exon 50 skipping.[95] The local delivery of nSpCas9-ABEmax components into the skeletal muscles of DMD ΔEx51 mice can induce dystrophin restoration in nearly all skeletal myofibers and thereby prevent muscle pathology. At the very same time another study by Ryu S.M. et al (2017) corrected a nonsense point mutation in exon 20 by introducing an A-to-G substitution.[96] The system has been found to cause genomic correction at ~3.3% efficiency and restore dystrophin expression in up to 17% of skeletal myofibers in the DMD mice.

Yuan J. et al (2018) employed cytosine base editors (CBEs) fused with nSaCas9 to induce exon skipping by converting G-to-A at splice sites in human DMD ΔEx51 iPSC-derived cardiomyocytes.[97] The study demonstrated ~90% at the genomic level, which brings about nearly complete restorations of dystrophin and β-dystroglycan in the

corrected cardiomyocytes. Li et al (2021) further demonstrated that a single-dose AAV9-CBE treatment targeting exon 4 in neonatal *Dmd*^{E4} mice resulted in over 50% exon skipping and up to 90% dystrophin restoration in the heart, with therapeutic effects persisting for over a year.[98] Although cytosine base editors (CBEs) have demonstrated success in genome editing, multiple studies suggest that adenine base editors (ABEs) offer a safer alternative due to their higher specificity and lower off-target activity. This raises the critical need to investigate the potential risks associated with in vivo use of CBEs, particularly in the context of DMD animal models, to evaluate whether they might lead to unforeseen adverse effects such as oncogenesis.[99–101]

Xu et al (2021) addressed key challenges such as large size of base editors, their inherent off-target activities, and the dependency on a nearby protospacer adjacent motif (PAM) by developing the NG-targeting adenine base editor (iABE-NGA).[102] Systemic delivery of AAV9-iABE-NGA in adult *mdx*^{4cv} mice achieved near-complete dystrophin restoration in the heart and up to 15% in skeletal muscle fibers after 10 months, with minimal off-target activity and no detectable toxicity. Additional advancements include the study by Qiu H. et al (2023) who developed targeted activation-induced cytidine deaminase (AID)-mediated mutagenesis (TAM) to achieve exon skipping in patient-derived iPSC cardiomyocytes.[103] This approach precisely edits conserved guanines at splice sites in genomic DNA, abrogates exon recognition, and skips targeted exons. Using a targeted AID-mediated mutagenesis cytosine base editor (TAM-CBE), they achieved near-complete skipping of these exons in patient-derived iPSC cardiomyocytes. Chai et al (2023) employed an ABE-mediated "single-swap" editing strategy in human iPSC-derived cardiomyocytes and ΔEx44 mice.[104] The "single-swap" editing approach corrects the frameshift mutation by substituting a single nucleotide, thereby restoring dystrophin expression in skeletal and cardiac muscles at rates of 19–31% and 53–60%, respectively. Similarly, transfection of ABE and a guide RNA (gRNA) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with a large deletion (ΔE48–54) enabled exon 55 skipping and subsequently achieving 42.5% dystrophin restoration.[105]

In a very recent study, intramuscular administration of ABEs, driven by ubiquitous or muscle-specific promoters, was employed to target and correct the nonsense mutations in the dystrophin gene of two humanized mouse models expressing human dystrophin gene exons with nonsense mutations in exon 23 or exon 30. The base editing treatment successfully restored dystrophin expression up to 59% in the skeletal muscle fibers of the humanized DMD mice.[106] Most notably, Lin J et al (2024) demonstrated systemic delivery of ABE via AAV in the humanized DMD mice improves the muscle function to the similar level of wild type with up to 96% restoration of dystrophin expression in critical muscle tissues, including the heart, tibialis anterior, and diaphragm muscles.[107]

Although these studies collectively underscore the transformative potential of base editing in treating DMD, limited target specificity, off-target changes to the genome, large size of dCas9-deaminase fusion protein are the current major weakness of base editing.[96,108,109] Additionally, newly engineered prime editing can also function as BEs, allowing for all possible base conversions at a specific position of the genome.

Prime editing gRNA (pegRNA) in combination with nickase Cas9 and a spacer complementary to the target site can perform targeted and precise small insertions, deletions, or base changing[62,110] (Figure 1D). Chemello et al (2021) were the first to evaluate prime editing in the context of DMD.[95] The study utilized PE in cardiomyocytes derived from human DMD ΔEx51 iPSCs to restore the open reading frame (ORF) of dystrophin transcripts by inserting two nucleotides into downstream exon 52. This editing resulted in functional dystrophin protein expression at 24.8–39.7% of normal levels in the corrected cardiomyocytes. Additionally, contractile function was improved in cardiomyocytes differentiated from the edited iPSCs.

A major limitation of prime editing is the rapid decline in editing efficiency when the target nucleotide is distant from the nick site, reducing flexibility in target site selection.[111] To address this limitation, Happi MC et al optimized the reverse transcription template (RTT) length to enhance editing efficiency.[111] This optimization was applied to correct the c.8713C>T mutation in DMD exon 59 in patient-derived myoblasts. The approach led to a 3.8-fold improvement in the correction of the target nucleotide located at position +13 from the nick site. Collectively, the optimized prime editing achieved a 22% mutation correction rate, restoring 42% dystrophin expression in differentiated myotubes. In a related study, PE was used to correct the c.428 G>A point mutation in exon 6 of the DMD gene in patient-derived myoblasts.[112] Electroporation of myoblasts yielded up to 28% modifications, resulting in detectable dystrophin expression in myotubes. These findings highlight the potential of prime editing as a precise and versatile tool for correcting mutations in the *DMD* gene.

Exon Knock-In

In contrast to the previously discussed strategies, the exon knock-in approach uses CRISPR-induced homology-dependent repair (HDR) to incorporate a DNA donor template, enabling the restoration of full-length dystrophin protein[12] (Figure 1E and F). This approach is particularly critical for mutations in the N- or C-terminal regions of dystrophin, which are functionally important and cannot tolerate CRISPR-mediated deletions.[19] However, exon knock-in via HDR is generally unsuitable for restoration dystrophin expression in DMD due to its low efficiency in post-mitotic cells.[61] Nevertheless, HDR-based strategies have been utilized to address point mutations in DMD animal models and single-exon deletions in human DMD iPSCs.[61]

In one of the earliest studies employing HDR-mediated correction of the *DMD* gene, Long et al (2014) injected a sgRNA targeting exon 23 of the *Dmd* gene and a single-stranded oligodeoxynucleotide (ssODN) as a donor template for HDR-mediated gene repair into zygote of *mdx* mice.[68] The study reported a 41% correction rate of the *mdx* alleles through HDR, leading to dystrophin restoration in various muscle tissues, albeit at different rates in 7- to 9-week-old mice. Despite the remarkable efficiency of HDR-mediated gene editing, it was still only half of what was achieved using non-homologous end joining (NHEJ)-mediated in-frame correction. In a similar study, the authors employed a 180-bp ssODN with LbCpf1 and sgRNA in zygotes to correct a point mutation in exon 23.[113] The correction rates ranged from 8% to 50%, resulting in full restoration of dystrophin protein in multiple skeletal muscle groups, heart, and brain, with no signs of fibrosis or inflammatory infiltration. Moreover, the SpCas9 system and donor templates have been used to insert the missing exon 44 into DMD ΔEx44 iPSCs, enabling the generation of full-length human dystrophin protein in derived cells.[71]

A study by Bengtsson NE et al (2017) utilized CRISPR/Cas9-mediated HDR to target exon 53 in *mdx*^{4Cv} mice.[90] AAV6-encoded SpCas9, sgRNA, and a donor template were delivered intramuscularly to restore full-length wild-type dystrophin. HDR-mediated gene editing achieved an efficacy of 0.18% in total genomes, approximately 47.7% lower than the efficiency of NHEJ for the same target region. Although HDR was not the primary focus of this study, it highlighted the limited applicability of HDR in post-mitotic cells, such as mature muscle fibers, due to its reliance on the cell cycle.

In addition to mouse models, HDR-mediated correction of the *Dmd* gene has been explored in canine models. A study by Mata López S et al (2020) demonstrated intramuscular injections of HDR-CRISPR/Cas9 in GRMD dogs resorted up to 16% dystrophin protein expression of normal levels.[114] However, the treatment did not yield significant improvements in histopathologic analysis or muscle force measurements. The findings underscore the necessity of further optimization of HDR-based gene editing strategies to enhance their efficacy for the treatment of DMD.

To address the limitations of conventional approaches, a recently developed method known as homology-independent targeted integration (HITI) provides a more efficient alternative for exon knock-in in post-mitotic cells.[115] HITI is an NHEJ-based knock-in technique that uses an exogenous donor template flanked by Cas9 cleavage sites. Cas9-mediated cleavage of the genomic target and donor template enables the NHEJ machinery to integrate the donor DNA precisely and efficiently. HITI-mediated exon knock-in was recently applied to insert the missing human exon 52 into hDMDΔ52/*mdx* mice.[116] Both intramuscular and systemic delivery of the SaCas9 system and AAV-encoded DNA templates restored full-length dystrophin expression in skeletal and cardiac muscles, with up to 50% dystrophin-positive cells observed in one mouse. Additionally, the study demonstrated the potential of delivering a "superexon" encompassing the last 28 exons of the *DMD* gene, which could be applicable to a broader range of DMD mutations, potentially benefiting over 20% of DMD patients.[116]

Alternative Approaches

Modulating disease-modifier genes offers a promising therapeutic approach for all DMD patients, regardless of their specific dystrophin mutation. One such modifier, utrophin (*UTRN*), a dystrophin homolog,[117,118] has shown potential to partially compensate for the lack of dystrophin in DMD.[119] While it plays a crucial membrane-stabilizing role in fetal muscle cells, utrophin is typically replaced by dystrophin during adulthood.[120,121] Studies have demonstrated that increasing *UTRN* expression can alleviate dystrophinopathy symptoms in a dose-dependent manner without inducing toxicity, highlighting its therapeutic potential.[122,123]

Given the large size of utrophin cDNA, which exceeds the packaging capacity of most viral vectors, alternative strategies have been explored to upregulate *UTRN* expression. CRISPR activation (CRISPRa) systems have shown significant promise in this regard (Figure 1G). For instance, Wojtal et al (2016) employed a catalytically inactive SpCas9 fused to a VP160 transcriptional activator (dCas9-VP160) to target the *UTRN* promoter in Δexon 45–52 DMD myoblasts.[124] This approach led to a 1.7- to 6.9-fold increase in utrophin expression compared to basal levels. Similarly, Liao et al used a different CRISPRa system in *mdx* mice, achieving a 3-4-fold increase in *UTRN* expression in vivo, along with improved hind-limb grip strength compared to Cas9/*mdx* controls after two months of treatment.[125]

In a recent study, Andrysiak et al (2024) applied a CRISPR-dCas9-VP64 system with sgRNA targeting the *UTRN* promoter in DMD hiPSC-CMs. This approach resulted in a nearly 4-fold increase in *UTRN* mRNA levels, which preserved the physiological functions of dystrophic hiPSC-CMs, including intracellular Ca^{2+} handling.[126]

Another strategy for upregulating utrophin involves CRISPR/Cas9-mediated genome editing to delete the inhibitory miRNA target region (IMTR) within the *UTRN* 3′ UTR. Sengupta et al (2020) demonstrated this approach in DMD patient-derived human induced pluripotent stem cells (DMD-hiPSCs), resulting in a 2-fold increase in utrophin protein levels.[127] Functional improvements were also observed in myotubes differentiated from the edited iPSCs.

