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# Challenges in developing gene therapy against Duchenne muscular dystrophy

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ABSTRACT

Duchenne muscular dystrophy is a progressive X-linked recessive neuromuscular disorder resulting from pathogenic mutations in the *DMD* gene, which codes dystrophin. It is one of the essential structural proteins of muscle cells that maintains the integrity of cross-striated muscles. Duchenne muscular dystrophy causes progressive muscular weakness and, as a consequence, reduces life expectancy due to respiratory failure and/or heart failure.

Glucocorticoids are considered the standard of care in Duchenne muscular dystrophy, although they are not highly effective and may lead to numerous adverse effects. For decades, many studies have been focused on finding an effective therapy for Duchenne muscular dystrophy; however, no etiology-oriented product is currently available for patients with Duchenne muscular dystrophy. That being said, the latest studies demonstrate that promising effective gene therapy for Duchenne muscular dystrophy is possible in the near future. The ongoing studies include approaches such as replacement therapy with shortened dystrophin forms and genome editing. Despite high efficacy of the approaches *in vitro* and in animal models, there is a number of challenges when it comes to treating human patients with Duchenne muscular dystrophy. The first challenge is the gene size — *DMD* is one of the largest genes, which makes it difficult to load it into viral vectors for delivery. Second, Duchenne muscular dystrophy is caused by over 7000 mutations, so creating universal gene therapies applicable to wide patient populations is problematic. Besides, low efficacy of genetic structure delivery and immune responses — both to the transgene and the viral vector — are a concern. Moreover, long-term sequelae of dystrophin deficiency could persist even if the protein expression is restored. The ongoing studies offer strategies to overcome the limitations above.

This review aims to discuss the current challenges, the solutions to which may become a breakthrough in gene therapy for Duchenne muscular dystrophy and other hereditary diseases.

**Keywords:** Duchenne muscular dystrophy; gene therapy; dystrophin; treatment strategy.

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# Препятствия на пути к разработке генной терапии мышечной дистрофии Дюшенна

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АННОТАЦИЯ

Мышечная дистрофия Дюшенна (МДД) — это прогрессирующее нервно-мышечное Х-сцепленное рецессивное заболевание, возникающее в результате появления патогенных мутаций в гене *DMD*, кодирующем белок дистрофин. Это важный структурный белок мышечных клеток, который поддерживает целостность поперечнополосатой мускулатуры. МДД приводит к прогрессирующей мышечной слабости и, как результат, сокращению продолжительности жизни из-за дыхательной и/или сердечной недостаточности.

Стандартом лечения МДД считается применение глюкокортикоидов, которые не являются высокоэффективными и могут быть причиной многих побочных эффектов. В течение десятилетий множество исследований было направлено на поиск эффективного метода терапии, однако в настоящее время для пациентов с МДД не существует лекарства, способного полностью устранить причину заболевания. Тем не менее последние исследования демонстрируют, что создание эффективной и перспективной генной терапии МДД возможно в ближайшем будущем. Такие подходы, как заместительная терапия укороченными формами дистрофина и редактирование генома, активно изучаются в настоящее время, но, хотя некоторые из этих подходов показали высокую эффективность на клеточных культурах и модельных животных, существует ряд препятствий для их эффективного использования при лечении миодистрофии Дюшенна у человека. В первую очередь к этим препятствиям относится размер гена (*DMD* является одним из крупнейших), что затрудняет его упаковку в вирусные векторы для доставки. Более 7000 различных мутаций служат причиной МДД, что осложняет создание универсальных препаратов генной терапии, которые могли бы быть применимы к большим группам пациентов. Кроме того, серьёзными проблемами являются низкая эффективность доставки генетических конструкций и иммунные ответы как на трансген, так и на вирусный вектор. А долгосрочные последствия дефицита дистрофина могут сохраняться даже при восстановлении экспрессии белка. Несмотря на перечисленные проблемы, в текущих исследованиях предлагаются различные стратегии для преодоления этих ограничений.

Целью данного обзора является обсуждение существующих проблем, решение которых может стать значительным шагом к разработке генной терапии МДД и многих других наследственных заболеваний.

**Ключевые слова:** мышечная дистрофия Дюшенна; генная терапия; дистрофин; терапевтические стратегии.

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## INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe, progressive, X-linked recessive neuromuscular disorder caused by pathogenic variants in the nucleotide sequence of the *DMD* gene. This gene is the largest in the human genome, with a size of approximately 2.5 Mb, consisting of 79 exons and 78 introns [1]. It contains seven distinct promoters and undergoes alternative splicing, resulting in the expression of multiple dystrophin isoforms in various tissue types [2, 3]. In addition to skeletal and cardiac muscle, dystrophin expression is observed in cortical neurons of the brain, Purkinje cells of the cerebellum, the retina, neurons of the central nervous system, the kidneys, and Schwann cells [2–5]. A full-length mRNA transcript of 14 kb encodes a protein with a molecular mass of 427 kDa, comprising 3685 amino acids [6]. The dystrophin protein consists of four domains: an N-terminal actin-binding domain, a central rod domain, a cysteine-rich domain, and a C-terminal domain, as well as hinge regions [7]. Dystrophin has been shown to be a part of the dystrophin-associated glycoprotein complex [8]. It also includes dystroglycans, sarcoglycans, syntrophins, and dystrobrevins. These proteins can be properly localized only in the presence of dystrophin at the cell membrane. The dystrophin-associated glycoprotein complex is essential for muscle fiber contraction and plays a key role in maintaining the stability of the muscle cell membrane [9].

Pathogenic variants in the *DMD* gene that cause DMD often result in a frameshift and the generation of a premature stop codon, leading to the absence of dystrophin protein in cells. This results in disruption of sarcolemma integrity and muscle tissue damage during contraction [10]. Individuals with DMD exhibit progressive skeletal muscle dystrophy, respiratory failure, cardiomyopathy, and varying degrees of cognitive dysfunction. As the disease progresses, muscle fibers are replaced by fibrous and adipose tissue [11]. Patients typically lose the ability to walk independently by an average age of 12 years, and after the age of 20, the risk of premature death due to cardiac and/or respiratory failure increases [12]. Deletions and duplications that do not cause a frameshift, as well as some missense variants, underlie a milder and more slowly progressive phenotype—Becker muscular dystrophy (BMD)—in which partially functional dystrophin protein or lower levels of its expression may be produced [13, 14].

According to various data, DMD affects approximately 1 in 5000 live male births, making it one of the most common hereditary disorders. About two-thirds of DMD cases result from the transmission of a pathogenic gene variant from mother to son, whereas one-third of cases arise from spontaneous (*de novo*) mutations [15].

