REVIEW ARTICLE

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Current advances and future directions in Duchenne muscular dystrophy treatment – review

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Abstract

Introduction and Objective. Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder characterized by the absence or severe deficiency of functional dystrophin, a key component of the muscle cytoskeleton and dystrophin-associated protein complex. The aim of the review is to summarize emerging therapeutic approaches currently being investigated for Duchenne muscular dystrophy, and to evaluate their potential advantages and limitations.

Review Methods. A literature search was conducted using the PubMed database. Original articles, review articles, and clinical trials published predominantly between 2020–2026 (95%) were included. Publications addressing the pathogenesis, genetic basis, and emerging therapeutic approaches for Duchenne muscular dystrophy were identified and analyzed following an initial screening process.

Brief description of the state of knowledge. Duchenne muscular dystrophy is a progressive and degenerative disease that can lead to premature death. The disease primarily affects the muscular, cardiovascular, and respiratory systems. Early clinical manifestations include delayed motor development, frequent falls, and difficulty climbing stairs, which progressively contribute to the loss of independent ambulation.

Summary. Advances in the understanding of the molecular mechanisms underlying the disease have led to the development of therapies targetting the mutation itself. In recent years, considerable attention has been focused on antisense oligonucletides, CRISPR-Cas therapy, microdystrophin gene replacement, utrophin modulation, stem cell treatment, and many more. Despite these promising developments, significant challenges remain, including immunological safety concerns, efficient delivery of genetic material, and the long-term therapeutic effects.

Key words

stem cells, CRISPR/Cas9, Duchenne muscular dystrophy, genome editing, microdystophin, utrophin modulation

INTRODUCTION AND OBJECTIVE

Duchenne muscular dystrophy (DMD) is a severe, progressive X-linked recessive neuromuscular disorder characterized by progressive skeletal muscle weakness, respiratory failure, and cardiomyopathy [1]. It is one of the most common inherited childhood myopathies, affecting approximately 1 in 5,000 live male births worldwide [1,2]. The disease is caused by more than 7,000 mutations in the large sequence of DMD gene located on chromosome Xp21. It is the largest known human gene, spanning approximately 2.2–2.5 million base pairs and comprising 79 exons [1,3,4]. Owing to its large size, the gene is highly susceptible to mutations, with approximately one-third of cases resulting from *de novo* mutations, while the remainder are linked with germline mosaicism or inheritance from a carrier mother [1,5]. The most common genetic alterations are exon deletions, accounting for approximately 60–65% of cases, whereas duplications represent about 11% of all mutations [1].

The DMD gene encodes dystrophin, a protein with a molecular weight of approximately 427 kDa that plays a crucial role in maintaining the structural integrity of muscle fiber membranes [1,6]. In healthy muscle, dystrophin links the intracellular actin cytoskeleton to the extracellular matrix (ECM) through the dystrophin-glycoprotein complex (DGC) and the integrin complex. These structural connections stabilize the sarcolemma during muscle contraction and stretching, protecting muscle fibres from mechanical damage and maintaining membrane integrity. The lack of dystrophin and disorganization of many dystrophin-associated proteins, disrupts these links. As a result, the sarcolemma becomes leaky and highly susceptible to injury. Characteristic sarcolemmal microruptures, referred to as 'delta lesions', develop in dystrophic muscle fibres, leading to recurrent inflammations, muscle cell death and fibrosis. Loss of membrane integrity also promotes abnormal calcium influx into muscle cells. The intracellular calcium levels are further amplified by sarcoplasmic reticulum leakage and reactive oxygen species (ROS) involvement. Calcium overload activates multiple pathological signalling pathways, impairs muscle repair mechanisms, and affects mitochondrial function. Consequently, mitochondrial respiration and ATP production decline, membrane potential is lost, and mitochondrial homeostasis becomes compromised. These mitochondrial abnormalities result in increased electrons leakage from the electron transport chain and excessive ROS generation. Elevated ROS levels stimulate muscle fibrosis, promote cell death, and contribute to disease progression. Normally, inflammation is limited, controlled, and quickly resolved. However, in