In addition to CRISPR-mediated utrophin upregulation, efforts have focused on enhancing the expression of dystrophin variants. For instance, Cure Rare Disease, Inc. initiated the first CRISPR-based n-of-1 clinical trial for DMD (NCT05514249), targeting a 27-year-old patient with an exon 1 deletion.[128] The trial employed AAV9-dCas9-VP64 to upregulate dystrophin expression. However, the patient experienced severe adverse events, including cardiac dysfunction and acute respiratory distress syndrome, which were later found to be attributed to the high AAV dose rather than the CRISPR technology itself.

DMD Animal Models Generated by CRISPR-Mediated Genome Editing

In addition to its therapeutic applications, CRISPR/Cas9 technology has transformed the creation of animal models for DMD by providing a rapid, cost-effective, and precise method for introducing specific genetic mutations. Unlike traditional methods that take years, CRISPR reduces the timeline to months and allows simultaneous targeting of multiple genes using guide RNAs (gRNAs).[129,130] With minimal reagents and direct microinjection into zygotes, it eliminates the need for complex processes like embryonic stem cell use. The high efficiency and ease of use associated with CRISPR/Cas9 make it an invaluable tool for developing animal models that closely mimic human diseases like DMD.[131–134] These models are crucial for understanding disease mechanisms and testing potential treatments, significantly advancing research in neuromuscular disorders.

To date, over 60 different animal models of DMD have been identified or generated across various species, including *Caenorhabditis elegans*, Drosophila, zebrafish, rodents, rabbits, dogs, pigs, and nonhuman primates.[135]

Mice Model

ΔEx50 Mouse Model: Created by Amoasii et al (2017), this model carries an out-of-frame deletion of exon 50 in the *Dmd* gene, leading to dystrophin absence in skeletal and cardiac muscles and exhibiting dystrophic symptoms.[74] A variant with a luciferase construct was later developed to enable non-invasive monitoring of dystrophin restoration.[136]

ΔEx44 Mouse Model: Developed by Min et al (2019), this model features exon 44 deletion, showing a lack of dystrophin in skeletal and cardiac muscles and severe dystrophic pathology, making it suitable for testing exon-skipping therapies targeting distal exon 43–55 hotspots.[76]

ΔEx8-34 Model: Generated by Egorova et al (2019), this model harbors the largest deletion created in vivo, spanning exons 8–34. It displays severe dystrophic features and is used for testing therapies targeting the proximal exon 1–22 mutation hotspot.[137]

Exon 23 Frameshift Models: Koo et al (2018) created models with a 14 bp deletion or 1 bp insertion in exon 23, resulting in dystrophin loss and muscle dysfunction. These models demonstrated the potential of smaller Cas9 variants like CjCas9 for DMD therapies.[138]

Point Mutation Model (D108): Kim et al (2017) introduced a point mutation in exon 20 using base-editing technology, leading to a premature stop codon.[139] While phenotypic analysis was limited, this model serves as proof of concept for base-editing therapeutic approaches.

hDMD del45: This humanized model carries an exon 45 deletion, mimicking common patient mutations. It exhibits dystrophic pathology when crossed with *mdx* backgrounds and is used to test therapies like exon 45–55 skipping.[89]

Δ52-54 mouse model: Wong et al (2020) generated a DMD mouse model which carries a deletion of exons 52 to 54 in the dystrophin gene.[140] This genetic alteration leads to the absence of dystrophin protein, resulting in progressive muscle deterioration and weakened muscle strength. Notably, these mice also present with early-onset hypertrophic cardiomyopathy, a condition that is absent in some existing DMD mouse models.

$DMD^{\Delta mE5051,KIhE50/Y}$ mice: In this model, mouse exons 50 and 51 of the *Dmd* gene are replaced with the human exon 50 sequence, effectively mimicking the human condition.[107] Lin et al (2024) knocked in the human exon 50 sequence to replace both exon 50 and 51 of mouse *Dmd* gene in a single step. This humanized DMD mouse model exhibited disorganized muscle fiber and severe fibrosis in muscle but not heart tissue.

Other Animal Models

Although DMD mice model are easily reproducible and cost-effective, they sometimes show mild symptoms compared to human DMD patients. For example, for *mdx* model they do not develop pronounced pathological characteristics until around 15 months of age and have a relatively minor reduction in lifespan of about 25%.[61] One possible reason behind this mild phenotype can be attributed to their strong muscle regeneration capabilities and the compensatory upregulation of utrophin.[141,142] To address these limitations, researchers have sought to replicate the DMD phenotype in other animal models.

Nakamura et al (2014) developed several DMD rat models using CRISPR/Cas9 technology.[143] By targeting exons 3 and 16 of the *Dmd* gene with two gRNAs and Cas9 mRNA, the authors generated mutations characterized by deletions (1–577 bp) or insertions (1–4 bp), with deletions being more frequent. Some rats exhibited mosaicism. Out-of-frame mutants demonstrated complete dystrophin loss in the tibialis anterior (TA) muscle, while in-frame mutants showed reduced dystrophin levels. Both mutant types exhibited impaired muscle function and significantly elevated serum creatine kinase (CK) levels.

In addition to skeletal muscle pathology, the diaphragm and heart displayed dystrophic changes, with the cardiac abnormalities representing a notable advantage of this model over the ***mdx*** mouse model, although further investigation is required.[144] The larger body size and continuous disease progression in these rats make them a promising alternative for studying DMD and its therapeutic interventions.[144–146]

A DMD rabbit model was developed by Sui et al (2018) using CRISPR/Cas9 technology.[147] Cas9 mRNA and two gRNAs targeting exon 51 of the *Dmd* gene were co-injected into rabbit zygotes, resulting in 26 mutant rabbits out of 33 live births from 128 injected zygotes. These mutants displayed various indels, including cases with multiple mutations in the same or both alleles. Mutant rabbits showed significantly reduced DMD transcript levels and a complete loss of dystrophin protein. Reduced levels of α-sarcoglycan and glycosylated α-dystroglycan were also observed at skeletal muscle membranes. Dystrophic phenotypes included reduced muscle fiber, increased fibrosis and abnormal cardiac function with reduced ejection fraction (EF) and fractional shortening (FS) by 4 months. Additionally, rabbits are highly productive, with year-round breeding capabilities, shorter gestation periods, and relatively low maintenance costs compared to larger animals.[147–149] However, despite its physiological relevance and potential for studying DMD progression and cardiac pathology, the DMD rabbit model has not yet been utilized for therapeutic testing, limiting its application in preclinical studies.[150]

Pigs have emerged as a promising model for DMD research due to their genetic, physiological, and anatomical similarities to humans, particularly in cardiac structure and function. The first CRISPR/Cas9-generated DMD pig model was developed by Klymiuk et al (2013) through exon 52 deletions.[151] However, this model exhibited early mortality, with most piglets dying within the first week of life. Subsequently, in 2016, Yu et al employed CRISPR/Cas9 technology to target exon 27 of the *Dmd* gene in Diannan miniature pigs.[152] They microinjected Cas9 mRNA and a single-guide RNA (sgRNA) into zygotes, resulting in the birth of two piglets, one of which carried multiple indel mutations in the

Dmd gene. This mutant pig exhibited reduced dystrophin levels in skeletal muscles and the heart, along with typical dystrophic features such as decreased myofiber size and necrosis. However, again both piglets died shortly after birth, with the mutant surviving only 52 days. The early mortality observed in these models presents a significant challenge for utilizing porcine DMD models in preclinical studies, as the short lifespan limits the ability to comprehensively evaluate disease progression and therapeutic interventions. Despite these limitations, the close anatomical and physiological parallels between pigs and humans make porcine models particularly valuable for studying the cardiac aspects of DMD. Additional challenges include the longer gestational period of pigs (approximately 114 days), their substantial space requirements, higher maintenance costs, and regulatory restrictions on the use of genetically engineered pigs.[148,153,154] Despite these limitations, the anatomical and physiological similarities between pigs and humans, particularly in cardiac structure and function, make them valuable for studying the cardiac aspects of DMD.[150]

Although no CRISPR/Cas9-generated canine model for DMD currently exists, canine DMD models closely replicate the disease progression and severity observed in human patients, including limb muscle fibrosis and cardiomyopathy.[155–157] In Japan, studies utilizing canine DMD models, such as the Golden Retriever model of DMD (GRMD)[155] and Canine X-linked Muscular Dystrophy in Japan (CXMDJ) dogs,[158] have demonstrated that these models may be more suitable for preclinical translational research compared to other large animal models. However, the widespread use of canine DMD models in therapeutic translation is significantly constrained by the substantial economic costs and the extended time required to breed these animals in sufficient numbers.[148,153,154]

The first CRISPR/Cas9-generated monkey model for DMD was developed by Chen et al in 2015.[159] The researchers used CRISPR/Cas9 to introduce mutations in exons 4 and 46 of the *DMD* gene in rhesus monkeys (*Macaca mulatta*). A total of 179 embryos were injected, resulting in two stillborn and nine live monkeys carrying the desired mutations. For stillborn monkeys, three different frameshifting mutations in exon 4: a 2 bp deletion and 2 bp and 20 bp insertions was found. Histological analysis revealed dystrophic features, including reduced fiber size, hypertrophic myofiber clusters, 12.5–17.5% centrally nucleated fibers (CNFs), and increased interstitial space. These findings suggest early-onset dystrophic symptoms before birth. The live monkeys displayed various frameshifting mutations in exons 4 and/or 46, but no phenotypic analysis was conducted. A subsequent study by the same group confirmed the absence of off-target edits in coding regions for two live mutant monkeys, with minimal off-target indels detected in intergenic and intronic regions.[160] While monkeys share the highest genetic and physiological similarity to humans, challenges such as high costs, long gestational periods, the lack of phenotypic data for live mutants, and ethical considerations make the use of this model in therapeutic testing for DMD highly challenging.[150,159]

Challenges and Future Direction

CRISPR-based therapies have gained significant attention in recent years for their potential to revolutionize the treatment of genetic diseases. Approval of the first CRISPR/Cas9-based therapy for sickle cell disease on December 8, 2023,[161] highlights the transformative potential of genome editing in treating genetic disorders. In the context of DMD, CRISPR holds promise as a ground-breaking approach to address the root genetic causes of the disease. However, its application remains experimental and is still in the early stages of development due to several challenges in this field. This discussion aims to outline the key challenges associated with the use of CRISPR for DMD therapy and explore potential solutions to enable therapeutic genome editing to become a reality for DMD patients.