## CURRENT APPROACHES TO THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

At present, there is no drug that completely eliminates the cause of DMD/BMD; therefore, all available treatments aim to improve the quality of life and slow disease progression. Primarily, symptomatic and pathogenetic therapy is used.

Glucocorticoid therapy is the gold standard for treating patients with DMD. Numerous studies have demonstrated its ability to slow disease progression [16, 17]. These drugs can improve muscle strength and reduce inflammation [18]. Prednisolone and deflazacort have been shown to slow the loss of muscle mass and prolong the ability to walk independently [19]. However, long-term use of corticosteroids may lead to multiple adverse side effects [20]. Some patients experience impaired growth and maturation, diabetes mellitus, and adrenal insufficiency. In addition, corticosteroid administration, in combination with the natural deficiency of vitamin D, contributes to the development of osteoporosis [21]. Clinical studies of new steroid drugs are currently underway, with the aim of making long-term use safer by reducing adverse side effects [22].

One of the most common causes of DMD is deletions in the exon 45–55 region [23], which lead to a frameshift in the reading frame. In such deletions, the skipping of an additional exon is often possible, which can potentially restore the disrupted reading frame. As a pathogenetic therapy for deletions, drugs that modify splicing are used. These agents are antisense oligonucleotides (ASOs) that, by the principle of complementarity, can bind to pre-mRNA and influence the splicing process by blocking spliceosome activity. As a result, the exon along with the adjacent introns is removed from the mature mRNA [24], restoring dystrophin expression in muscle cells. For example, viltolarsen and golodirsen[1]* promote exon 53 skipping [25, 26]. Eteplirsen* and casimersen* are aimed at skipping exons 51 and 45, respectively [27, 28]. This approach is highly specific, as different exon skipping strategies are required depending on the location of the deletion. Each of these drugs must undergo all phases of clinical studies independently. Skipping of exons 51, 53, and 45 can be applied in 14%, 8%, and 9% of patients, respectively. The proportion of patients eligible for other ASOs is much smaller: 4% (exon 50 skipping), 3% (exon 43 skipping), and 2% (exon 8 skipping) [23]. To date, the United States Food and Drug Administration (FDA) has approved four ASO-based drugs mentioned above. It has been shown that these drugs can induce dystrophin synthesis

[1] Hereinafter, an asterisk denotes drugs that are not registered in the State Register of Medicinal Products of the Russian Federation.

at levels of <1% (eteplirsen), 1% (golodirsen and casimersen), and 5% (viltolarsen), respectively [29]. However, an increase in dystrophin expression levels alone is not a direct indicator of treatment efficacy, as the primary expected outcome is the halting of the degenerative process or improvement of motor functions, whereas ASOs have shown only a slight slowing of disease progression in clinical studies. Moreover, such results were obtained with concurrent corticosteroid therapy [27]. In addition, therapy can cause numerous adverse effects such as headache, fever, and nausea. Since these drugs must be administered weekly, most patients require venous catheter placement, which can lead to various complications, including infections, thrombosis, and septicemia [30, 31]. Considering the overall challenges of lifelong administration and the far-from-ideal patient compliance, the efficacy of this therapy is further reduced.

Another drug used in Russia is ataluren. It is prescribed to patients with a nonsense mutation in the *DMD* gene. This drug acts on the process of protein translation in ribosomes, enabling the reading of mRNA information even in the presence of a premature stop codon, thus producing a full-length protein [32, 33]. Studies have shown that ataluren increases the expression of full-length dystrophin [34] and prolongs the ability to walk independently [35]. In practice, however, ataluren has not demonstrated sufficiently high clinical efficacy and has been associated with adverse effects such as nausea, vomiting, headache, fever, and many others [36]. This drug has been conditionally approved for use in the EU, Brazil, and Russia, but has not been approved by the FDA.

The development of therapeutic strategies based on the gene therapy principles offers significant prospects in clinical practice due to a number of advantages. Etiopathogenetic action aimed at correcting primary molecular defects ensures inhibition of further progression of the pathological condition. The most advanced towards widespread clinical implementation is microdystrophin—a truncated form of the dystrophin protein delivered into the cell as a transgene within an adeno-associated viral (AAV) vector. In 2023, the FDA approved Elevidys (delandistrogene moxeparvovec)*, which successfully completed the second phase of clinical studies; however, the results of the third phase were inconclusive. Elevidys did not lead to a significant improvement in the North Star Ambulatory Assessment score at week 52 compared with the placebo group. Some of the secondary efficacy endpoints, such as time to rise, 10/100 m walk/run, stride velocity, and 4-stair climb, showed improvements during treatment but without statistical significance [37].

Thus, to date, none of the symptomatic or pathogenetic treatment methods is highly effective or suitable for all patients with DMD. Some etiological and pathogenetic features of DMD (Fig. 1), discussed in this review, constitute significant barriers to the development of effective gene therapy.

# OBSTACLES TO THE DEVELOPMENT OF GENE THERAPY

## Gene size

*DMD* is one of the largest human genes. It measures 2.3 Mb and consists of 79 exons. The dystrophin protein has a molecular weight of 427 kDa and contains more than 3600 amino acids. The large size of the gene is the reason for frequent rearrangements such as deletions (about 60%), duplications (about 6%), translocations, and point mutations. Currently, the global TREAT-NMD DMD database contains more than 7000 described mutations, most of which are located in two hotspots encompassing exons 2–20 and 45–55 [23].

It has been shown that the N-terminal and C-terminal domains are critical for proper protein function; therefore, missense variants located in the exons encoding these domains often lead to severe disease forms [38]. At the same time, large deletions that do not cause a reading frame shift and affect the structure of central domains do not lead to severe forms of muscular dystrophy. A rare case was identified in which a patient had a deletion of the central part of the gene covering 46% of the coding sequence, yet only mild BMD was observed [39]. Conversely, relatively short deletions that disrupt the reading frame result in very severe forms of DMD.

Functional analysis of dystrophin structural domains, as well as genotype studies in patients with mild forms of BMD and DMD, have shown that several protein regions can be deleted in various combinations (Fig. 2). Such truncated forms of dystrophin as mini- and microdystrophins are sufficiently functional and perform most of the roles of the full-length protein [40]. In addition, unlike the full-length gene, the shortened form does not exceed the packaging capacity of AAV. Most often, studies use the *mdx* mouse model, in which a nonsense mutation leads to the absence of dystrophin expression [41]. This model has demonstrated that expression of shortened *DMD* gene forms can almost completely prevent dystrophic symptoms [42, 43]. Based on these findings, various microdystrophin constructs approximately 3.6–4.9 kb in length have been developed [40, 44, 45].