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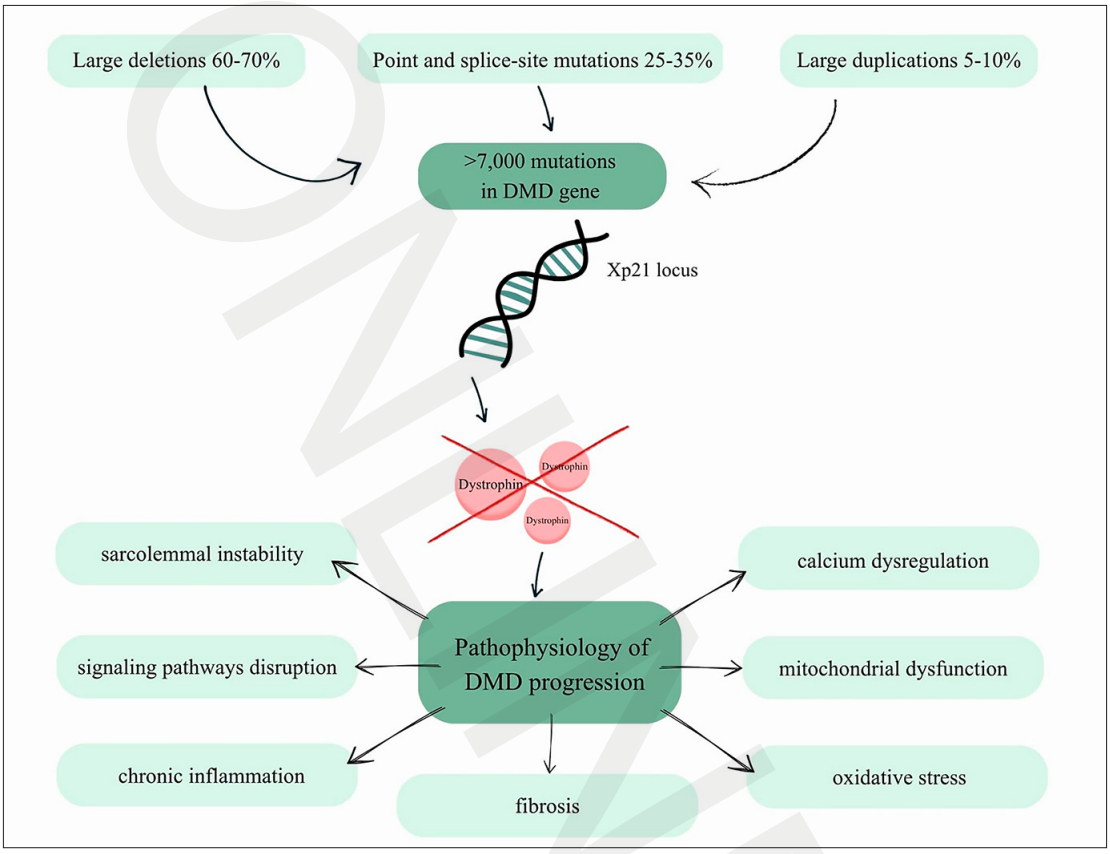


Figure 1. Genetic basis and pathophysiology of DMD. The figure shows the percentage distribution of mutation types responsible for the development of DMD - among them are large deletions, point and splice-site mutations, and large duplications in the Xp21 locus. This disrupts the formation of the dystrophin and subsequently leads to the development of disease symptoms underlying mechanisms such as sarcolemmal instability, disruption of signaling pathways, chronic inflammation, fibrosis, oxidative stress, mitochondrial dysfunction and calcium dysregulation [1, 4, 7–10]

DMD membrane instability leads to the continuous release of cytoplasmic content that triggers downstream signalling cascades. A key mediator of this process is the nuclear factor kappa B (NF-κB) signaling pathway. In affected muscles, it is constantly active due to ongoing muscle damage and stimulates expression of pro-inflammatory factors e.g. TNF-α, IL-1β, and IL-6. Consequently, chronic inflammation persists and attracts immune cells, mostly macrophages and T lymphocytes that accelerate muscle degradation and promote fibrosis [1,7,8]. Moreover, increased oxidative stress and transforming growth factor β (TGF-β) expression driven by NF-κB, exacerbates deposition of ECM components and collagen. The renin–angiotensin system (RAS) further enhances this process, resulting in substantial collagen accumulation, increased stiffness, and progressive muscle weakness. Moreover, NF-κB impairs muscle regeneration by inducing degradation and inhibition of protein synthesis, ultimately leading to the loss of muscle mass and strength [8]. In addition, epigenetic regulators such as histone deacetylases (HDACs) also contribute to DMD progression by modulating muscle regeneration and inflammatory responses. Their increased activity promotes impaired myogenic gene expression and also enhances pro-fibrotic remodelling [9]. Consequently, patients experience a gradual loss of both mass and function of skeletal, respiratory, and cardiac muscles [1]. A graphical representation of the pathogenesis of DMD and its genetic basis is shown in Figure 1.