Editing Efficiency and Durability

Optimizing CRISPR technology through the precise design of editing strategies and efficient delivery of CRISPR components to various tissues is pivotal for developing effective therapies for DMD. Over the years, researchers have developed different strategies to enhance this technology. For instance, the development of single-cut editing approaches has resolved challenges associated with double-cut methods. Innovative techniques such as Homology-Independent Targeted Integration (HITI)[115,116] and Single Homology Arm Donor-Mediated Intron-Targeting Integration (SATI)[162] have been established to enable robust DNA knock-in in both proliferating and quiescent cells. Alternative strategies focusing on overexpressing utrophin, a paralog of dystrophin, have also been explored to compensate for dystrophin's functional deficiency.[127,163] Additionally, refining newly developed technologies such as CRISPR-associated

transposases, guide RNA-assisted targeting of transposable elements, twin prime editors, and programmable site-specific integration methods could pave the way for therapies capable of fully restoring dystrophin protein levels, offering transformative potential for DMD treatment.[164–167]

Given the multinucleated nature of myofibers, complete correction of all nuclei may not be necessary to achieve therapeutic benefits. Clinical findings suggest that restoring dystrophin expression to levels between 4% and 50% can shift a DMD phenotype to the milder Becker Muscular Dystrophy (BMD) phenotype.[168] However, due to the high turnover rates of skeletal muscles in DMD patients, corrected myofibers may eventually be replaced by dystrophic ones, limiting the long-term effectiveness of treatment.[169,170] Since muscle regeneration is driven by satellite cells undergoing de novo myogenesis, targeting these cells with CRISPR components could theoretically ensure durable therapeutic benefits. Several studies have shown that genomic editing in muscle satellite cells can maintain restored dystrophin expression for up to 18 months in the skeletal muscles of *mdx* mice.[171–174]

An alternative strategy involves co-delivering micro-dystrophin with CRISPR gene-editing components to achieve prolonged dystrophin restoration.[175] A recent study reported that systemic administration of an AAV6-encoded micro-dystrophin gene in DMD *mdx*^{4Cv} mice stabilizes skeletal myofibers, prevents the loss of CRISPR components, and ensures persistent dystrophin expression in skeletal muscles.[175] Given the success of micro-dystrophin gene therapy in clinical trials and its subsequent FDA approval, this dual approach may provide sustained therapeutic benefits, addressing key challenges in DMD management.

Immunogenicity

Adeno-associated virus (AAV) vectors are currently the most effective means for systemic delivery of CRISPR components.[176,177] However, a significant challenge in using AAV vectors is the activation of innate and adaptive immune responses. Pre-existing anti-AAV antibodies are present in a substantial proportion of the population, rendering some DMD patients with high levels of AAV-neutralizing antibodies ineligible for AAV-based therapies.[178,179] Additionally, the high doses of AAV, typically ranging from 5.5×10^{14} to 1.8×10^{15} vector genomes (vg)/kg, required to targ et al l muscles in the human body can lead to adverse effects.[76,83,90,180] The administration of high-dose AAV9 (at least 1.5×10^{114} vg/kg) has been associated with adverse effects, including liver toxicity and kidney injury, in animal models such as dogs, piglets, and nonhuman primates.[181,182] Furthermore, systemic delivery of AAV9 carrying the human micro-dystrophin gene in clinical trials for DMD patients, at doses between 5×10^{13} and 3×10^{14} vg/kg, has been linked to severe adverse events such as cardiopulmonary insufficiency and thrombocytopenia, with a recent patient fatality raising additional concerns.[183] To address these issues, interventions such as plasmapheresis or immunosuppressive treatments may be employed to lower anti-AAV antibody titers or suppress the host immune system before initiating therapy.[64,184] Moreover, novel AAV capsid variants like AAVMYO and MyoAAV, which exhibit enhanced muscle tropism, are being developed.[185–187] These variants have the potential to reduce the required viral dose by at least tenfold, significantly improving safety. Self-complementary AAV (scAAV) vectors have been developed to deliver sgRNA expression cassettes to skeletal and cardiac muscles.[188] Unlike single-stranded AAV (ssAAV) vectors, scAAV bypasses the need for second-strand synthesis, is more resistant to degradation, and requires at least 20-fold lower doses to achieve effective genome modification.[188–190]

While the dual-AAV system offers an advantage in achieving the necessary sgRNA-to-Cas9 ratio for efficient systemic editing, the development of "all-in-one" AAV vectors that combine small Cas9 variants and sgRNAs into a single vector could further reduce the required viral dose and simplify manufacturing processes.[13,138,191]

Beyond AAV vectors, immune responses specific to Cas proteins, which are derived from bacterial and archaeal sources, have been observed in both animal models and humans. For example, antibodies and T cells targeting SaCas9 and SpCas9 have been detected in approximately 78% and 58–67% of healthy individuals, respectively.[192] However, systemic delivery of AAV-SaCas9 components in neonatal mice did not elicit cellular or humoral immune responses against AAV vectors or Cas proteins, possibly due to the lower AAV dosage requirement, better-preserved muscle condition, and absence of pre-existing immunity in young animals.[11,83] Furthermore, engineered Cas9 proteins with reduced immunogenicity have been developed, providing a promising strategy to minimize immune activation.[193] Another component of CRISPR/Cas gene editing, sgRNAs, has been shown to stimulate innate immune responses

in vitro, though their immunogenicity in vivo remains uncertain.[194,195] Further research is needed to explore post-transcriptional modifications of sgRNAs to reduce potential immunogenic effects in vivo.

Since corticosteroids are commonly used in DMD patients to manage inflammation, their concurrent administration with CRISPR gene therapy presents a practical strategy to mitigate immune responses, enhancing the feasibility and safety of the treatment. Additionally, concerns regarding immune responses to restored dystrophin protein appear minimal, as DMD patients typically exhibit a low frequency of spontaneous "revertant fibers" in which the reading frame is naturally corrected.[196] Consequently, dystrophin is unlikely to be recognized as foreign by the immune system, further supporting the therapeutic potential of CRISPR-based approaches for DMD.

Off-Target Activity

The potential for off-target activity in CRISPR systems remains a significant challenge for clinical applications. Despite efforts to design precise single-guide RNAs (sgRNAs), the CRISPR/Cas9 system can tolerate minor mismatches, potentially causing double-stranded breaks (DSBs) at unintended genomic locations. Additionally, SpCas9 has been reported to exhibit relatively high off-target activity due to its tolerance for up to five mismatches between the sgRNA sequence and the target site.[197–199] While most off-target events are observed in highly proliferative cells in vitro, studies suggest that off-target genome editing is minimal in post-mitotic skeletal and cardiac cells.[11,12]

To reduce this potential outcome, different high-fidelity Cas enzymes, such as HypaCas9, evoCas9, SpCas9-HF1, and enAsCas12a-HF1, have been developed).[[61,199–204]] Cas12 proteins, in particular, demonstrate lower off-target activity compared to Cas9 variants, making them a safer alternative for therapeutic applications.[60,205] Another approach involves paired nicking, where a nickase Cas9 (nCas9) induces offset single-stranded breaks instead of DSBs. These single-stranded breaks are repaired via base-excision repair, a high-fidelity DNA repair pathway, significantly reducing off-target mutagenesis.[206]

Optimizing sgRNAs also plays a crucial role in improving specificity. Truncated sgRNAs, shorter than 20 nucleotides, have been shown to decrease genome-wide off-target events by up to fivefold.[207] Additionally, muscle-specific promoters can be employed to restrict CRISPR component expression exclusively to muscle tissue, reducing the risk of unintended editing in non-target tissues such as the liver and kidneys.[74,90,208]

Computational tools, including Cas-OFFinder and CRISPRscan, assist in designing sgRNAs with high efficiency and minimal off-target potential. Furthermore, advanced techniques such as Digenome-seq, GUIDE-seq, and CIRCLE-seq enable the identification of a broader range of potential off-target sites, facilitating comprehensive assessments of CRISPR-mediated genome editing.[14] These strategies collectively enhance the precision and safety of CRISPR-based therapeutics, paving the way for more effective clinical applications.

Delivery

Adeno-associated virus (AAV) vectors are widely utilized for delivering CRISPR components in DMD studies due to their ability to efficiently transduce both dividing and non-dividing cells. Their high transduction efficiency, low immunogenicity, and tissue tropism toward skeletal and cardiac muscles make them particularly suitable for DMD therapy.[14,209] However, AAV vectors face significant limitations, including a packaging capacity of approximately 4.7 kilobases (kb).[57] Since the SpCas9 protein alone (~4 kb) nearly fills this limit, adding components such as sgRNAs, transcriptional activators, or reverse transcriptases becomes impractical. To address this limitation, smaller Cas9 orthologs like SaCas9 and CjCas9 or dual AAV vectors are used to deliver separate components.[178,210] However, the dual vector system often requires higher viral doses, increasing the risk of toxicity. Additionally, AAV vectors pose risks such as integration into double-stranded DNA break (DSB) sites, potentially leading to unintended mutagenesis, and their accumulation in the liver raises concerns about dose-dependent toxicity and adverse effects.[11,211]

Lentiviral vectors (LVs) have also been explored as delivery vehicles for CRISPR components in DMD patient-derived myoblasts. With a relatively large packaging capacity (~8 kb) and the ability to transduce both mitotic and post-mitotic cells, LVs present an alternative option.[212–214] However, their potential for insertional mutagenesis and limited systemic bioavailability in skeletal muscle hinder their broader application in DMD models.[215] Adenovirus (AdV), another viral vector, has been employed for delivering CRISPR components in DMD therapy.[216,217] While AdV

efficiently transduces immature or regenerative muscle cells, its efficacy in mature skeletal muscle fibers remains limited.[218] Despite this, AdV has demonstrated promise in in vitro studies by removing large fragments of the DMD mutational hotspot and restoring dystrophin expression up to 50% in intramuscularly treated *mdx* mice, improving muscle function.[216,217]

Non-viral vectors, including Lipid Nanoparticles (LNPs), Polymers, Gold Nanoparticles (GNPs), and Virus-Like Particles (VLPs), provide alternative approaches for CRISPR component delivery in DMD therapy.[212] These nanoparticles are internalized by cells via endocytosis and offer several advantages, such as reduced immunogenicity, lower costs, and minimized off-target effects.[12,219,220] Among these, LNPs have been extensively studied. Initially approved for siRNA delivery,[221] LNPs gained prominence with their use in the first U.S.-approved SARS-CoV-2 vaccines.[222,223] In DMD therapy, Kenjo E. et al (2021) demonstrated that LNP-encapsulated Cas9 mRNA and gRNA restored functional dystrophin expression by excising exon 45 in a Δexon 44 humanized mouse model and DMD patient-derived myoblasts.[224] Similarly, Mata López S. et al (2020) showed that a commercial LNP encapsulating plasmid expressing Cas9 and sgRNAs successfully restored dystrophin expression through HDR-mediated insertion of exon 7 in the GRMD model.[114] Wei et al (2020) reported a 4.2% restoration of dystrophin protein following LNP delivery in ΔEx44 DMD mice.[225]

Gold Nanoparticles (GNPs) have also been explored for delivering CRISPR/Cas9 components in DMD therapy due to their diverse surface coatings,[226] nontoxic core, and the range of their core sizes.[227] Lee et al pioneered the use of CRISPR-Gold for DMD-related gene editing, demonstrating minimal broad immune activation with no upregulation of pro-inflammatory cytokines in plasma.[228] However, localized inflammation was observed two weeks post-injection, with increased recruitment of innate immune cells. Additionally, the gene editing efficiency with CRISPR-Gold was reported to be around 1%.

Last but the not the least, virus-Like Particles (VLPs), composed of viral proteins but lacking a viral genome, have emerged as a promising delivery system for CRISPR/Cas9 components[229,230] in DMD therapy. VLPs are modular and capable of packaging mRNA, RNPs, or proteins, allowing for high editing efficiency while avoiding the risks of viral genome integration.[229,230] For DMD, a VLP system containing a VSV-G protein and an RNP targeting exon 53 restored dystrophin expression in the TA muscles of the del52hDMD/*mdx* model.[231] Additionally, the NanoMEDIC VLP delivery system achieved over 90% exon skipping in DMD patient-derived iPS-myoblasts, sustained exon skipping in a luciferase reporter mouse model, and 1.6% exon 23 skipping in the *mdx* mouse model.[232] These findings highlight the potential of VLPs as a non-viral delivery platform for CRISPR therapeutics in DMD.