Despite their advantages, the shortened form, unlike the full-length version, is not fully functional, and higher levels of microdystrophin are required to achieve a pronounced clinical effect. In studies using the *mdx* mouse model, expression of full-length dystrophin at about 20% of normal levels resulted in complete restoration of diaphragm function, whereas expression of the shortened form at the same level provided only partial functional recovery [42]. Thus, the clinical effect of microdystrophin therapy depends not only on the level of expression but also on the molecular structure. Further mouse studies have demonstrated that various shortened dystrophin forms can improve functional parameters.

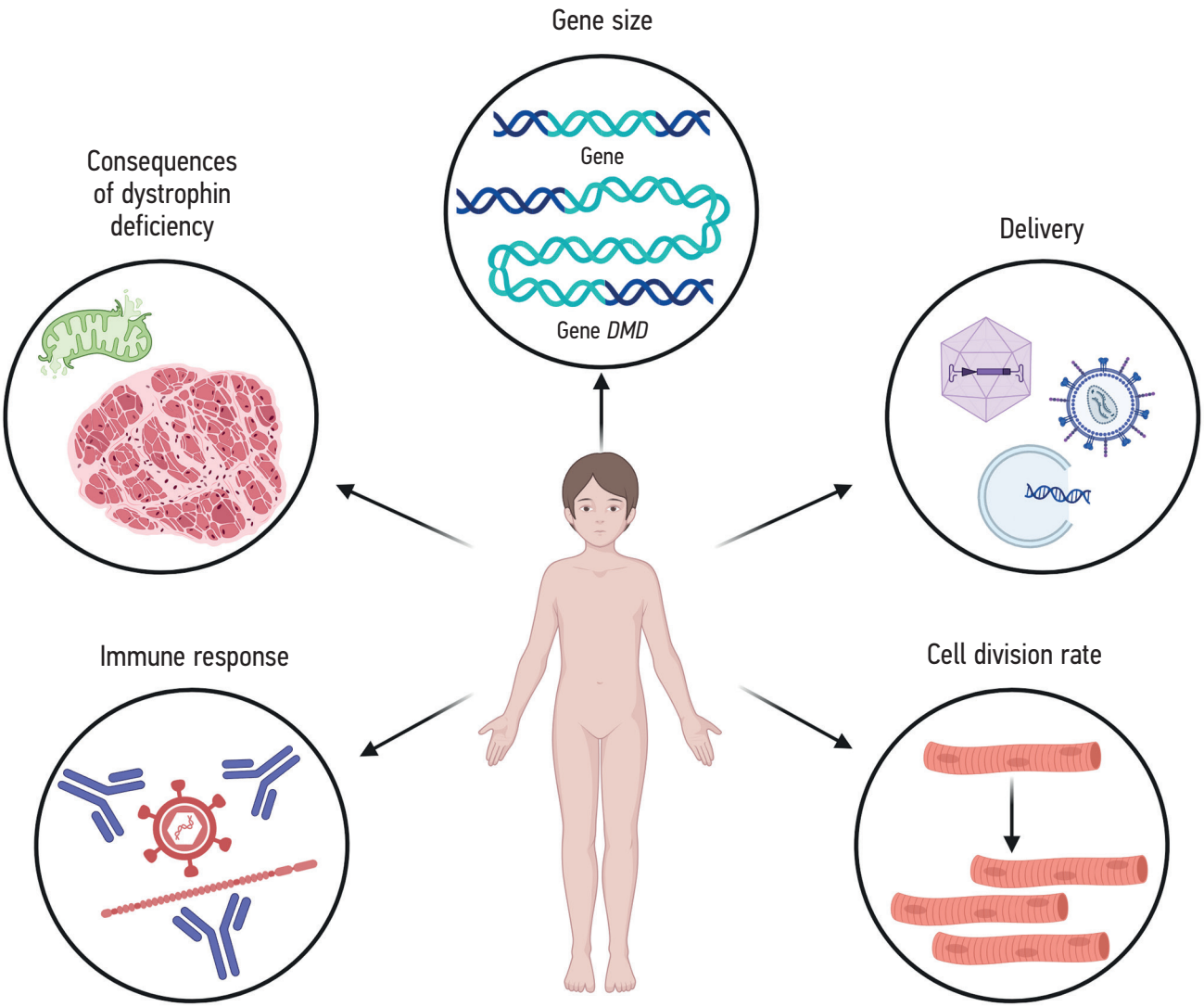


**Fig. 1.** Challenges in developing gene therapies.

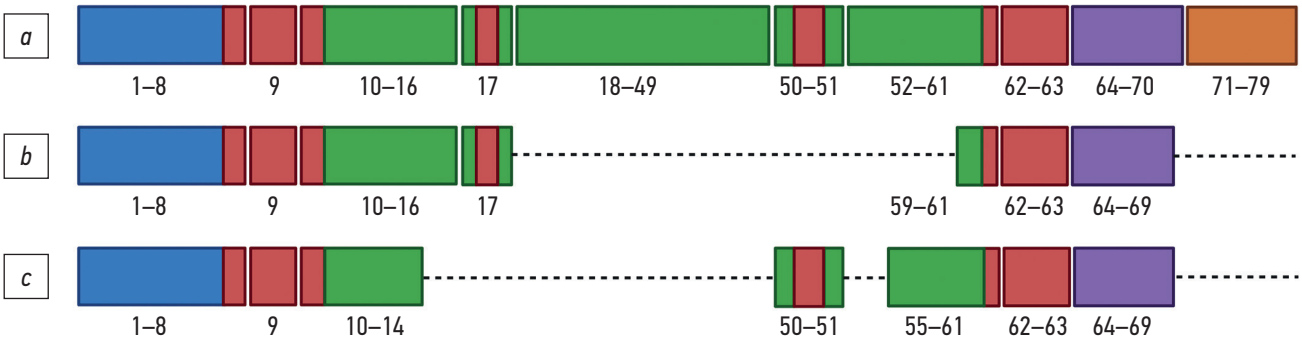


**Fig. 2.** Illustration: *a*, full-size *DMD*; *b*, shortened *DMD* form evaluated in clinical studies by Sarepta-Roche; *c*, shortened *DMD* form evaluated in clinical studies by Pfizer. Every exon is colored to match the protein domain it encodes: blue for actin-binding domain; red for hinge regions; green for central rod domain; purple for cysteine-rich region; orange for C-terminal domain.