Current diagnosis of DMD is based on clinical features and genetic testing, and is typically established by the age of four years. Genetic analysis of the DMD gene is commonly used for screening, particularly for the detection of large mutations, including deletions and duplications. These variants can be identified in approximately 70% of patients using multiplex ligation-dependent probe amplification (MLPA). Smaller variants, including point mutations and splice-site mutations, require different techniques using next-generation sequencing-based approaches, such as targeted gene panels or whole-genome sequencing (WGS) [8,10].

DMD is a progressive, deliberating and degenerative condition that is ultimately fatal [1]. Initially, the disease primarily affects the proximal muscles of the lower limbs and trunk [8]. The first clinical manifestations of DMD typically appear between the ages of 2 and 3 years and include delayed motor development, frequent falls, difficulty climbing stairs, waddling gait and the characteristic Gower's sign (a difficulty standing up from a sitting position) [1,2,5]. The disease follows an inevitably progressive course, with most patients losing independent ambulation between 10 and 12 years of age [1,2,11]. Further progression of DMD demonstrates multiple issues that need proper clinical attention. The most challenging are cardiac and respiratory management. Life-threatening consequences of cardiac muscle dystrophin deficiency include heart hypertrophy, dilated cardiomyopathy, arrhythmias, and congestive heart failure. As for pulmonary impairment, respiratory muscle weakness leads to severe ventilatory insufficiency, restrictive lung disease, higher risk of upper respiratory tract infections and pneumonia. Moreover, immobility leads to lower bone density and higher risk of fractures [1,7,8]. Dystrophin

deficiency may also affect the central nervous system and is associated with an increased prevalence of cognitive impairment, attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorders [1]. However, these manifestations vary depending on the involved isoforms as well as clinical phenotype between DMD and Becker Muscular dystrophy (BMD) [12].

Despite the lack of a curative treatment, patient outcomes have improved considerably over recent decades. Before 1970, the average life expectancy of individuals with DMD was approximately 14 years; however, owing to advances in multidisciplinary care, many patients now survive into their third or even fourth decade of life [13]. Current standard-of-care management includes physiotherapy, glucocorticoid therapy, respiratory support, and cardiological care [13,14]. Existing treatments are focused on delaying disease progression and improving patient life quality. Physical therapy and glucocorticosteroids (GKS) use is the standard practice in disease control. GKS such as deflazacort or prednisone, help decrease inflammation and muscle degradation, improve strength and mobility, enhance pulmonary function and slow cardiomyopathy development [1,4,15]. Nevertheless, their long-term use is associated with numerous adverse effects, including obesity, osteoporosis, growth retardation, and delayed puberty [1].

Advances in the understanding of the molecular mechanisms underlying DMD have led to the development of therapies targetting the root cause of the disease. In recent years, considerable attention has been focused on exon-skipping strategies, nonsense mutation read-through therapies, microdystrophin gene replacement, and genome-editing technologies such as Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins (CRISPR-Cas) [3,6,11,16,17]. A major milestone was the approval of the first microdystrophin-based gene therapy, marking the beginning of a new era of disease-modifying treatment for DMD [2,3]. Despite these promising developments, significant challenges remain, including immunological safety concerns, efficient delivery of genetic material, and the long-term durability of therapeutic effects [2,3,6,16].

The aim of this review is to summarize the current advances in DMD treatment and to discuss the most promising future therapeutic directions, with particular emphasis on gene therapy, genome-editing approaches, and other innovative molecular strategies that may further improve the prognosis of patients with DMD.

REVIEW METHODS

A narrative review of the literature published between 2020 – 2026 was conducted using the PubMed database. Less than 5% of the included publications were published before 2020. The literature search was performed using the following key words: 'Duchenne muscular dystrophy', 'genome editing', 'microdystrophin', 'CRISPR/Cas9', 'utrophin modulation' and 'stem cells'. Publications with paid access and published in languages other than English were excluded. Original studies, clinical trials, review articles and book chapters were included in the analysis. The relevant publications were selected based on their clinical significance in relation to the topic under discussion

DESCRIPTION OF THE STATE OF KNOWLEDGE

Genome editing. In recent years, particular attention has been paid to the transfer of the dystrophin gene via an adeno-associated virus (AAV) vector [18]. AAV is a small, non-enveloped virus containing a single-stranded linear DNA [19]. Due to the size of the dystrophin protein-coding region and the limited packaging capacity of AAV, efforts have been made to create recombinant genes encoding minidystrophin and microdystrophin, allowing for gene transfer and subsequent production of a protein with preserved biological function and alleviating the DMD symptoms despite the aforementioned limitations. However, this approach has so far been tested primarily in animal models [18].