All these studies suggest that, while viral vectors remain prominent tools for delivering CRISPR components for DMD therapy, their inherent limitations, such as size constraints, potential immunogenicity, and toxicity risks, necessitate the exploration of alternative delivery systems. Non-viral vectors have the potential to revolutionize CRISPR-based DMD therapies and address key challenges in clinical translation. However, further study is needed to increase their efficacy and specificity.

Concluding Remarks

CRISPR/Cas9-mediated gene editing has emerged as a transformative therapeutic strategy for Duchenne muscular dystrophy (DMD), offering the prospect of a one-time, potentially curative intervention that addresses the disease at its genetic root. This stands in contrast to current FDA-approved therapies such as exon-skipping antisense oligonucleotides and micro-dystrophin gene therapy which remain prohibitively expensive and inaccessible to patients in developing nations. For instance, the annual cost of exon-skipping therapies such as eteplirsen (Exondys 51) exceeds $1 million per patient, while delandistrogene moxeparvovec is priced at $3.2 million for a single infusion (FDA, 2023; Institute for Clinical and Economic Review, 2023). Such exorbitant costs severely limit global accessibility, particularly in resource-limited regions. In contrast, CRISPR-based interventions aim to permanently restore dystrophin expression through a single administration, eliminating the need for lifelong treatments and their cumulative financial burden. Encouragingly, the recent approval of the first CRISPR-based therapy for sickle cell disease marks a pivotal milestone in the field of gene editing, demonstrating the feasibility of translating this technology into clinical applications. This achievement highlights the potential of CRISPR-based therapies to address other monogenic disorders, including DMD. Given the substantial

progress in preclinical research involving patient-derived cell lines and animal models, CRISPR/Cas9-based therapies are poised to become a viable treatment for DMD within the next decade. Advances in the precision, safety, and delivery of CRISPR systems are likely to address current challenges, such as efficient systemic delivery, consistent editing across affected tissues, and minimizing potential risks like off-target effects and immunogenicity. As the field continues to evolve, the collaboration of scientists, clinicians, and regulatory agencies will be paramount in navigating the complexities of gene-editing therapies. With continued innovation and concerted efforts, CRISPR-based treatments hold the potential to revolutionize the management of DMD and significantly improve patient outcomes.

Disclosure

Prof. Dr. Toshifumi Yokota reports being co-founder and shareholder of OligomicsTx which aims to commercialize antisense technology. The authors report no other conflicts of interest in this work.

References

1. O'Brien KF, Kunkel LM. Dystrophin and muscular dystrophy: past, present, and future. *Mol Gene Metabol*. 2001;74(1–2):75–88. doi:10.1006/mgme.2001.3220.
2. Henricson EK, Abresch RT, Cnaan A, et al. The cooperative international neuromuscular research group Duchenne natural history study: glucocorticoid treatment preserves clinically meaningful functional milestones and reduces rate of disease progression as measured by manual muscle testing and other commonly used clinical trial outcome measures. *Muscle Nerve*. 2013;48(1):55–67. doi:10.1002/mus.23808
3. Yiu EM, Kornberg AJ. Duchenne muscular dystrophy. *J Paediatr Child Health*. 2015;51(8):759–764. doi:10.1111/jpc.12868.
4. Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol*. 2018;17(3):251–267. doi:10.1016/s1474-4422(18)30024-3
5. Passamano L, Taglia A, Palladino A, et al. Improvement of survival in Duchenne Muscular Dystrophy: retrospective analysis of 835 patients. *Acta Myol*. 2012;31(2):121–125.
6. Hoffman EP, Brown Jr RH, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell*. 1987;51(6):919–928. doi:10.1016/0092-8674(87)90579-4.
7. Gloss D, Moxley RT, Ashwal S, Oskoui M. Practice guideline update summary: corticosteroid treatment of Duchenne muscular dystrophy. *Neurology*. 2016;86(5):465–472. doi:10.1212/wnl.0000000000002337.
8. McDonald CM, Henricson EK, Abresch RT, et al. Long-term effects of glucocorticoids on function, quality of life, and survival in patients with Duchenne muscular dystrophy: a prospective cohort study. *Lancet*. 2018;391(10119):451–461. doi:10.1016/s0140-6736(17)32160-8
9. Salmaninejad A, Valilou SF, Bayat H, et al. Duchenne muscular dystrophy: an updated review of common available therapies. *Int J Neurosci*. 2018;128(9):854–864. doi:10.1080/00207454.2018.1430694
10. Nelson CE, Robinson-Hamm JN, Gersbach CA. Genome engineering: a new approach to gene therapy for neuromuscular disorders. *Nat Rev Neurol*. 2017;13(11):647–661. doi:10.1038/nrneurol.2017.126.
11. Olson EN. Toward the correction of muscular dystrophy by gene editing. *Proc Natl Acad Sci*. 2021;118(22):. doi:10.1073/pnas.2004840117
12. Min Y-L, Bassel-Duby R, Olson EN. CRISPR correction of Duchenne Muscular Dystrophy. *Annl Rev Med*. 2019;70(1):239–255. doi:10.1146/annurev-med-081117-010451.
13. Chemello F, Olson EN, Bassel-Duby R. CRISPR-editing therapy for Duchenne Muscular Dystrophy. *Human Gene Ther*. 2023;34(9–10):379–-387. doi:10.1089/hum.2023.053.
14. Chemello F, Bassel-Duby R, Olson EN. Correction of muscular dystrophies by CRISPR gene editing. *J Clin Investig*. 2020;130(6):2766–2776. doi:10.1172/jci136873.
15. Bladen CL, Salgado D, Monges S, et al. The TREAT-NMD DMD Global Database: analysis of More than 7,000 Duchenne Muscular Dystrophy Mutations. *Human Mutation*. 2015;36(4):395–402. doi:10.1002/humu.22758
16. White SJ, Aartsma-Rus A, Flanigan KM, et al. Duplications in theDMD gene. *Human Mutation*. 2006;27(9):938–945. doi:10.1002/humu.20367
17. Nakamura A, Fueki N, Shiba N, et al. Deletion of exons 3−9 encompassing a mutational hot spot in the DMD gene presents an asymptomatic phenotype, indicating a target region for multiexon skipping therapy. *J Human Genet*. 2016;61(7):663–667. doi:10.1038/jhg.2016.28
18. Nakamura A, Shiba N, Miyazaki D, et al. Comparison of the phenotypes of patients harboring in-frame deletions starting at exon 45 in the Duchenne muscular dystrophy gene indicates potential for the development of exon skipping therapy. *J Human Genet*. 2016;62(4):459–463. doi:10.1038/jhg.2016.152
19. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol*. 2003;2(12):731–740. doi:10.1016/s1474-4422(03)00585-4.
20. England SB, Nicholson LVB, Johnson MA, et al. Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. *Nature*. 1990;343(6254):180–182. doi:10.1038/343180a0
21. Matsumura K, Burghes AH, Mora M, et al. Immunohistochemical analysis of dystrophin-associated proteins in Becker/Duchenne muscular dystrophy with huge in-frame deletions in the NH2-terminal and rod domains of dystrophin. *J Clin Investig*. 1994;93(1):99–105. doi:10.1172/jci116989
22. Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics*. 1988;2(1):90–95. doi:10.1016/0888-7543(88)90113-9.
23. Anwar S, He M, Lim KRQ, Maruyama R, Yokota T. A genotype-phenotype correlation study of Exon skip-equivalent in-frame deletions and Exon skip-amenable out-of-frame deletions across the DMD gene to simulate the effects of Exon-skipping therapies: a meta-analysis. *J Personalized Med*. 2021;11(1):46. doi:10.3390/jpm11010046.