Based on these findings, many ongoing clinical studies are evaluating various microdystrophin drugs (see Fig. 2) [46].

Research into different microdystrophin variants using AAV vectors of various serotypes is ongoing [47]. For this therapy to be highly effective, as described above, relatively high doses of the drug are required, which means high doses of viral vectors. This is because the number of viral vectors is calculated based on body weight, and the average age of

a DMD patient receiving treatment is 4–5 years. In Russia, the average age at diagnosis is 6.5 years [48], and thus, older patients require even greater viral volumes, which are more likely to trigger an undesirable immune response (discussed in detail below). In phase I clinical studies of microdystrophin (delandistrogene moxeparvovec*), sustained expression in most muscle fibers was observed [45]. However, phase III results showed no statistically significant differences between the treatment and placebo groups. Given these findings, it can be assumed that even a significant increase in expression of the shortened form of dystrophin is insufficient to compensate for the function of the full-length protein [37]. Nevertheless, such conclusions require results from clinical studies of other microdystrophin-based drugs, and longer patient follow-up may help achieve statistical significance. In addition, alternative delivery methods such as nanovesicles are actively being developed. They offer high biocompatibility and can overcome various tissue barriers. Nanovesicles also have high packaging capacity, which may allow for potential delivery of cDNA encoding full-length dystrophin [49].

## Cell division rate

The ability to maintain dystrophin expression at the required level over a prolonged period following therapy remains uncertain. Cardiomyocytes are considered long-lived cells with a low turnover rate; therefore, dystrophin correction in this muscle type is expected to be long-lasting. In adult skeletal muscle, however, the cell turnover rate is difficult to assess, particularly in DMD. Regeneration of muscle fibers occurs through the fusion of myosatellite cells with damaged myofibrils [50], whereas the formation of new fibers is mediated by the proliferation of myosatellite cells, which may be difficult to edit effectively [51]. Consequently, there is concern that with each regeneration cycle, edited myoblasts will be diluted by unedited cells derived from satellite cells, leading to the loss of AAV DNA encoding the microdystrophin transgene, a gradual decline in protein expression, and disease progression. Recent studies, however, have demonstrated that AAV can efficiently transduce muscle satellite cells, and that the CRISPR/Cas system can edit the pathogenic nucleotide variant of the *DMD* gene [52, 53]. This has been shown to restore dystrophin expression and partially recover the function of dystrophic muscles [54]. Editing of satellite cells would also help to avoid immune complications associated with repeated drug administration, as described below.

## Delivery

Striated muscle tissue affected in DMD accounts for nearly 40% of human body mass [55]. Both skeletal and cardiac muscles are difficult targets for the delivery of genetic constructs, due to their physical separation by fasciae and the high degree of organization of the sarcolemma, which contains numerous invaginations known as T-tubules. Therefore, effective delivery of gene therapy components to affected muscle tissues remains an unresolved challenge [56].

Currently, both viral and non-viral delivery methods are employed in DMD therapy. Viral vectors include adenoviral vectors, AAV, and lentiviral vectors. Adenoviral vectors have the highest packaging capacity (up to 34 kb) and do not integrate into the genome, but they are characterized by high cytotoxicity and immunogenicity [57]. Lentiviral vectors integrate into the genome, have a medium packaging capacity (up to 8 kb), and require a lower viral titer for efficient delivery compared to adenoviruses [58]. To date, the most studied and widely used vectors are AAV [59]. They can efficiently transduce postmitotic tissues, exhibit natural tropism for muscle tissue, enable long-term expression of the target transgene, and are safer than adenoviruses or lentiviruses due to their lower immunogenicity and near-complete lack of genome integration [60]. This method is used not only for the delivery of truncated forms of dystrophin, but also for components of genome-editing systems such as CRISPR/Cas9. Importantly, with this delivery method, selective expression of the Cas9 nuclease in muscle cells can be achieved through the use of specific promoters. Combined with the selective muscle tropism of certain virus serotypes, a high degree of cell specificity can be obtained [61]. A limitation of the approach is that AAV has a lower packaging capacity compared with other viral vectors. The open reading frame of SpCas9 is about 4.2 kb, which is close to the maximal AAV packaging limit (optimally 4.1–4.9 kb) [62]. Therefore, for this type of Cas9 protein, an additional vector carrying the single-guide RNA for nuclease targeting is required [63]. Alternatively, a smaller Cas9 variant can be used, allowing the use of a single vector. With the SaCas9 protein, which is approximately 3.2 kb in size, single-vector delivery enabled gene editing in *mdx* mouse models [54]. However, SaCas9 has significantly lower editing efficiency compared with SpCas9 [64].

Gene therapy components can also be delivered via non-viral methods. Electroporation enables the delivery of genetic constructs by creating nanometer-sized pores in the cell membrane using high-voltage electric pulses. This method has been used to deliver the CRISPR/Cas9 system directly into the skeletal muscles of *mdx* mice, demonstrating functional restoration of dystrophin expression [54]. The major limitation of this approach is high cell mortality caused by electric pulses, which restricts its *in vivo* application. Another non-viral delivery method is lipofection, in which effector molecules are encapsulated in lipid vesicles capable of penetrating the cell membrane [65]. In this case, transfection agents are non-immunogenic and less cytotoxic compared with electroporation but lack the ability for targeted delivery to specific tissues or organs. Approaches based on the transient delivery of genome-editing components in the form of mRNA and ribonucleoproteins are also being investigated, as these may reduce cytotoxicity and off-target effects [66]. At present, new and more efficient delivery strategies are being actively developed, including

nanosized extracellular vesicles, which have considerable therapeutic potential. However, the production of such vesicles is costly and subject to numerous technological limitations, which significantly hinder implementation of this method into clinical practice [67].

## Immune response

In DMD, full-length dystrophin is absent from birth and is essentially a foreign antigen when it appears in the body, including in the form of microdystrophin. Intravenous administration of an AAV vector carrying the microdystrophin transgene has been shown, in some patients, to cause adverse reactions such as weakness of the proximal and distal limb muscles and respiratory muscles. Signs of myositis, myocarditis, and muscle edema with T-cell infiltration on biopsy have also been observed. The duration of these adverse reactions corresponded to the duration of transgene expression, suggesting an immune response against dystrophin. Enzyme-linked immunosorbent assay and antibody epitope mapping demonstrated immunological reactivity to a peptide region encoded by exons 8–11 in patients with deletions spanning from exon 8 to exon 21 [68]. Similar findings were reported in a patient with a deletion of exons 3–17 [69].