One example of AAV-vector-based gene transfer therapy encoding microdystrophin transgene is delandistrogene moxeparvovec protein administration. In the single-arm, open-label ENDEAVOR study involving male patients aged from 4 – 8 years with genetically confirmed DMD, treatment with delandistrogene moxeparvovec was associated with improvements in motor function or stabilization of disease progression. Effects were documented on the 17-item North Star Ambulatory Assessment scale (NSAA), that evaluates whether patients can perform motor activities without assistance, as well as other functional measures such as supine-to-stand and the 10 meter walk/run test [20]. In the future, the use of dual- or triple-vector systems, as well as non-viral delivery approaches together with trans-splicing strategies, may offer a promising solution for delivering the full-length dystrophin gene to DMD patients [3].

CRISPR-Cas is a technology that enables precise genome editing. In gene therapy, it allows for the introduction of targetted changes in genetic material to correct mutations responsible for the development of diseases. The most important strategies utilizing the CRISPR-Cas system include: exon skipping through the modification of splicing sites, restoration of the correct reading frame by inserting a single nucleotide before exon, inducing INDEL mutations (microdeletions or microinsertions) to eliminate a premature STOP codon, single-nucleotide editing leading to the reversal of a STOP codon, and the creation of hybrid exons, enabling the restoration of a functional protein [21]. An additional advantage of CRISPR-Cas is the permanence of the genomic repair and the fact that repeated cycles of therapy are not required to achieve satisfactory results, as is the case with antisense oligonucleotides. For example, Iyombe-Engembe et al. successfully applied a CRISPR-induced deletion (CinDel) approach to restore the dystrophin reading frame in DMD patient-derived myoblasts carrying a frameshift deletion of exons 51–53. This strategy generated a hybrid exon by inducing targetted deletions at the splicing sites and restored the reading frame [22].

However, it is also important to note that serious and potentially fatal adverse events have been reported following CRISPR-based therapies. In one case, a 27-year-old patient with diagnosed DMD who received recombinant AAV treatment containing the CRISPR-based therapeutic agent, experienced cardiac arrest on the sixth day of treatment. The event was considered to be potentially associated with the high dose of the AAV vector [23]. Another important application of CRISPR-Cas technology is the generation of cell lines and animal models carrying disease-associated mutations. These models facilitate the investigation of disease

pathogenesis and the development and preclinical evaluation of novel therapeutic strategies [24].

Antisense oligonucleotides. Another approach to treating DMD may be exon skipping using antisense oligonucleotides [25]. Antisense oligonucleotides are short, synthetic, single-stranded oligonucleotides carrying distinct chemical modifications (such as phosphorodiamidate morpholino oligomers or 2'-O-methyl phosphorothioate backbones) designed to enhance stability and hybridization affinity [16,25]. Similarly to some CRISPR-Cas9 methods, the goal of this treatment is to convert a frameshift mutation into an in-frame mutation by skipping exons, thereby producing dystrophin with partial functionality [25]. Antisense oligonucleotides target the primary transcript at the splice donor or splice acceptor site which is located at the adjacent exon to the mutation site [22]. Moreover, internal hybridization can target exonic splicing enhancer sequences within an exon resulting in its exclusion [26].

It is estimated that frameshift mutation accounts for approximately 6–8% of DMD cases, with the most common locations in the genome being exons 44–55 [25]. Examples of antisense oligonucleotide therapies include Casimersen, which induces exon 45 skipping; Viltolarsen and Golodirsen, which target exon 53 skipping; SRP-5051 and Eteplirsen – exon 51, and Brogidirsen – exon 44. The therapy was evaluated in Phase 1/2 clinical trials to assess its safety, dosing and pharmacokinetic profile before clinical implementation. Moreover, some Phase 3 clinical trials are currently recruiting participants [25, 27].

Glucocorticoid receptor agonists. As mentioned above, GKS are the standard treatment regimen for DMD. In response to this, strong emphasis has been placed on investigating similar substances, such as vamorolone, which belongs to the class of dissociative glucocorticoid receptor agonists. Assessment was made using 5 motor outcomes: 6-minute walk test distance, time to run/walk 10 m velocity, time to stand up from supine velocity, time to climb 4 stairs velocity and NSAA. When administered to boys aged 4 – 7 years with a molecular DMD diagnosis, vamorolone at the dose of 6 mg/kg, compared with prednisone at the dose of 0.75 mg/kg, demonstrated similar motor outcomes in all investigated tests and had a more favourable safety profile [28].