24. Malhotra SB, Hart KA, Klamut HJ, et al. Frame-shift deletions in patients with Duchenne and Becker Muscular Dystrophy. *Science*. 1988;242 (4879):755–759. doi:10.1126/science.3055295
25. Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet*. 1989;45(4):498–506.
26. Sabrina Haque U, Kohut M, Yokota T. Comprehensive review of adverse reactions and toxicology in ASO-based therapies for Duchenne Muscular Dystrophy: from FDA-approved drugs to peptide-conjugated ASO. *Curr Res Toxicol*. 2024;7(100182):. doi:10.1016/j.crtox.2024.100182
27. Lim KR, Maruyama R, Yokota T. Eteplirsen in the treatment of Duchenne muscular dystrophy. *Drug Des Devel Ther*. 2017;11:533–545. doi:10.2147/DDDT.S97635
28. FDA. FDA grants accelerated approval to first drug for Duchenne Muscular Dystrophy. Secondary FDA grants accelerated approval to first drug for Duchenne Muscular Dystrophy. 2016. Available from: https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-first-drug-duchenne-muscular-dystrophy. Accessed April 9, 2025.
29. FDA. FDA grants accelerated approval to first targeted treatment for rare Duchenne muscular dystrophy mutation. Secondary FDA grants accelerated approval to first targeted treatment for rare Duchenne muscular dystrophy mutation 2019. Available from: https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-first-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation#:~:text=The%20U,with%20DMD%20have%20this%20mutation. Accessed April 9, 2025.
30. MDA. Muscular Dystrophy Association Celebrates FDA Approval of Viltolarsen for Treatment of Duchenne Muscular Dystrophy Amenable to Exon 53 Skipping. Secondary Muscular Dystrophy Association Celebrates FDA Approval of Viltolarsen for Treatment of Duchenne Muscular Dystrophy Amenable to Exon 53 Skipping. 2020. Available from: https://www.mda.org/press-releases/muscular-dystrophy-association-celebrates-fda-approval-viltolarsen-treatment-duchenne#:~:text=DMD%20is%20caused%20by%20mutations,independently%2C%20and%20breathe%20without%20assistance. Accessed April 9, 2025.
31. FDA. FDA approves targeted treatment for rare Duchenne Muscular Dystrophy Mutation. Secondary FDA approves targeted treatment for rare Duchenne Muscular Dystrophy mutation 2020. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation#:~:text=Viltepso%20,amenable%20to%20exon%2053%20skipping. Accessed April 9, 2025.
32. Serepta. Sarepta therapeutics announces FDA approval of AMONDYS 45™ (casimersen) injection for the treatment of Duchenne Muscular Dystrophy (DMD) in patients amenable to skipping Exon 45. Secondary Sarepta therapeutics announces FDA Approval of AMONDYS 45™ (casimersen) Injection for the Treatment of Duchenne Muscular Dystrophy (DMD) in Patients Amenable to Skipping Exon 45. 2021. Available from: https://www.globenewswire.com/news-release/2021/02/25/2182845/0/en/Sarepta-Therapeutics-Announces-FDA-Approval-of-AMONDYS-45-casimersen-Injection-for-the-Treatment-of-Duchenne-Muscular-Dystrophy-DMD-in-Patients-Amenable-to-Skipping-Exon-45.html#:~:text=today%20announced%20that%20the%20U,approval%20of%20AMONDYS%2045%20may. Accessed April 9, 2025.
33. Vasterling ME, Maitski RJ, Davis BA, et al. AMONDYS 45 (Casimersen), a Novel Antisense Phosphorodiamidate Morpholino Oligomer: clinical Considerations for Treatment in Duchenne Muscular Dystrophy. *Cureus*. 2023;15(12):e51237. doi:10.7759/cureus.51237
34. FDA. FDA approves targeted treatment for rare Duchenne Muscular Dystrophy Mutation. Secondary FDA approves targeted treatment for rare Duchenne Muscular Dystrophy Mutation. 2021. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation-0#:~:text=For%20Immediate%20Release%3A%20February%2025%2C,2021. Accessed April 9, 2025.
35. Mendell JR, Muntoni F, McDonald CM, et al. AAV gene therapy for Duchenne muscular dystrophy: the EMBARK Phase 3 randomized trial. *Nat Med*. 2025;31(1):332–341. doi:10.1038/s41591-024-03304-z
36. FDA. FDA expands approval of gene therapy for patients with Duchenne Muscular Dystrophy. Secondary FDA expands approval of gene therapy for patients with Duchenne Muscular Dystrophy. 2024. Available from: https://www.fda.gov/news-events/press-announcements/fda-expands-approval-gene-therapy-patients-duchenne-muscular-dystrophy#:~:text=Duchenne%20muscular%20dystrophy%20,mutation%20in%20the%20DMD%20gene. Accessed April 9, 2025.
37. FDA. FDA approves first gene therapy for treatment of certain patients with Duchenne Muscular Dystrophy. Secondary FDA approves first gene therapy for treatment of certain patients with Duchenne Muscular Dystrophy. 2023. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapy-treatment-certain-patients-duchenne-muscular-dystrophy#:~:text=braces%20or%20a%20wheelchair%20as,mutations%20and%20require%20repeated%20administration. Accessed April 9, 2025.
38. Mullard A. FDA approves first gene therapy for Duchenne muscular dystrophy, despite internal objections. *Nat Rev Drug Discov*. 2023;22 (8):610. doi:10.1038/d41573-023-00103-y.
39. Shoti J, Qing K, Keeler GD, Duan D, Byrne BJ, Srivastava A. Development of capsid- and genome-modified optimized AAVrh74 vectors for muscle gene therapy. *Mol Ther Methods Clin Dev*. 2023;31:101147. doi:10.1016/j.omtm.2023.101147
40. Heier CR, Damsker JM, Yu Q, et al. VBP15, a novel anti-inflammatory and membrane-stabilizer, improves muscular dystrophy without side effects. *EMBO Mol Med*. 2013;5(10):1569–1585. doi:10.1002/emmm.201302621
41. Reeves EKM, Hoffman EP, Nagaraju K, Damsker JM, McCall JM. VBP15: preclinical characterization of a novel anti-inflammatory delta 9,11 steroid. *Bioorg Med Chem*. 2013;21(8):2241–2249. doi:10.1016/j.bmc.2013.02.009.
42. Hoffman EP, Schwartz BD, Mengle-Gaw LJ, et al. Vamorolone trial in Duchenne muscular dystrophy shows dose-related improvement of muscle function. *Neurology*. 2019;93(13). doi:10.1212/wnl.0000000000008168
43. Quattrocelli M, Zelikovich AS, Jiang Z, et al. Pulsed glucocorticoids enhance dystrophic muscle performance through epigenetic-metabolic reprogramming. *JCI Insight*. 2019;4(24). doi:10.1172/jci.insight.132402
44. Guglieri M, Bushby K, McDermott MP, et al. Effect of different corticosteroid dosing regimens on clinical outcomes in boys with Duchenne Muscular Dystrophy: a randomized clinical trial. *JAMA*. 2022;327(15):1456–1468. doi:10.1001/jama.2022.4315
45. Keam SJ. Vamorolone: first approval. *Drugs*. 2024;84(1):111–117. doi:10.1007/s40265-023-01986-2.
46. Lamb YN. Givinostat: first approval. *Drugs*. 2024;84(7):849–856. doi:10.1007/s40265-024-02052-1.
47. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816–821. doi:10.1126/science.1225829.

48. Cong L, Ran FA, Cox D, et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science*. 2013;339(6121):819–823. doi:10.1126/science.1231143
49. Mali P, Yang L, Esvelt KM, et al. RNA-Guided Human Genome Engineering via Cas9. *Science*. 2013;339(6121):823–826. doi:10.1126/science.1232033
50. Kakarougkas A, Jeggo PA. DNA DSB repair pathway choice: an orchestrated handover mechanism. *Br J Radiol*. 2014;87(1035):20130685. doi:10.1259/bjr.20130685.
51. Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nat Rev Mol Cell Biol*. 2010;11(3):196–207. doi:10.1038/nrm2851.
52. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem*. 2010;79(1):181–211. doi:10.1146/annurev.biochem.052308.093131.
53. Makarova KS, Wolf YI, Iranzo J, et al. Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. *Nat Rev Microbiol*. 2019;18(2):67–83. doi:10.1038/s41579-019-0299-x
54. Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature*. 2014;507(7490):62–67. doi:10.1038/nature13011.
55. Xie H, Tang L, He X, et al. SaCas9 requires 5′-NNGRRT-3′ PAM for sufficient cleavage and possesses higher cleavage activity than SpCas9 or FnCpf1 in human cells. *Biotechnol J*. 2018;13(4). doi:10.1002/biot.201700561
56. Kim E, Koo T, Park SW, et al. In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni. *Nat Commun*. 2017;8(1):14500. doi:10.1038/ncomms14500
57. Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. *Mol Ther*. 2010;18(1):80–86. doi:10.1038/mt.2009.255.
58. Zetsche B, Gootenberg Jonathan S, Abudayyeh Omar O, et al. Cpf1 is a single RNA-guided endonuclease of a Class 2 CRISPR-Cas system. *Cell*. 2015;163(3):759–771. doi:10.1016/j.cell.2015.09.038
59. Tran MH, Park H, Nobles CL, et al. A more efficient CRISPR-Cas12a variant derived from Lachnospiraceae bacterium MA2020. *Mol Ther Nucleic Acids*. 2021;24:40–53. doi:10.1016/j.omtn.2021.02.012;.
60. Kim D, Kim J, Hur JK, Been KW, S-h Y, Kim J-S. Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. *Nature Biotechnol*. 2016;34(8):863–868. doi:10.1038/nbt.3609.
61. Chen G, Wei T, Yang H, Li G, Li H. CRISPR-based therapeutic gene editing for Duchenne muscular dystrophy: advances, challenges and perspectives. *Cells*. 2022;11(19):2964. doi:10.3390/cells11192964.
62. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. *Nature Biotechnol*. 2020;38(7):824–844. doi:10.1038/s41587-020-0561-9.
63. Pickar-Oliver A, Gersbach CA. The next generation of CRISPR–Cas technologies and applications. *Nat Rev Mol Cell Biol*. 2019;20(8):490–507. doi:10.1038/s41580-019-0131-5.
64. Zhang Y, Long C, Bassel-Duby R, Olson EN. Myoediting: toward prevention of muscular dystrophy by therapeutic genome editing. *Physiol Rev*. 2018;98(3):1205–1240. doi:10.1152/physrev.00046.2017.
65. Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. *Nat Methods*. 2011;8(9):765–770. doi:10.1038/nmeth.1670.
66. Sander JD, Ramirez CL, Linder SJ, et al. In silico abstraction of zinc finger nuclease cleavage profiles reveals an expanded landscape of off-target sites. *Nucl Acids Res*. 2013;41(19):e181–e81. doi:10.1093/nar/gkt716
67. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *J Clin Investig*. 2014;124(10):4154–4161. doi:10.1172/jci72992.
68. Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN. Prevention of muscular dystrophy in mice by CRISPR/Cas9–mediated editing of germline DNA. *Science*. 2014;345(6201):1184–1188. doi:10.1126/science.1254445.
69. Chakrabarti AM, Henser-Brownhill T, Monserrat J, Poetsch AR, Luscombe NM, Scaffidi P. Target-specific precision of CRISPR-mediated genome editing. *Mol Cell*. 2019;73(4):699–713.e6. doi:10.1016/j.molcel.2018.11.031.
70. Fletcher S, Adams AM, Johnsen RD, Greer K, Moulton HM, Wilton SD. Dystrophin Isoform Induction In Vivo by Antisense-mediated Alternative Splicing. *Mol Ther*. 2010;18(6):1218–1223. doi:10.1038/mt.2010.45.
71. Li Hongmei L, Fujimoto N, Sasakawa N, et al. Precise correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9. *Stem Cell Rep*. 2015;4(1):143–154. doi:10.1016/j.stemcr.2014.10.013
72. Long C, Li H, Tiburcy M, et al. Correction of diverse muscular dystrophy mutations in human engineered heart muscle by single-site genome editing. *Sci Adv*. 2018;4(1):9004. doi:10.1126/sciadv.aap9004
73. Lemoine J, Dubois A, Dorval A, et al. Correction of exon 2, exon 2–9 and exons 8–9 duplications in DMD patient myogenic cells by a single CRISPR/Cas9 system. *Sci Rep*. 2024;14(1). doi:10.1038/s41598-024-70075-5
74. Amoasii L, Long C, Li H, et al. Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. *Sci Trans Med*. 2017;9(418):8081. doi:10.1126/scitranslmed.aan8081
75. Amoasii L, Hildyard JCW, Li H, et al. Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. *Science*. 2018;362(6410):86–91. doi:10.1126/science.aau1549
76. Min Y-L, Li H, Rodriguez-Caycedo C, et al. CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells. *Sci Adv*. 2019;5(3):4324. doi:10.1126/sciadv.aav4324
77. Rok M, Wong TWY, Maino E, et al. Prevention of early-onset cardiomyopathy in Dmd exon 52–54 deletion mice by CRISPR-Cas9-mediated exon skipping. *Mol Ther*. 2023;30:246–258. doi:10.1016/j.omtm.2023.07.004;.
78. Min Y-L, Chemello F, Li H, et al. Correction of three prominent mutations in mouse and human models of Duchenne Muscular Dystrophy by single-cut genome editing. *Mol Ther*. 2020;28(9):2044–2055. doi:10.1016/j.ymthe.2020.05.024
79. Young Courtney S, Hicks Michael R, Ermolova Natalia V, et al. A single CRISPR-Cas9 deletion strategy that targets the majority of DMD patients restores Dystrophin Function in hiPSC-derived muscle cells. *Cell Stem Cell*. 2016;18(4):533–540. doi:10.1016/j.stem.2016.01.021
80. Ousterout DG, Kabadi AM, Thakore PI, Majoros WH, Reddy TE, Gersbach CA. Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. *Nat Commun*. 2015;6(1):7244. doi:10.1038/ncomms7244.