During clinical studies of the gene therapy product Elevidys (delandistrogene moxeparvovec*), immune-mediated reactions were identified in patients with deletions in exons 8–9. Symptoms of immune-mediated myositis included muscle weakness, dysphagia, respiratory impairment (cough, dyspnea), fever, fatigue, and weight loss. This immune reaction in children has an especially acute onset. Therefore, this therapy is contraindicated in patients with deletions in exons 8–9. In addition, the drug is restricted for use in patients with deletions in exons 1–17 and/or 59–71, as these cases are also at risk of developing severe immune-mediated myositis.[2]

It is noteworthy that approximately half of patients with DMD exhibit dystrophin-positive revertant (normal) fibers, which may account for up to 7% of the total fibers [70]. The mechanism underlying this phenomenon remains insufficiently understood, but the most likely explanation is somatic mosaicism or the occurrence of an additional mutation restoring the reading frame [71]. Accordingly, in such patients, dystrophin should not be recognized as a foreign protein. However, in cases of large deletions, restoring the reading frame will not reintroduce previously lost epitopes, to which the immune system may still respond if they are introduced into the body as part of gene therapy [70].

Since some patients have T cells specific to dystrophin and are pre-immunized against various dystrophin epitopes [72], numerous studies are currently aimed at identifying dystrophin paralogous genes encoding functionally similar proteins. One alternative therapeutic approach may involve increasing the expression of the closely related protein utrophin. This protein is a structural and functional autosomal paralog of dystrophin encoded by the *UTRN* gene [73]. In fetal muscle cells, it can assemble into a large transmembrane glycoprotein complex and bind actin filaments [74], but in adulthood it is typically replaced by dystrophin [75]. Increasing utrophin levels can help maintain muscle cell integrity and alleviate some dystrophic symptoms. As this protein is endogenous, several approaches have been explored to enhance its expression, including transgene delivery and indirect stimulation of the gene promoter activity [76]. In a study using the *mdx* mouse model lacking dystrophin, high expression of a truncated utrophin transgene in skeletal and diaphragmatic muscles significantly alleviated pathological symptoms [77]. Moreover, utrophin upregulation can be achieved using the CRISPR/Cas9 system: Cas9 protein lacking endonuclease activity enables targeted delivery of a transcriptional activator to the *UTRN* gene promoter [78]. This system has been shown to increase utrophin expression in myoblasts by 1.7–6.9-fold [79]. Another approach to upregulate utrophin expression is the administration of small molecules that activate *UTRN* gene transcription. In 2017, clinical studies of ezutromid* were conducted; however, they failed to show efficacy in patients with DMD. The studies were discontinued after phase II[3].

In addition to dystrophin, AAV, which is widespread in human populations, can also serve as an antigen for the immune system [80]. As a result, both humoral and cellular immunity to this virus develop over a human's lifetime. Pre-existing antibodies in the human body can block vector transduction, thereby reducing the efficacy of therapy, whereas the administered AAV can cause hyperstimulation of the immune system [81]. Therefore, before administering AAV-based drugs, it is important to ensure the absence of antibodies to specific viral serotypes. After the application of gene therapy, monitoring for expected adverse effects is necessary. AAV vectors can accumulate in the liver, creating a risk of dose-dependent toxicity [82]. Furthermore, the administration of AAV induces the formation of new antibodies, which precludes the possibility of re-administration of the viral preparation [83]. Possible approaches to overcoming the immune response to AAV include plasmapheresis, immunomodulatory drugs, or the selection of an alternative viral serotype. The effectiveness of these approaches is under active investigation [82].

The components of genome-editing systems are also foreign to the human body. Among human infectious diseases, there are some caused by bacteria possessing nucleases of the CRISPR/Cas9 family. This leads to the development

[2] https://www.fda.gov/ [Internet]. Elevidys. Available at: https://www.fda.gov/vaccines-blood-biologics/tissue-tissue-products/elevidys Accessed on: November 13, 2024.

[3] https://clinicaltrials.gov/ [Internet]. Phaseout DMD: a phase 2 clinical study to assess the activity and safety of utrophin modulation with ezutromid in ambulatory paediatric male subjects with duchenne muscular dystrophy (SMT C11005): clinical trial registration NCT02858362. 2019. Accessed on: https://cdn.clinicaltrials.gov/large-docs/62/NCT02858362/Prot_000.pdf

of immunity to the Cas9 protein. Using enzyme-linked immunosorbent assay, antibodies against SaCas9 and SpCas9 were detected in 78% and 58% of donors, respectively. It has been demonstrated that both humoral and cell-mediated immunity to Cas9 proteins exist in humans, and this must be taken into account when developing gene therapy [84]. This problem can be addressed by using modified Cas9 proteins in which specific immunodominant epitopes are altered to reduce immune recognition. At the same time, the protein's functionality and specificity are fully preserved [85].

Corticosteroids, routinely prescribed in patients with DMD, generally reduce the risk of immune response by acting on T cells [72]. However, the broad range of potential antigens in gene therapy imposes significant limitations on its clinical applicability.

### Consequences of dystrophin deficiency

Another obstacle to the use of gene therapy for DMD is the irreversible pathological changes that may have occurred in the patient's body before the initiation of treatment. Currently, approaches aimed at mitigating the secondary pathological mechanisms associated with dystrophin deficiency are actively being developed. The absence of this protein leads to the disruption of the dystrophin-associated complex, increasing the susceptibility of the sarcolemma to damage occurring during muscle fiber contraction. Calcium regulation is also impaired, leading to chronic inflammation and fibrosis [11]. Elevated calcium concentrations stimulate the production of reactive oxygen species, which in turn increases oxidative stress, exacerbating calcium dysregulation, causing mitochondrial dysfunction and inflammation [86]. This results in impaired adenosine triphosphate production and metabolic dysfunction. Many drugs targeting the consequences of dystrophin deficiency are undergoing clinical studies [87]. Thus, in cases of late diagnosis, the combination of gene therapy with agents aimed at eliminating the consequences of dystrophin absence may improve treatment efficacy. It is also important to continue advancing the capabilities for earlier clinical and molecular genetic diagnosis.

## CONCLUSION

Gene therapy is a promising and potentially effective approach to the treatment of DMD. However, despite significant progress, this technology is associated with a number of substantial limitations. Some are related to the specifics of DMD pathogenesis, gene structure, and the involvement of not only muscle tissue but also other body systems. Others are associated with adverse reactions to the components of gene therapy products. It is also important to note that delayed diagnosis and late initiation of treatment limit the ability to reverse accumulated pathological changes. On the other hand, the shortcomings of currently available treatment methods necessitate continued efforts to improve DMD gene therapy despite these challenges. A deeper understanding of these issues will facilitate their resolution.