Histone deacetylase inhibitors. In addition to therapies targetting specific genetic mutations, approved treatments that can be used regardless of the underlying mutation include GKS and givinostat, a class I and II histone deacetylase inhibitor. During clinical trials, givinostat has demonstrated anti-inflammatory, anti-fibrotic, and regenerative properties [29], as well as the ability to slow disease progression [30]. Inhibition of HDACs causes distancing of the DNA chromatin and enables easier attachment of transcriptional factors. This post-translational modification leads to gene expression up-regulation. In the case of DMD, expression of the myostatin inhibitor, follistatin, is enhanced [31].

Ataluren. Recent studies suggest that ataluren, the first orally administered disease-modifying therapy developed for DMD, promotes translational readthrough of premature stop codons, thereby enabling the synthesis of functional dystrophin. This effect appears to be most pronounced for the UGA stop codon [32,33]. Clinical studies, including the Strategic Targetting of Registries and International Database of Excellence (STRIDE) trial, have evaluated the long-term safety and effectiveness of ataluren in a broader population of patients after its introduction into clinical practice [33].

Utrophin Modulation. Utrophin is a protein that is structurally and functionally homologous to dystrophin. It is encoded by the *UTRN* gene and shares approximately 80% structural homology with dystrophin. Similar to dystrophin, utrophin can interact with components of the DGC and contribute to sarcolemmal stabilization. During embryonic development, utrophin is widely expressed in the muscle fibre membrane; however, after birth, its expression becomes primarily restricted to specialized structures, such as neuromuscular and myotendinous junctions [6,34–36]**.** In DMD, the absence of dystrophin induces an increase in utrophin expression as a compensatory mechanism. Utrophin can partially replace dystrophin functions and stabilize the sarcolemma; however, its physiological expression level is insufficient to prevent disease progression. Therefore, increasing utrophin expression throughout the sarcolemma represents an attractive therapeutic strategy [34]**.** One of the major advantages of utrophin modulation is its potential independence from the specific type of *DMD* gene mutation [6]**.**

One of the initial approaches involved the use of small molecules capable of increasing *UTRN* gene transcription. Ezutromid (SMT C1100) attracted particular interest because of its ability to increase utrophin expression and promote its localization within the sarcolemma. In preclinical studies, this compound demonstrated protective effects in the *mdx* mouse model, reducing muscle damage and improving muscle function. Ezutromid underwent phase I clinical trials, in which it demonstrated good tolerability. However, a subsequent phase II trial failed to demonstrate convincing clinical efficacy, and further development of the compound was discontinued. This does not necessarily invalidate the concept of utrophin modulation. Rather, it suggests that more effective compounds are needed to increase utrophin expression to a sufficiently high level and ensure its uniform distribution throughout the sarcolemma [6,34,36,37].

Other compounds investigated in preclinical models include AICAR, resveratrol, heregulin, and PPAR-β/δ agonists. These agents may enhance utrophin expression through different mechanisms involving the AMPK, SIRT1, and PGC-1α pathways, which promote an oxidative and more fatigue-resistant muscle phenotype. AICAR activates AMPK, leading to increased PGC-1α expression and improved mitochondrial biogenesis and oxidative metabolism, while PPAR-β/δ agonists similarly promote oxidative muscle fibre formation and the expression of genes involved in aerobic metabolism. Heregulin may increase utrophin expression through epigenetic mechanisms involving MSK1/2 activation and histone H3 phosphorylation. Resveratrol may act through the SIRT1/PGC-1α pathway, potentially enhancing utrophin expression while also improving mitochondrial function [34]**.**

However, it is important to recognize that utrophin is not a fully functional equivalent of dystrophin. Although it can stabilize the sarcolemma, it differs from dystrophin in several aspects, including its interactions with neuronal nitric oxide synthase (nNOS). Consequently, utrophin over-expression alone may not be sufficient to fully restore all dystrophin functions, particularly those related to the regulation of