81. Maggio I, Liu J, Janssen JM, Chen X, Gonçalves MAFV. Adenoviral vectors encoding CRISPR/Cas9 multiplexes rescue dystrophin synthesis in unselected populations of DMD muscle cells. *Sci Rep*. 2016;6(1):37051. doi:10.1038/srep37051.
82. Kyrychenko V, Kyrychenko S, Tiburcy M, et al. Functional correction of dystrophin actin binding domain mutations by genome editing. *JCI Insight*. 2017;2(18):95918. doi:10.1172/jci.insight.95918
83. Nelson CE, Wu Y, Gemberling MP, et al. Long-term evaluation of AAV-CRISPR genome editing for Duchenne muscular dystrophy. *Nat Med*. 2019;25(3):427–432. doi:10.1038/s41591-019-0344-3
84. Xu L, Lau YS, Gao Y, Li H, Han R. Life-long AAV-mediated CRISPR genome editing in dystrophic heart improves cardiomyopathy without causing serious lesions in mdx mice. *Mol Ther*. 2019;27(8):1407–1414. doi:10.1016/j.ymthe.2019.05.001.
85. Poyatos-García J, Soblechero-Martín P, Liquori A, et al. Deletion of exons 45 to 55 in the DMD gene: from the therapeutic perspective to the in vitro model. *Skeletal Muscle*. 2024;14(1). doi:10.1186/s13395-024-00353-3
86. Maggio I, Stefanucci L, Janssen JM, et al. Selection-free gene repair after adenoviral vector transduction of designer nucleases: rescue of dystrophin synthesis in DMD muscle cell populations. *Nucl Acids Res*. 2016;44(3):1449–1470. doi:10.1093/nar/gkv1540
87. Chen M, Shi H, Gou S, et al. In vivo genome editing in mouse restores dystrophin expression in Duchenne muscular dystrophy patient muscle fibers. *Genome Med*. 2021;13(1). doi:10.1186/s13073-021-00876-0
88. Duchêne BL, Cherif K, Iyombe-Engembe J-P, et al. CRISPR-induced deletion with SaCas9 restores Dystrophin expression in Dystrophic models in vitro and in vivo. *Mol Ther*. 2018;26(11):2604–2616. doi:10.1016/j.ymthe.2018.08.010
89. Young CS, Mokhonova E, Quinonez M, Pyle AD, Spencer MJ. Creation of a novel humanized Dystrophic Mouse Model of Duchenne Muscular Dystrophy and application of a CRISPR/Cas9 gene editing therapy. *J Neuromuscular Dis*. 2017;4(2):139–145. doi:10.3233/jnd-170218.
90. Bengtsson NE, Hall JK, Odom GL, et al. Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. *Nat Commun*. 2017;8(1):14454. doi:10.1038/ncomms14454
91. Moretti A, Fonteyne L, Giesert F, et al. Somatic gene editing ameliorates skeletal and cardiac muscle failure in pig and human models of Duchenne muscular dystrophy. *Nat Med*. 2020;26(2):207–214. doi:10.1038/s41591-019-0738-2
92. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016;533(7603):420–424. doi:10.1038/nature17946.
93. Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*. 2017;551(7681):464–471. doi:10.1038/nature24644
94. Tan J, Zhang F, Karcher D, Bock R. Engineering of high-precision base editors for site-specific single nucleotide replacement. *Nat Commun*. 2019;10(1):. doi:10.1038/s41467-018-08034-8
95. Chemello F, Chai AC, Li H, et al. Precise correction of Duchenne muscular dystrophy exon deletion mutations by base and prime editing. *Sci Adv*. 2021;7(18):4910. doi:10.1126/sciadv.abg4910
96. Ryu S-M, Koo T, Kim K, et al. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. *Nature Biotechnol*. 2018;36(6):536–539. doi:10.1038/nbt.4148
97. Yuan J, Ma Y, Huang T, et al. Genetic Modulation of RNA Splicing with a CRISPR-Guided Cytidine Deaminase. *Mol Cell*. 2018;72(2):380–94. e7. doi:10.1016/j.molcel.2018.09.002
98. Li J, Wang K, Zhang Y, et al. Therapeutic Exon Skipping Through a CRISPR-Guided Cytidine Deaminase Rescues Dystrophic Cardiomyopathy in Vivo. *Circulation*. 2021;144(22):1760–1776. doi:10.1161/circulationaha.121.054628
99. Zhou C, Sun Y, Yan R, et al. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. *Nature*. 2019;571 (7764):275–278. doi:10.1038/s41586-019-1314-0
100. Grünewald J, Zhou R, Garcia SP, et al. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. *Nature*. 2019;569(7756):433–437. doi:10.1038/s41586-019-1161-z
101. Jin S, Zong Y, Gao Q, et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science*. 2019;364 (6437):292–295. doi:10.1126/science.aaw7166
102. Xu L, Zhang C, Li H, et al. Efficient precise in vivo base editing in adult dystrophic mice. *Nat Commun*. 2021;12(1). doi:10.1038/s41467-021-23996-y
103. Qiu H, Li G, Yuan J, et al. Efficient exon skipping by base-editor-mediated abrogation of exonic splicing enhancers. *Cell Rep*. 2023;42 (11):113340. doi:10.1016/j.celrep.2023.113340
104. Chai AC, Chemello F, Li H, et al. Single-swap editing for the correction of common Duchenne muscular dystrophy mutations. *Mol Ther Nucleic Acids*. 2023;32:522–535. doi:10.1016/j.omtn.2023.04.009;.
105. Wang P, Li H, Zhu M, Han RY, Guo S, Han R. Correction of DMD in human iPSC-derived cardiomyocytes by base-editing-induced exon skipping. *Mol Ther Methods Clin Dev*. 2023;28:40–50. doi:10.1016/j.omtm.2022.11.010
106. Jin M, Lin J, Li H, et al. Correction of human nonsense mutation via adenine base editing for Duchenne muscular dystrophy treatment in mouse. *Mol Ther Nucl Acids*. 2024;35(2):102165. doi:10.1016/j.omtn.2024.102165
107. Lin J, Jin M, Yang D, et al. Adenine base editing-mediated exon skipping restores dystrophin in humanized Duchenne mouse model. *Nat Commun*. 2024;15. doi:10.1038/s41467-024-50340-x
108. Lai Y, Yue Y, Liu M, et al. Efficient in vivo gene expression by trans-splicing adeno-associated viral vectors. *Nat Biotechnol*. 2005;23 (11):1435–1439. doi:10.1038/nbt1153
109. Sun L, Li J, Xiao X. Overcoming adeno-associated virus vector size limitation through viral DNA heterodimerization. *Nat Med*. 2000;6 (5):599–602. doi:10.1038/75087.
110. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 2019;576 (7785):149–157. doi:10.1038/s41586-019-1711-4
111. Happi Mbakam C, Rousseau J, Lu Y, et al. Prime editing optimized RTT permits the correction of the c.8713C>T mutation in DMD gene. *Mol Ther Nucl Acids*. 2022;30:272–285. doi:10.1016/j.omtn.2022.09.022;.
112. Happi Mbakam C, Rousseau J, Tremblay G, Yameogo P, Tremblay JP. Prime editing permits the introduction of specific mutations in the gene responsible for Duchenne Muscular Dystrophy. *Int J Mol Sci*. 2022;23(11):6160. doi:10.3390/ijms23116160.
113. Zhang Y, Long C, Li H, et al. CRISPR-Cpf1 correction of muscular dystrophy mutations in human cardiomyocytes and mice. *Sci Adv*. 2017;3 (4):1602814. doi:10.1126/sciadv.1602814

114. Mata López S, Balog-Alvarez C, Vitha S, et al. Challenges associated with homologous directed repair using CRISPR-Cas9 and TALEN to edit the DMD genetic mutation in canine Duchenne muscular dystrophy. *PLoS One*. 2020;15(1):e0228072. doi:10.1371/journal.pone.0228072
115. Suzuki K, Tsunekawa Y, Hernandez-Benitez R, et al. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. *Nature*. 2016;540(7631):144–149. doi:10.1038/nature20565
116. Pickar-Oliver A, Gough V, Bohning JD, et al. Full-length dystrophin restoration via targeted exon integration by AAV-CRISPR in a humanized mouse model of Duchenne muscular dystrophy. *Mol Ther*. 2021;29(11):3243–3257. doi:10.1016/j.ymthe.2021.09.003
117. Love DR, Hill DF, Dickson G, et al. An autosomal transcript in skeletal muscle with homology to dystrophin. *Nature*. 1989;339(6219):55–58. doi:10.1038/339055a0
118. Tinsley JM, Blake DJ, Roche A, et al. Primary structure of dystrophin-related protein. *Nature*. 1992;360(6404):591–593. doi:10.1038/360591a0
119. Tinsley J, Deconinck N, Fisher R, et al. Expression of full-length utrophin prevents muscular dystrophy in mdx mice. *Nat Med*. 1998;4(12):1441–1444. doi:10.1038/4033
120. Lim KRQ, Yoon C, Yokota T. Applications of CRISPR/Cas9 for the treatment of Duchenne Muscular Dystrophy. *J Personalized Med*. 2018;8(4):38. doi:10.3390/jpm8040038.
121. Clerk A, Morris GE, Dubowitz V, Davies KE, Sewry CA. Dystrophin-related protein, utrophin, in normal and dystrophic human fetal skeletal muscle. *Histochem J*. 1993;25(8):554–561. doi:10.1007/bf02388063.
122. Squire S. Prevention of pathology in mdx mice by expression of utrophin: analysis using an inducible transgenic expression system. *Human Mol Genetics*. 2002;11(26):3333–3344. doi:10.1093/hmg/11.26.3333.
123. Fisher R, Tinsley JM, Phelps SR, et al. Non-toxic ubiquitous over-expression of utrophin in the mdx mouse. *Neuromuscul Disord*. 2001;11(8):713–721. doi:10.1016/s0960-8966(01)00220-6
124. Wojtal D, Kemaladewi Dwi U, Malam Z, et al. Spell checking nature: versatility of CRISPR/Cas9 for developing treatments for inherited disorders. *Am J Hum Genet*. 2016;98(1):90–101. doi:10.1016/j.ajhg.2015.11.012
125. Liao H-K, Hatanaka F, Araoka T, et al. In vivo target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation. *Cell*. 2017;171(7):1495–507.e15. doi:10.1016/j.cell.2017.10.025
126. Andrysiak K, Ferdek PE, Sanetra AM, et al. Upregulation of utrophin improves the phenotype of Duchenne muscular dystrophy hiPSC-derived CMs. *Mol Ther Nucleic Acids*. 2024;35(3):102247. doi:10.1016/j.omtn.2024.102247
127. Sengupta K, Mishra MK, Loro E, Spencer MJ, Pyle AD, Khurana TS. Genome editing-mediated Utrophin Upregulation in Duchenne Muscular Dystrophy Stem Cells. *Mol Ther Nucleic Acids*. 2020;22:500–509. doi:10.1016/j.omtn.2020.08.031
128. Lek A, Wong B, Keeler A, et al. Death after high-dose rAAV9 gene therapy in a patient with Duchenne's Muscular Dystrophy. *N Engl J Med*. 2023;389(13):1203–1210. doi:10.1056/nejmoa2307798
129. Tu Z, Yang W, Yan S, Guo X, Li X-J. CRISPR/Cas9: a powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. *Mol Neurodegen*. 2015;10(1):. doi:10.1186/s13024-015-0031-x
130. Mou H, Kennedy Z, Anderson DG, Yin H, Xue W. Precision cancer mouse models through genome editing with CRISPR-Cas9. *Genome Med*. 2015;7(1):. doi:10.1186/s13073-015-0178-7
131. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell*. 2013;153(4):910–918. doi:10.1016/j.cell.2013.04.025
132. Burgio G. Redefining mouse transgenesis with CRISPR/Cas9 genome editing technology. *Genome Biol*. 2018;19(1):. doi:10.1186/s13059-018-1409-1
133. Qin W, Kutny PM, Maser RS, et al. Generating mouse models using CRISPR-Cas9-mediated genome editing. *Curr Protocols Mouse Biol*. 2016;6(1):39–66. doi:10.1002/9780470942390.mo150178
134. Capecchi MR. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat Rev Genet*. 2005;6(6):507–512. doi:10.1038/nrg1619.
135. Laurent M, Geoffroy M, Pavani G, Guiraud S. CRISPR-based gene therapies: from preclinical to clinical treatments. *Cells*. 2024;13(10):800. doi:10.3390/cells13100800.
136. Amoasii L, Li H, Zhang Y, et al. In vivo non-invasive monitoring of dystrophin correction in a new Duchenne muscular dystrophy reporter mouse. *Nat Commun*. 2019;10(1):4537. doi:10.1038/s41467-019-12335-x
137. Egorova TV, Zotova ED, Reshetov DA, et al. CRISPR/Cas9-generated mouse model of Duchenne muscular dystrophy recapitulating a newly identified large 430 kb deletion in the human *DMD* gene. *Dis Models Mech*. 2019;12(4). doi:10.1242/dmm.037655
138. Koo T, Lu-Nguyen NB, Malerba A, et al. Functional rescue of dystrophin deficiency in mice caused by frameshift mutations using Campylobacter jejuni Cas9. *mol Ther*. 2018;26(6):1529–1538. doi:10.1016/j.ymthe.2018.03.018
139. Kim K, Ryu S-M, Kim S-T, et al. Highly efficient RNA-guided base editing in mouse embryos. *Nature Biotechnol*. 2017;35(5):435–437. doi:10.1038/nbt.3816
140. Wong TWY, Ahmed A, Yang G, et al. A novel mouse model of Duchenne muscular dystrophy carrying a multi-exonic *Dmd* deletion exhibits progressive muscular dystrophy and early-onset cardiomyopathy. *Dis Models Mech*. 2020;13(9). doi:10.1242/dmm.045369
141. Deconinck AE, Rafael JA, Skinner JA, et al. Utrophin-Dystrophin-deficient mice as a model for Duchenne Muscular Dystrophy. *Cell*. 1997;90(4):717–727. doi:10.1016/s0092-8674(00)80532-2
142. Perkins KJ, Davies KE. The role of utrophin in the potential therapy of Duchenne muscular dystrophy. *Neuromuscul Disord*. 2002;12:S78–S89. doi:10.1016/s0960-8966(02)00087-1
143. Nakamura A. Mutation-based therapeutic strategies for Duchenne Muscular Dystrophy: from genetic diagnosis to therapy. *J Personal Med*. 2019;9(1):16. doi:10.3390/jpm9010016.
144. Nakamura K, Fujii W, Tsuboi M, et al. Generation of muscular dystrophy model rats with a CRISPR/Cas system. *Sci Rep*. 2014;4(1). doi:10.1038/srep05635
145. Ellenbroek B, Youn J. Rodent models in neuroscience research: is it a rat race? *Dis Models Mech*. 2016;9(10):1079–1087. doi:10.1242/dmm.026120.
146. Abbott A. The Renaissance rat. *Nature*. 2004;428(6982):464–466. doi:10.1038/428464a.
147. Sui T, Lau YS, Liu D, et al. A novel rabbit model of Duchenne muscular dystrophy generated by CRISPR/Cas9. *Dis Models Mech*. 2018;11(6). doi:10.1242/dmm.032201