## ADDITIONAL INFORMATION

**Authors' contributions.** E.V. Kurshakova — collection and analysis of literary sources, preparation and writing of the text of the article; O.A. Levchenko — collection and analysis of literary sources, preparation and writing of the text of the article, writing the text and editing the article; A.V. Lavrov — writing the text and editing the article. All authors approved the manuscript (version for publication) and agreed to take responsibility for all aspects of the work, ensuring the proper consideration and resolution of any issues related to the accuracy and integrity of any part of the study.

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## REFERENCES | СПИСОК ЛИТЕРАТУРЫ

**1.** Cohn RD, Campbell KP. Molecular basis of muscular dystrophies. *Muscle Nerve*. 2000;23(10):1456–1471. doi: 10.1002/1097-4598(200010)23:10<1456::aid-mus2>3.0.co;2-t

**2.** Chelly J, Hamard G, Koulakoff A, et al. Dystrophin gene transcribed from different promoters in neuronal and glial cells. *Nature*. 1990;344(6261):64–65. doi: 10.1038/344064a0

**3.** Górecki DC, Monaco AP, Derry JM, et al. Expression of four alternative dystrophin transcripts in brain regions regulated by different promoters. *Hum Mol Genet*. 1992;1(7):505–510. doi: 10.1093/hmg/1.7.505 EDN: IPBULT

**4.** D'Souza VN, Nguyen TM, Morris GE, et al. A novel dystrophin isoform is required for normal retinal electrophysiology. *Hum Mol Genet*. 1995;4(5):837–842. doi: 10.1093/hmg/4.5.837

**5.** Byers TJ, Lidov HG, Kunkel LM. An alternative dystrophin transcript specific to peripheral nerve. *Nat Genet*. 1993;4(1):77–81. doi: 10.1038/ng0593-77

**6.** Emery AE. The muscular dystrophies. *Lancet*. 2002;359(9307):687–695. doi: 10.1016/S0140-6736(02)07815-7 EDN: DRTGLN

**7.** Gao QQ, McNally EM. The dystrophin complex: structure, function, and implications for therapy. *Compr Physiol*. 2015;5(3):1223–1239. doi: 10.1002/cphy.c140048

**8.** Ervasti JM, Campbell KP. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J Cell Biol*. 1993;122(4):809–823. doi: 10.1083/jcb.122.4.809

**9.** Omairi S, Hau KL, Collins-Hooper H, et al. Regulation of the dystrophin-associated glycoprotein complex composition by the metabolic properties of muscle fibres. *Sci Rep*. 2019;9(1):2770. doi: 10.1038/s41598-019-39532-4 EDN: FHHGIH
**10.** Meyers TA, Townsend D. Cardiac pathophysiology and the future of cardiac therapies in duchenne muscular dystrophy. *Int J Mol Sci*. 2019;20(17):4098. doi: 10.3390/ijms20174098 EDN: VPMTFR
**11.** Blake DJ, Weir A, Newey SE, Davies KE. Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev*. 2002;82(2):291–329. doi: 10.1152/physrev.00028.2001
**12.** Emery AEH, Muntoni F, Quinlivan RCM. *Duchenne muscular dystrophy. Fourth edition*. Oxford, New York: Oxford University Press; 2015. 320 p. doi: 10.1093/med/9780199681488.003.0007
**13.** 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.
**14.** Le Rumeur E. Dystrophin and the two related genetic diseases, Duchenne and Becker muscular dystrophies. *Bosn J Basic Med Sci*. 2015;15(3):14–20. doi: 10.17305/bjbms.2015.636
**15.** Pikó H, Vancsó V, Nagy B, et al. Dystrophin gene analysis in Hungarian Duchenne/Becker muscular dystrophy families — detection of carrier status in symptomatic and asymptomatic female relatives. *Neuromuscul Disord*. 2009;19(2):108–112. doi: 10.1016/j.nmd.2008.10.011
**16.** 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
**17.** Balaban B, Matthews DJ, Clayton GH, Carry T. Corticosteroid treatment and functional improvement in Duchenne muscular dystrophy: long-term effect. *Am J Phys Med Rehabil*. 2005;84(11):843–850. doi: 10.1097/01.phm.0000184156.98671.d0
**18.** McDonald CM, Sajeev G, Yao Z, et al. Deflazacort vs prednisone treatment for Duchenne muscular dystrophy: A meta-analysis of disease progression rates in recent multicenter clinical trials. *Muscle Nerve*. 2020;61(1):26–35. doi: 10.1002/mus.26736 EDN: MRXTVB
**19.** Griggs RC, Miller JP, Greenberg CR, et al. Efficacy and safety of deflazacort vs prednisone and placebo for Duchenne muscular dystrophy. *Neurology*. 2016;87(20):2123–2131. doi: 10.1212/WNL.0000000000003217
**20.** Ward LM, Hadjiyannakis S, McMillan HJ, et al. Bone health and osteoporosis management of the patient with duchenne muscular dystrophy. *Pediatrics*. 2018;142(Suppl. 2):S34–S42. doi: 10.1542/peds.2018-0333E
**21.** 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):e1312–e1323. doi: 10.1212/WNL.0000000000008168
**22.** Keam SJ. Vamorolone: first approval. *Drugs*. 2024;84(1):111–117. doi: 10.1007/s40265-023-01986-2 EDN: MRCROY
**23.** Bladen CL, Salgado D, Monges S, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. *Hum Mutat*. 2015;36(4):395–402. doi: 10.1002/humu.22758 EDN: UFQPHB
**24.** Bennett CF. Therapeutic antisense oligonucleotides are coming of age. *Annu Rev Med*. 2019;70:307–321. doi: 10.1146/annurev-med-041217-010829
**25.** Komaki H, Takeshima Y, Matsumura T, et al. Viltolarsen in Japanese Duchenne muscular dystrophy patients: A phase 1/2 study. *Ann Clin Transl Neurol*. 2020;7(12):2393–2408. doi: 10.1002/acn3.51235 EDN: RHXIMB
**26.** Heo YA. Golodirsen: first approval. *Drugs*. 2020;80(3):329–333. doi: 10.1007/s40265-020-01267-2 EDN: IHMPCR
**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 EDN: YXQCCH
**28.** Shirley M. Casimersen: first approval. *Drugs*. 2021;81(7):875–879. doi: 10.1007/s40265-021-01512-2 EDN: TOCOFI
**29.** Aartsma-Rus A, De Waele L, Houwen-Opstal S, et al. The dilemma of choice for duchenne patients eligible for exon 51 skipping the European experience. *J Neuromuscul Dis*. 2023;10(3):315–325. doi: 10.3233/JND-221648 EDN: RIGSIJ
**30.** *Eteplirsen. LiverTox: clinical and research information on drug-induced liver injury*. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555333/
**31.** *Viltolarsen. LiverTox: Clinical and research information on drug-induced liver injury*. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK588132/
**32.** Peltz SW, Morsy M, Welch EM, Jacobson A. Ataluren as an agent for therapeutic nonsense suppression. *Annu Rev Med*. 2013;64:407–425. doi: 10.1146/annurev-med-120611-144851 EDN: RLANYJ
**33.** Roy B, Friesen WJ, Tomizawa Y, et al. Ataluren stimulates ribosomal selection of near-cognate tRNAs to promote nonsense suppression. *Proc Natl Acad Sci U S A*. 2016;113(44):12508–12513. doi: 10.1073/pnas.1605336113
**34.** Finkel RS, Flanigan KM, Wong B, et al. Phase 2a study of ataluren-mediated dystrophin production in patients with nonsense mutation Duchenne muscular dystrophy. *PLoS One*. 2013;8(12):e81302. doi: 10.1371/journal.pone.0081302
**35.** Mercuri E, Muntoni F, Osorio AN, et al. Safety and effectiveness of ataluren: comparison of results from the STRIDE Registry and CINRG DMD Natural History Study. *J Comp Eff Res*. 2020;9(5):341–360. doi: 10.2217/cer-2019-0171 EDN: CZHIPM
**36.** McDonald CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;390(10101):1489–1498. doi: 10.1016/S0140-6736(17)31611-2
**37.** 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 EDN: XMKPEH
**38.** Sweeney HL, Barton ER. The dystrophin-associated glycoprotein complex: what parts can you do without? *Proc Natl Acad Sci U S A*. 2000;97(25):13464–13466. doi: 10.1073/pnas.011510597
**39.** England SB, Nicholson LV, 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
**40.** Harper SQ, Hauser MA, DelloRusso C, et al. Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy. *Nat Med*. 2002;8(3):253–261. doi: 10.1038/nm0302-253