Table 1. Emerging treatment methods and outcomes reported in clinical trials

Trial	Type of study	Substance/ method	Main statement
ENDEAVOR Study [20]	single-arm, open-label	delandistrogene moxeparvovec - microdystrofin	– good safety profile – an increase in the NSAA score from 22 points at baseline to 26 points at the 1-year follow-up
Efficient Restoration of the Dystrophin Gene Reading Frame and Protein Structure in DMD Myoblasts Using the CinDel Method [22]	in vivo cell study	CRISPR-Cas – modification of spicling sites	– reading frame restoration in 64% of the in vivo cells
Safety, tolerability, and pharmacokinetics of casimersen in patients with DMD amenable to exon 45 skipping [39]	randomized, double-blind, placebo-controlled, dose-titration trial, phase I/II trial	casimersen – antisense oligonucleotide	– most of the adverse events observed during casimersen therapy, regardless of dose, were mild and not related to the drug – in the group of boys aged 7 to 21 years, targeting exon 45 with various doses of casimersen was characterized by a high level of safety and tolerability
Phase 1/2 trial of brogidirsen [27]	phase I/II trial	brogidirsen – antisense oligonucleotide	– upregulation of ciliary neurotrophic factor and downregulation of peptidyl arginine deiminase 2 were observed – brogidirsen demonstrated a favorable safety and tolerability profile at doses of up to 80 mg/kg administered over 24 week – mean dystrophin expression levels reaching 16.63% (40mg/kg dosage) and 24.47% (80 mg/kg dosage) of normal healthy levels.
Efficacy and Safety of Vamorolone Over 48 Weeks in Boys With Duchenne Muscular Dystrophy [28]	randomized, double-blind, controlled trial	vamorolone – glucocorticoid receptor agonist	– most common adverse events in vamorolone groups were upper respiratory tract infections, vomiting and Cushingoid features – the 6 mg/kg dose of vamorolone resulted in superior outcomes in three of the five assessed motor function tests compared with the 2 mg/kg dose – reversal of bone morbidities was observed following the transition from prednisone to vamorolone
EPIDYS study [30]	randomized, double-blind, multicentre, placebo-controlled trial	givinostat – histone deacetylase inhibitor	– motor functions deterioration was observed after 72 weeks of treatment in both placebo and givinostat group (four-stair climb assessment), but in givinostat group the progression over time was slower
Study 041 [32]	randomized, double-blind, placebo-controlled, phase III trial	ataluren	– after 72 weeks of treatment the outcomes in 6 minutes walk distance slowed the deterioration by 21% – ataluren was beneficial to secondary end-points (NSAA, 10 meters run/walk time, time to descend four stairs) in comparison to placebo group

blood flow during muscle contraction [34,38].

The detailed results of the clinical trials are presented in Table 1.

Stem/progenitor cell-based therapies. Stem/progenitor cell-based therapies represent a promising therapeutic strategy for DMD, with the primary goals of restoring dystrophin expression and promoting muscle regeneration and functional recovery. A wide range of cellular approaches has been investigated, including transplantation of myoblasts, muscle stem cells (MuSCs), induced pluripotent stem cell (iPSC)-derived myogenic progenitors, dystrophin-expressing chimeric (DEC) cells, mesenchymal stem/stromal cells (MSCs) and cardiosphere-derived cells (CDCs). Despite encouraging results obtained in preclinical models, the successful translation of these approaches into clinical practice remains challenging [34,40,41].

One of the major factors limiting the clinical translation of stem/progenitor cell therapies is the discrepancy between preclinical and human outcomes. GKS modulates inflammatory responses and macrophage activity and may therefore influence the survival and integration of transplanted cells [9,31].

Preconditioning of stem/progenitor cells prior to transplantation represents one strategy for improving therapeutic efficacy. This approach may involve epigenetic modification, exposure to hypoxic conditions, cytokines, or other factors capable of increasing cell survival, migration, and integration. For example, treatment of iPSC-derived myogenic progenitors with the histone deacetylase inhibitor givinostat has been shown to enhance their proliferative and migratory properties and improve muscle regeneration and dystrophin expression in animal models. Similarly, hypoxic preconditioning or exposure to interferon-γ may increase the survival of transplanted cells and reduce their immune-mediated elimination. Secreted factors, including extracellular vesicles and microRNAs, may also play an important role in promoting tissue remodelling and modifying the muscle microenvironment [42,43].

The most important findings and key conclusions from recent clinical trials investigating cell-based therapies are presented in Table 2.

Nitric oxide donors and NSAID-based combination therapy. In DMD, the absence of dystrophin disrupts the proper localization and function of nNOS, leading to reduced nitric oxide (NO) production and impaired blood flow regulation in skeletal muscles during exercise. This contributes to muscle hypoxia and disruption of the NO–sGC–cGMP signalling pathway. Consequently, phosphodiesterase-5 (PDE5) inhibitors, such as sildenafil and tadalafil, have been investigated as potential therapeutic agents because they increase cGMP levels. Although these drugs have shown promising effects in mdx mouse models, clinical trials have not demonstrated significant benefits in improving skeletal muscle or cardiac function. Some studies were discontinued because of a lack of efficacy or adverse effects [34,48].