148. Wells DJ. Tracking progress: an update on animal models for Duchenne muscular dystrophy. *Dis Models Mech*. 2018;11(6):. doi:10.1242/dmm.035774
149. Wang Y, Fan N, Song J, et al. Generation of knockout rabbits using transcription activator-like effector nucleases. *Cell Regener*. 2014;3(1):3. doi:10.1186/2045-9769-3-3
150. Lim KRQ, Nguyen Q, Dzierlega K, Huang Y, Yokota T. CRISPR-generated animal models of Duchenne Muscular Dystrophy. *Genes*. 2020;11(3):342. doi:10.3390/genes11030342.
151. Klymiuk N, Blutke A, Graf A, et al. Dystrophin-deficient pigs provide new insights into the hierarchy of physiological derangements of dystrophic muscle. *Human Mol Genetics*. 2013;22(21):4368–4382. doi:10.1093/hmg/ddt287
152. Yu -H-H, Zhao H, Qing Y-B, et al. Porcine Zygote injection with Cas9/sgRNA results in DMD-modified Pig with muscle Dystrophy. *Int J Mol Sci*. 2016;17(10):1668. doi:10.3390/ijms17101668
153. Selsby JT, Ross JW, Nonneman D, Hollinger K. Porcine models of Muscular Dystrophy. *ILAR J*. 2015;56(1):116–126. doi:10.1093/ilar/ilv015.
154. Maxmen A. Model pigs face messy path. *Nature*. 2012;486(7404):453. doi:10.1038/486453a.
155. Kornegay JN. The golden retriever model of Duchenne muscular dystrophy. *Skeletal Muscle*. 2017;7(1):. doi:10.1186/s13395-017-0124-z
156. Brinkmeyer-Langford C, Chu C, Balog-Alvarez C, et al. Expression profiling of disease progression in canine model of Duchenne muscular dystrophy. *PLoS One*. 2018;13(3):e0194485. doi:10.1371/journal.pone.0194485
157. Cozzi F, Cerletti M, Luvoni GC, et al. Development of muscle pathology in canine X-linked muscular dystrophy. II. Quantitative characterization of histopathological progression during postnatal skeletal muscle development. *Acta Neuropathol*. 2001;101(5):469–478. doi:10.1007/s004010000308
158. Shimatsu Y, Katagiri K, Furuta T, et al. Canine X-linked muscular dystrophy in Japan (CXMDJ). *Exp Anim*. 2003;52(2):93–97. doi:10.1538/expanim.52.93
159. Chen Y, Zheng Y, Kang Y, et al. Functional disruption of the dystrophin gene in rhesus monkey using CRISPR/Cas9. *Human Mol Genetics*. 2015;24(13):3764–3774. doi:10.1093/hmg/ddv120
160. Wang S, Ren S, Bai R, et al. No off-target mutations in functional genome regions of a CRISPR/Cas9-generated monkey model of muscular dystrophy. *J Biol Chem*. 2018;293(30):11654–11658. doi:10.1074/jbc.ac118.004404
161. Singh A, Irfan H, Fatima E, Nazir Z, Verma A, Akilimali A. Revolutionary breakthrough: FDA approves CASGEVY, the first CRISPR/Cas9 gene therapy for sickle cell disease. *Ann Med Surg Lond*. 2024;86(8):4555–4559. doi:10.1097/MS9.0000000000002146.
162. Suzuki K, Yamamoto M, Hernandez-Benitez R, et al. Precise in vivo genome editing via single homology arm donor mediated intron-targeting gene integration for genetic disease correction. *Cell Res*. 2019;29(10):804–819. doi:10.1038/s41422-019-0213-0
163. Guiraud S, Edwards B, Babbs A, et al. The potential of utrophin and dystrophin combination therapies for Duchenne muscular dystrophy. *Human Mol Genetics*. 2019;28(13):2189–2200. doi:10.1093/hmg/ddz049
164. Klompe SE, Vo PLH, Halpin-Healy TS, Sternberg SH. Transposon-encoded CRISPR–Cas systems direct RNA-guided DNA integration. *Nature*. 2019;571(7764):219–225. doi:10.1038/s41586-019-1323-z.
165. Strecker J, Ladha A, Gardner Z, et al. RNA-guided DNA insertion with CRISPR-associated transposases. *Science*. 2019;365(6448):48–53. doi:10.1126/science.aax9181
166. Anzalone AV, Gao XD, Podracky CJ, et al. Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. *Nature Biotechnol*. 2021;40(5):731–740. doi:10.1038/s41587-021-01133-w
167. Yarnall MTN, Ioannidi EI, Schmitt-Ulms C, et al. Drag-and-drop genome insertion of large sequences without double-strand DNA cleavage using CRISPR-directed integrases. *Nat Biotechnol*. 2023;41(4):500–512. doi:10.1038/s41587-022-01527-4
168. Hoffman EP, Kunkel LM, Angelini C, Clarke A, Johnson M, Harris JB. Improved diagnosis of Becker muscular dystrophy by dystrophin testing. *Neurology*. 1989;39(8):1011. doi:10.1212/wnl.39.8.1011.
169. Ancel S, Stuelsatz P, Feige JN. Muscle stem cell Quiescence: controlling stemness by staying asleep. *Trends Cell Biol*. 2021;31(7):556–568. doi:10.1016/j.tcb.2021.02.006.
170. Yin H, Price F, Rudnicki MA. Satellite cells and the muscle stem cell Niche. *Physiol Rev*. 2013;93(1):23–67. doi:10.1152/physrev.00043.2011.
171. Nance ME, Shi R, Hakim CH, et al. AAV9 edits muscle stem cells in normal and Dystrophic adult mice. *Mol Ther*. 2019;27(9):1568–1585. doi:10.1016/j.ymthe.2019.06.012
172. Kwon JB, Ettyreddy AR, Vankara A, et al. In vivo gene editing of muscle stem cells with Adeno-associated viral vectors in a mouse model of Duchenne Muscular Dystrophy. *Mol Ther Methods Clin Dev*. 2020;19:320–329. doi:10.1016/j.omtm.2020.09.016;.
173. Goldstein JM, Tabebordbar M, Zhu K, et al. In situ modification of tissue stem and Progenitor cell genomes. *Cell Rep*. 2019;27(4):1254–64.e7. doi:10.1016/j.celrep.2019.03.105
174. Tabebordbar M, Zhu K, Cheng JKW, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science*. 2016;351(6271):407–411. doi:10.1126/science.aad5177
175. Bengtsson NE, Tasfaout H, Hauschka SD, Chamberlain JS. Dystrophin gene-editing stability is dependent on Dystrophin levels in skeletal but not cardiac muscles. *Mol Ther*. 2021;29(3):1070–1085. doi:10.1016/j.ymthe.2020.11.003.
176. Samulski RJ, Muzyczka N. AAV-mediated gene therapy for research and therapeutic purposes. *Ann Rev Virol*. 2014;1(1):427–451. doi:10.1146/annurev-virology-031413-085355.
177. Crudele JM, Chamberlain JS. AAV-based gene therapies for the muscular dystrophies. *Human Molecular Genetics*. 2019;28(R1):R102–R07. doi:10.1093/hmg/ddz128.
178. Boutin S, Monteilhet V, Veron P, et al. Prevalence of serum IgG and neutralizing factors against Adeno-Associated Virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. *Human Gene Ther*. 2010;21(6):704–712. doi:10.1089/hum.2009.182
179. Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T. Pre-existing anti–adeno-associated virus antibodies as a challenge in AAV gene therapy. *Human Gene Ther Methods*. 2013;24(2):59–67. doi:10.1089/hgtb.2012.243.
180. Hakim CH, Wasala NB, Nelson CE, et al. AAV CRISPR editing rescues cardiac and muscle function for 18 months in dystrophic mice. *JCI Insight*. 2018;3(23):124297. doi:10.1172/jci.insight.124297
181. Kornegay JN, Li J, Bogan JR, et al. Widespread muscle expression of an AAV9 human mini-dystrophin vector after intravenous injection in neonatal dystrophin-deficient dogs. *Mol Ther*. 2010;18(8):1501–1508. doi:10.1038/mt.2010.94