**41.** Sicinski P, Geng Y, Ryder-Cook AS, et al. The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. *Science*. 1989;244(4912):1578–1580. doi: 10.1126/science.2662404 EDN: IEADHH

**42.** Phelps SF, Hauser MA, Cole NM, et al. Expression of full-length and truncated dystrophin mini-genes in transgenic mdx mice. *Hum Mol Genet*. 1995;4(8):1251–1258. doi: 10.1093/hmg/4.8.1251 EDN: IPCGZN

**43.** Wells DJ, Wells KE, Asante EA, et al. Expression of human full-length and minidystrophin in transgenic mdx mice: implications for gene therapy of Duchenne muscular dystrophy. *Hum Mol Genet*. 1995;4(8):1245–1250. doi: 10.1093/hmg/4.8.1245 EDN: IPCGZD

**44.** Hoy SM. Delandistrogene moxeparvovec: first approval. *Drugs.* 2023;83(14):1323–1329. doi: 10.1007/s40265-023-01929-x EDN: VMKDOA

**45.** Mendell JR, Sahenk Z, Lehman K, et al. Assessment of systemic delivery of raa-vrh74.mhck7.micro-dystrophin in children with duchenne muscular dystrophy: a nonrandomized controlled trial. *JAMA Neurol*. 2020;77(9):1122–1131. doi: 10.1001/jamaneurol.2020.1484 EDN: ONUKNY

**46.** Boehler JF, Brown KJ, Beatka M, et al. Clinical potential of microdystrophin as a surrogate endpoint. *Neuromuscul Disord*. 2023;33(1):40–49. doi: 10.1016/j.nmd.2022.12.007 EDN: FTFEGG

**47.** Duan D. Micro-dystrophin gene therapy goes systemic in duchenne muscular dystrophy patients. H*um Gene Ther*. 2018;29(7):733–736. doi: 10.1089/hum.2018.012

**48.** Gremyakova TA, Artemyeva SB, Baybarina EN, et al. Consensus concept of modern effective therapy for duchenne muscular dystrophy. *Neuromuscular Diseases*. 2023;13(2):10–19. doi: 10.17650/2222-8721-2023-13-2-10-19 EDN: RLIPTP

**49.** Oh SW, Han J, Park SS. Non-viral gene therapy for neuromuscular diseases including Duchenne muscular dystrophy using nanovesicles derived from human cells. *rdodj. OAE Publishing Inc*. 2024;3(4):N/A–N/A. doi: 10.20517/rdodj.2024.16 EDN: HHWVWJ

**50.** Min YL, Bassel-Duby R, Olson EN. CRISPR correction of duchenne muscular dystrophy. *Annu Rev Med*. 2019;70:239–255. doi: 10.1146/annurev-med-081117-010451 EDN: YTCVAS

**51.** Arnett AL, Konieczny P, Ramos JN, et al. Adeno-associated viral (AAV) vectors do not efficiently target muscle satellite cells. *Mol Ther Methods Clin Dev*. 2014;1:14038. doi: 10.1038/mtm.2014.38

**52.** 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 EDN: CQVRHJ

**53.** 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 EDN: JGZKVM

**54.** 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

**55.** Collins CA, Partridge TA. Self-renewal of the adult skeletal muscle satellite cell. *Cell Cycle*. 2005;4(10):1338–1341. doi: 10.4161/cc.4.10.2114

**56.** Padmaswari MH, Agrawal S, Jia MS, et al. Delivery challenges for CRISPR-Cas9 genome editing for Duchenne muscular dystrophy. *Biophys Rev (Melville)*. 2023;4(1):011307. doi: 10.1063/5.0131452 EDN: IKCXGB

**57.** Matsunaga W, Gotoh A. Adenovirus as a vector and oncolytic virus. *Curr Issues Mol Biol*. 2023;45(6):4826–4840. doi: 10.3390/cimb45060307 EDN: DUIQQN

**58.** Li X, Le Y, Zhang Z, et al. Viral vector-based gene therapy. *Int J Mol Sci*. 2023;24(9):7736. doi: 10.3390/ijms24097736