Table 2. Main findings of stem/progenitor stem cell-based therapies

Cell-based therapy	Cell source	Type of cell transplantation	Mechanism / therapeutic rationale	Key findings	Main limitations / challenges	Clinical status	Primary mechanism of action (paracrine effect or dystrophin expression restoration)
Myoblasts [40,41]	Muscle biopsy	Xenogeneic and allogeneic transplantation of human immortalized myoblasts into human and mice muscles	Integration with host myofibers and restoration of dystrophin expression	Dystrophin restoration and functional improvement demonstrated in mdx mice	Poor engraftment and survival; loss of potency during ex vivo expansion; limited efficacy in human trials	Clinical trials showed limited dystrophin restoration and functional benefit	Dystrophin expression restoration
MuSCs [40,41]	Muscle biopsy	Allogeneic or xenogeneic transplantation of mouse or human MuSCs into mice muscles	Self-renewal and differentiation into myogenic cells; repopulation of the muscle stem cell niche	Improved engraftment, increased dystrophin-positive fibers, and functional improvement in animal models	Limited number of cells obtained from biopsies; poor post-transplantation survival and engraftment; difficulties with systemic delivery	Preclinical development; limited clinical translation	Dystrophin expression restoration
iPSC-derived myogenic progenitors [40,41,44]	Human umbilical cord	Xenogeneic transplantation of human iPSC-derived myogenic progenitors into mice muscles	Generation of scalable myogenic cells capable of muscle integration and dystrophin restoration; the potential for introducing modifications to cells prior to transplantation	Engraftment, dystrophin restoration, muscle regeneration, and MuSC niche repopulation in animal models	Tumorigenicity risk; need for extensive quality control and elimination of undifferentiated pluripotent cells; complex manufacturing	Early clinical development	Dystrophin expression restoration
DEC [41,45]	Muscle biopsy	Possible xenogeneic-autologous and allogeneic-autologous transplatation (fusion of myoblasts) via intraosseous infusion	Fusion of healthy and DMD-derived myoblasts; delivery of dystrophin and potentially functional mitochondria	Improved dystrophin-positive muscle fibers, reduced inflammation and fibrosis; improved skeletal, respiratory, and cardiac muscle function in animal models	Potential allogeneic immune response; variable fusion efficiency; limited clinical cohort; inefficient systemic muscle delivery	First-in-human studies; preliminary evidence of safety and functional	Dystrophin expression restoration
MSCs [41,46]	Bone marrow, adipose tissue, umbilical cord, amniotic membrane, dental pulp and skeletal muscles	Allogeneic (both animal and human trials) intramuscular transplantation	Paracrine and immunomodulatory effects; stimulation of MuSCs, angiogenesis, and reduction of fibrosis	Improved muscle regeneration and function; anti-fibrotic and anti-inflammatory effects in animal models; low immunogenity	Limited evidence for direct dystrophin restoration; mechanisms predominantly paracrine; optimal dosing and cell source remain unclear	Phase I clinical studies, particularly with umbilical cord-derived mesenchymal stem/stromal cells	Paracrine effects
CDCs / Deramiocel (CAP-1002) [41,47]	Human donor hearts from organ procurement	Allogeneic transplantation via intravenous infusions	Paracrine effects and extracellular vesicle-mediated modulation of cardiac and skeletal muscle	Improved cardiac and upper-limb function; reduced fibrosis and improved muscle regeneration	Limited evidence of sustained dystrophin restoration; long-term efficacy and off-target effects require further evaluation	Clinical trials completed/ongoing; deramiocel has advanced in clinical development for DMD cardiomyopathy	Paracrine effects

L-arginine may also have therapeutic potential, as it serves as a substrate for NO synthesis. Studies in mdx mice have shown that L-arginine supplementation may reduce inflammation and support muscle regeneration. Furthermore, a phase III clinical trial suggested that combined treatment with L-arginine and metformin may delay the loss of muscle function in patients with DMD [38].

Follistatin. Myostatin, a negative regulator of muscle growth, is another potential therapeutic target. Inhibition of myostatin has been shown to increase muscle mass in mdx mice. Similarly, follistatin (FST), a myostatin-binding protein, may promote muscle growth and reduce myostatin-mediated inflammation. AAV-mediated delivery of follistatin improved muscle strength and reduced dystrophic pathology in mdx mice, while a phase I/II clinical trial investigating follistatin-344 gene therapy in patients with DMD has also been initiated. [34,49].