182. Hinderer C, Katz N, Buza EL, et al. Severe toxicity in nonhuman primates and piglets following high-dose intravenous administration of an Adeno-associated virus vector expressing human SMN. *Human Gene Ther*. 2018;29(3):285–298. doi:10.1089/hum.2018.015
183. Buscara L, Gross D-A, Daniele N. Of rAAV and men: from genetic neuromuscular disorder efficacy and toxicity preclinical studies to clinical trials and back. *J Personalized Med*. 2020;10(4):258. doi:10.3390/jpm10040258.
184. Moreno AM, Palmer N, Alemán F, et al. Immune-orthogonal orthologues of AAV capsids and of Cas9 circumvent the immune response to the administration of gene therapy. *Nat Biomed Eng*. 2019;3(10):806–816. doi:10.1038/s41551-019-0431-2
185. Weinmann J, Weis S, Sippel J, et al. Identification of a myotropic AAV by massively parallel in vivo evaluation of barcoded capsid variants. *Nat Commun*. 2020;11(1). doi:10.1038/s41467-020-19230-w
186. Tabebordbar M, Lagerborg KA, Stanton A, et al. Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across species. *Cell*. 2021;184(19):4919–38.e22. doi:10.1016/j.cell.2021.08.028
187. El Andari J, Renaud-Gabardos E, Tulalamba W, et al. Semirational bioengineering of AAV vectors with increased potency and specificity for systemic gene therapy of muscle disorders. *Sci Adv*. 2022;8(38). doi:10.1126/sciadv.abn4704
188. Zhang Y, Li H, Min Y-L, et al. Enhanced CRISPR-Cas9 correction of Duchenne muscular dystrophy in mice by a self-complementary AAV delivery system. *Sci Adv*. 2020;6(8). doi:10.1126/sciadv.aay6812
189. Ren C, Kumar S, Shaw DR, Ponnazhagan S. Genomic stability of self-complementary Adeno-associated virus 2 during early stages of transduction in mouse muscle in vivo. *Human Gene Ther*. 2005;16(9):1047–1057. doi:10.1089/hum.2005.16.1047.
190. McCart DM. Self-complementary AAV vectors advances and applications. *Mol Ther*. 2008;16(10):1648–1656. doi:10.1038/mt.2008.171.
191. Zhang Y, Nishiyama T, Li H, et al. A consolidated AAV system for single-cut CRISPR correction of a common Duchenne muscular dystrophy mutation. *Mol Ther Methods Clin Dev*. 2021;22:122–132. doi:10.1016/j.omtm.2021.05.014;.
192. Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat Med*. 2019;25(2):249–254. doi:10.1038/s41591-018-0326-x
193. Ferdosi SR, Ewaisha R, Moghadam F, et al. Multifunctional CRISPR-Cas9 with engineered immunosilenced human T cell epitopes. *Nat Commun*. 2019;10(1). doi:10.1038/s41467-019-09693-x
194. Kim S, Koo T, Jee H-G, et al. CRISPR RNAs trigger innate immune responses in human cells. *Genome Res*. 2018;28(3):367–373. doi:10.1101/gr.231936.117
195. Wienert B, Shin J, Zelin E, Pestal K, Corn JE. In vitro–transcribed guide RNAs trigger an innate immune response via the RIG-I pathway. *PLoS Biol*. 2018;16(7):e2005840. doi:10.1371/journal.pbio.2005840.
196. Thanh LT, Nguyen TM, Helliwell TR, Morris GE. Characterization of revertant muscle fibers in Duchenne muscular dystrophy, using exon-specific monoclonal antibodies against dystrophin. *Am J Hum Genet*. 1995;56(3):725–731.
197. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. 2013;31(9):822–826. doi:10.1038/nbt.2623
198. Lin Y, Cradick TJ, Brown MT, et al. CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. *Nucl Acids Res*. 2014;42(11):7473–7485. doi:10.1093/nar/gku402
199. Murugan K, Suresh SK, Seetharam AS, Severin AJ, Sashital DG. Systematic*in vitro*specificity profiling reveals nicking defects in natural and engineered CRISPR–Cas9 variants. *Nucl Acids Res*. 2021;49(7):4037–4053. doi:10.1093/nar/gkab163.
200. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. *Science*. 2016;351(6268):84–88. doi:10.1126/science.aad5227.
201. Chen JS, Dagdas YS, Kleinstiver BP, et al. Enhanced proofreading governs CRISPR–Cas9 targeting accuracy. *Nature*. 2017;550(7676):407–410. doi:10.1038/nature24268
202. Kulcsár PI, Tálas A, Huszár K, et al. Crossing enhanced and high fidelity SpCas9 nucleases to optimize specificity and cleavage. *Genome Biol*. 2017;18(1). doi:10.1186/s13059-017-1318-8
203. Casini A, Olivieri M, Petris G, et al. A highly specific SpCas9 variant is identified by in vivo screening in yeast. *Nat Biotechnol*. 2018;36(3):265–271. doi:10.1038/nbt.4066
204. Kleinstiver BP, Sousa AA, Walton RT, et al. Engineered CRISPR–Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. *Nat Biotechnol*. 2019;37(3):276–282. doi:10.1038/s41587-018-0011-0
205. Kleinstiver BP, Tsai SQ, Prew MS, et al. Genome-wide specificities of CRISPR-Cas Cpf1 nucleases in human cells. *Nat Biotechnol*. 2016;34(8):869–874. doi:10.1038/nbt.3620
206. Ran FA, Hsu Patrick D, Lin C-Y, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell*. 2013;154(6):1380–1389. doi:10.1016/j.cell.2013.08.021
207. Fu Y, Sander JD, Reyon D, Cascio VM, Joung JK. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nature Biotechnol*. 2014;32(3):279–284. doi:10.1038/nbt.2808.
208. Lau C-H, Suh Y. In vivo genome editing in animals using AAV-CRISPR system: applications to translational research of human disease. *F1000Research*. 2017;6:2153. doi:10.12688/f1000research.11243.1
209. Wang J-Z, Wu P, Shi Z-M, Xu Y-L, Liu Z-J. The AAV-mediated and RNA-guided CRISPR/Cas9 system for gene therapy of DMD and BMD. *Brain Dev*. 2017;39(7):547–556. doi:10.1016/j.braindev.2017.03.024.
210. Erkut E, Yokota T. CRISPR therapeutics for Duchenne Muscular Dystrophy. *Int J Mol Sci*. 2022;23(3):1832. doi:10.3390/ijms23031832.
211. Hanlon KS, Kleinstiver BP, Garcia SP, et al. High levels of AAV vector integration into CRISPR-induced DNA breaks. *Nat Commun*. 2019;10(1). doi:10.1038/s41467-019-12449-2
212. Padmaswari MH, Agrawal S, Jia MS, et al. Delivery challenges for CRISPR—Cas9 genome editing for Duchenne muscular dystrophy. *Biophys Rev*. 2023;4(1). doi:10.1063/5.0131452
213. Counsell JR, Asgarian Z, Meng J, et al. Lentiviral vectors can be used for full-length dystrophin gene therapy. *Sci Rep*. 2017;7(1). doi:10.1038/srep44775
214. Quenneville SP, Chapdelaine P, Skuk D, et al. Autologous transplantation of muscle precursor cells modified with a lentivirus for muscular Dystrophy: human cells and primate models. *Mol Ther*. 2007;15(2):431–438. doi:10.1038/sj.mt.6300047
215. White M, Whittaker R, Gándara C, Stoll EA. A guide to approaching regulatory considerations for lentiviral-mediated gene therapies. *Human Gene Ther Methods*. 2017;28(4):163–176. doi:10.1089/hgtb.2017.096.

216. Xu L, Park KH, Zhao L, et al. CRISPR-mediated genome editing restores dystrophin expression and function in mdx mice. *Mol Ther*. 2016;24(3):564–569. doi:10.1038/mt.2015.192
217. Zhu P, Wu F, Mosenson J, Zhang H, He T-C, Wu W-S. CRISPR/Cas9-mediated genome editing corrects Dystrophin mutation in skeletal muscle stem cells in a mouse model of muscle dystrophy. *Mol Ther Nucleic Acids*. 2017;7:31–41. doi:10.1016/j.omtn.2017.02.007
218. Nalbantoglu J, Larochelle N, Wolf E, Karpati G, Lochmuller H, Holland PC. Muscle-specific overexpression of the Adenovirus primary receptor CAR overcomes low efficiency of gene transfer to mature skeletal muscle. *J Virol*. 2001;75(9):4276–4282. doi:10.1128/jvi.75.9.4276-4282.2001.
219. Miller JB, Zhang S, Kos P, et al. Non-viral CRISPR/Cas gene editing in vitro and in vivo enabled by synthetic nanoparticle co-delivery of Cas9 mRNA and sgRNA. *Angew Chem Int Ed*. 2016;56(4):1059–1063. doi:10.1002/anie.201610209
220. Zuris JA, Thompson DB, Shu Y, et al. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. *Nature Biotechnol*. 2014;33(1):73–80. doi:10.1038/nbt.3081
221. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary Transthyretin Amyloidosis. *N Engl J Med*. 2018;379(1):11–21. doi:10.1056/NEJMoa1716153
222. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med*. 2020;383(27):2603–2615. doi:10.1056/nejmoa2034577
223. Chu L, McPhee R, Huang W, et al. A preliminary report of a randomized controlled Phase 2 trial of the safety and immunogenicity of mRNA-1273 SARS-CoV-2 vaccine. *Vaccine*. 2021;39(20):2791–2799. doi:10.1016/j.vaccine.2021.02.007
224. Kenjo E, Hozumi H, Makita Y, et al. Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice. *Nat Commun*. 2021;12. doi:10.1038/s41467-021-26714-w
225. Wei T, Cheng Q, Min Y-L, Olson EN, Siegwart DJ. Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nat Commun*. 2020;11(1):. doi:10.1038/s41467-020-17029-3
226. Siddique S, Chow JCL. Gold nanoparticles for drug delivery and cancer therapy. *Appl Sci*. 2020;10(11):1.
227. Haume K, Rosa S, Grellet S, et al. Gold nanoparticles for cancer radiotherapy: a review. *Cancer Nanotechnol*. 2016;7(1). doi:10.1186/s12645-016-0021-x
228. Lee K, Conboy M, Park HM, et al. Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nat Biomed Eng*. 2017;1(11):889–901. doi:10.1038/s41551-017-0137-2
229. Raguram A, Banskota S, Liu DR. Therapeutic in vivo delivery of gene editing agents. *Cell*. 2022;185(15):2806–2827. doi:10.1016/j.cell.2022.03.045.
230. Hamilton JR, Tsuchida CA, Nguyen DN, et al. Targeted delivery of CRISPR-Cas9 and transgenes enables complex immune cell engineering. *Cell Rep*. 2021;35(9):109207. doi:10.1016/j.celrep.2021.109207
231. Yao X, Lyu P, Yoo K, et al. Engineered extracellular vesicles as versatile ribonucleoprotein delivery vehicles for efficient and safe CRISPR genome editing. *J Extracell Vesicles*. 2021;10(5). doi:10.1002/jev2.12076
232. Gee P, Lung MSY, Okuzaki Y, et al. Extracellular nanovesicles for packaging of CRISPR-Cas9 protein and sgRNA to induce therapeutic exon skipping. *Nat Commun*. 2020;11(1). doi:10.1038/s41467-020-14957-y