**59.** Yuasa K, Miyagoe Y, Yamamoto K, et al. Effective restoration of dys-trophin-associated proteins in vivo by adenovirus-mediated transfer of truncated dystrophin cDNAs. *FEBS Lett*. 1998;425(2):329–336. doi: 10.1016/s0014-5793(98)00251-8 EDN: AAYNBB

**60.** Costa Verdera H, Kuranda K, Mingozzi F. AAV vector immunogenicity in humans: a long journey to successful gene transfer. *Mol Ther*. 2020;28(3):723–746. doi: 10.1016/j.ymthe.2019.12.010 EDN: WPXEZQ

**61.** Amoasii L, Long C, Li H, et al. Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. *Sci Transl Med*. 2017;9(418):eaan8081. doi: 10.1126/scitranslmed.aan8081 EDN: YFAEJV Erratum in: *Sci Transl Med*. 2018;10(425):eaat0240. doi: 10.1126/scitranslmed.aat0240

**62.** Dong JY, Fan PD, Frizzell RA. Quantitative analysis of the packaging capacity of recombinant adeno-associated virus. *Hum Gene Ther*. 1996;7(17):2101–2112. doi: 10.1089/hum.1996.7.17-2101

**63.** Grounds MD. Two-tiered hypotheses for Duchenne muscular dystrophy. *Cell Mol Life Sci*. 2008;65(11):1621–1625. doi: 10.1007/s00018-008-7574-8 EDN: PXALUH

**64.** Li F, Wing K, Wang JH, et al. Comparison of CRISPR/Cas endonucleases for in vivo retinal gene editing. *Front Cell Neurosci*. 2020;14:570917. doi: 10.3389/fncel.2020.570917 EDN: PYSCCB

**65.** 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 EDN: WTYTIR

**66.** Tsuchida CA, Wasko KM, Hamilton JR, Doudna JA. Targeted nonviral delivery of genome editors *in vivo*. *Proc Natl Acad Sci U S A*. 2024;121(11):e2307796121. doi: 10.1073/pnas.2307796121 EDN: VSVPYS

**67.** 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 Engl*. 2017;56(4):1059–1063. doi: 10.1002/anie.201610209 EDN: YWRCTZ

**68.** Bönnemann CG, Belluscio BA, Braun S, et al. Dystrophin immunity after gene therapy for duchenne's muscular dystrophy. *N Engl J Med*. 2023;388(24):2294–2296. doi: 10.1056/NEJMc2212912 EDN: VMQIQD

**69.** Mendell JR, Campbell K, Rodino-Klapac L, et al. Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med*. 2010;363(15):1429–1437. doi: 10.1056/NEJMoa1000228

**70.** Fanin M, Danieli GA, Cadaldini M, et al. Dystrophin-positive fibers in Duchenne dystrophy: origin and correlation to clinical course. *Muscle Nerve*. 1995;18(10):1115–1120. doi: 10.1002/mus.880181007

**71.** Klein CJ, Coovert DD, Bulman DE, et al. Somatic reversion/suppression in Duchenne muscular dystrophy (DMD): evidence supporting a frame-restoring mechanism in rare dystrophin-positive fibers. *Am J Hum Genet*. 1992;50(5):950–959.

**72.** Flanigan KM, Campbell K, Viollet L, et al. Anti-dystrophin T cell responses in Duchenne muscular dystrophy: prevalence and a glucocorticoid treatment effect. *Hum Gene Ther*. 2013;24(9):797–806. doi: 10.1089/hum.2013.092
**73.** 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
**74.** Rosenberg RN, Pascual JM. *Rosenberg's molecular and genetic basis of neurological and psychiatric disease*. Elsevier; 2020. P. 1–806.
**75.** Clerk A, Morris GE, Dubowitz V, et al. Dystrophin-related protein, utrophin, in normal and dystrophic human fetal skeletal muscle. *Histochem J.* 1993;25(8):554–561. doi: 10.1007/BF02388063
**76.** Soblechero-Martín P, López-Martínez A, de la Puente-Ovejero L, et al. Utrophin modulator drugs as potential therapies for Duchenne and Becker muscular dystrophies. *Neuropathol Appl Neurobiol*. 2021;47(6):711–723. doi: 10.1111/nan.12735 EDN: UDHHGO
**77.** Tinsley JM, Potter AC, Phelps SR, et al. Amelioration of the dystrophic phenotype of *mdx* mice using a truncated utrophin transgene. *Nature*. 1996;384(6607):349–353. doi: 10.1038/384349a0
**78.** Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*. 2013;152(5):1173–1183. doi: 10.1016/j.cell.2013.02.022 EDN: YAEWRE
**79.** Wojtal D, Kemaladewi DU, 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 EDN: WTLFVL
**80.** Gao G, Vandenberghe LH, Alvira MR, et al. Clades of adeno-associated viruses are widely disseminated in human tissues. *J Virol*. 2004;78(12):6381–6388. doi: 10.1128/JVI.78.12.6381-6388.2004
**81.** Yang Y, Haecker SE, Su Q, Wilson JM. Immunology of gene therapy with adenoviral vectors in mouse skeletal muscle. *Hum Mol Genet*. 1996;5(11):1703–1712. doi: 10.1093/hmg/5.11.1703 EDN: IPBAHX
**82.** Chicoine LG, Montgomery CL, Bremer WG, et al. Plasmapheresis eliminates the negative impact of AAV antibodies on microdystrophin gene expression following vascular delivery. *Mol Ther*. 2014;22(2):338–347. doi: 10.1038/mt.2013.244
**83.** Halbert CL, Rutledge EA, Allen JM, et al. Repeat transduction in the mouse lung by using adeno-associated virus vectors with different serotypes. *J Virol*. 2000;74(3):1524–1532. doi: 10.1128/jvi.74.3.1524-1532.2000
**84.** 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 EDN: WYAXYJ
**85.** Ferdosi SR, Ewaisha R, Moghadam F, et al. Multifunctional CRISPR-Cas9 with engineered immunosilenced human T cell epitopes. *Nat Commun*. 2019;10(1):1842. doi: 10.1038/s41467-019-09693-x EDN: SAABLB
**86.** Allen DG, Whitehead NP, Froehner SC. Absence of dystrophin disrupts skeletal muscle signaling: roles of $Ca2^{+}$, reactive oxygen species, and nitric oxide in the development of muscular dystrophy. *Physiol Rev*. 2016;96(1):253–305. doi: 10.1152/physrev.00007.2015 EDN: WPABZB
**87.** Pharmacological advances for treatment in Duchenne muscular dystrophy. *Current Opinion in Pharmacology*. *Elsevier*. 2017;34:36–48.

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