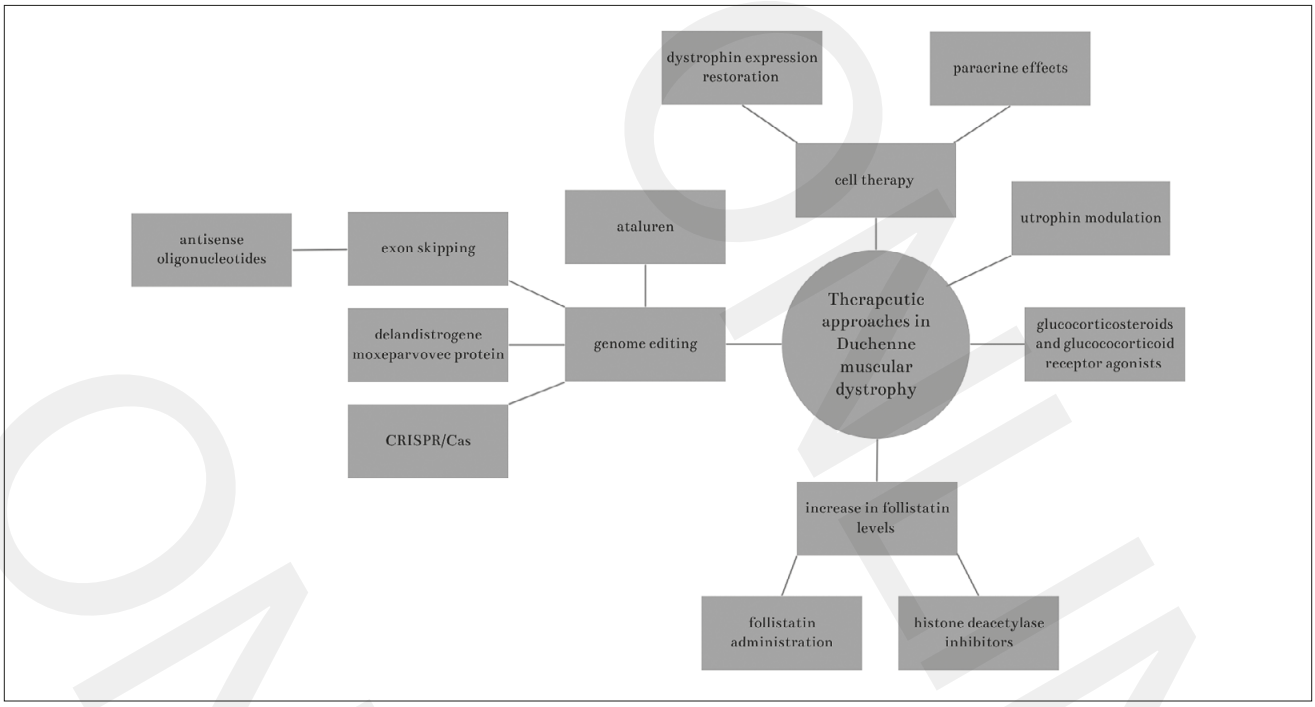


Figure 2. Therapeutic approaches for Duchenne muscular dystrophy. Schematic representation of emerging and established therapeutic strategies for DMD, including cell-based therapies, genome editing, systemic pharmacological treatment, utrophin modulation and targeting the follistatin

Urocortins. Urocortins (Ucns) are another potential therapeutic approach. By activating the CRF2 receptor and downstream cAMP signaling, urocortins may protect skeletal muscles, particularly the diaphragm, from progressive degeneration. Studies in mdx mice have demonstrated that urocortins may improve the structure and function of dystrophic muscles, suggesting their potential as a future therapeutic strategy for DMD [50].

Figure 2 shows main focuses that are investigated in DMD therapy and their mechanisms.

SUMMARY

Pathogenic variants in the DMD gene, located on chromosome Xp21.2–p21.1, disrupt dystrophin expression and lead to dystrophinopathies, most notably DMD. Dystrophin is a key component of the DGC which links the actin cytoskeleton to the extra-cellular matrix and protects the sarcolemma from mechanical damage generated during muscle contraction. The absence of dystrophin results in membrane destabilization, uncontrolled calcium influx, chronic inflammation, oxidative stress, and progressive fibro-fatty replacement of muscle tissue. These dysfunctions lead to gradual loss of both mass and function of skeletal, respiratory, and cardiac muscles. For this reason, in recent years there has been an increased focus on researching new therapeutic approaches that could alleviate the symptoms of the disease and slow the progression of motor impairment. Among these, several therapeutic strategies can be distinguished, including gene-editing approaches such as CRISPR-Cas, microdystrophin gene transfer, antisense oligonucleotides, ataluren, histone deacetylase inhibitors, and utrophin modulation. Other approaches include glucocorticoid receptor agonists, nitric oxide donors, follistatin, urocortins, and various stem/progenitor cell-based therapies.

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