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OBM Genetics

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Review

Gene Therapy Strategies for Muscular Dystrophies: Current Insights and Future Directions

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Academic Editor: Xingsi Xue

Special Issue: New Methods and Techniques for Genetic Research

OBM Genetics
2025, volume 9, issue 1
doi:10.21926/obm.genet.2501280

Received: September 04, 2024
Accepted: December 30, 2024
Published: January 08, 2025

Abstract

Gene therapy, a groundbreaking method for addressing genetic mutations, includes strategies such as gene repair, replacement, inactivation, or the introduction of therapeutic genes, circumventing traditional surgical or pharmacological approaches. Delivery through viral or non-viral vectors presents trade-offs in efficiency and immune response. Recent gene-editing technologies like ZFNs, TALENs, and CRISPR facilitate precise genome modifications by inducing targeted double-strand breaks, with CRISPR/Cas9 recognized for its versatility. Muscular dystrophies, marked by progressive muscle degeneration due to genetic mutations, are a significant focus for gene therapy. While a definitive cure remains elusive, gene therapy provides hope, with ongoing research investigating tailored approaches for various types of muscular dystrophy. This review highlights gene therapy's potential in treating muscular dystrophies, concentrating on the diverse strategies under exploration and contributing to the quest for effective therapeutic interventions and, potentially, cures for these debilitating conditions.

Keywords
Gene therapy; muscular dystrophies; CRISPR; gene editing

1. Background

Today Gene therapy has opened a new door for the treatment of diseases caused by mutations in genes [1]. Gene mutations often result in the absence or production of malfunctioning or nonfunctional proteins, leading to genetic diseases or cancers [2].

Gene therapy is a medical technique that uses gene modification instead of surgery or drugs to cure disease [2]. This technique follows 4 strategies for treatment: 1. Repairing of a mutated gene at its source, 2. Replacing a mutated gene with a normal copy of a gene, 3. Knock out or inactivation of the mutated gene and 4. Inserting a new gene to help moderate the effect of the mutated gene [3]. For these purposes, genetic material should be transferred to target cells by viral or non-viral vectors [4]. Both types of vectors have some advantages and disadvantages. For example, viral vectors are more efficient than non-viral vectors because of their ability to enter the cells, but they can induce inflammation and immunogenicity in their host [5]. The most common viral vectors that are used in gene delivery are Adeno-associated vectors (AAV) and Lenti-vectors [5, 6]. On the other hand, non-viral vectors which include chemical and physical methods are safe but have low delivery efficiency [6]. When a therapeutic gene is delivered to the somatic cells of the patient, any modification in the target gene can't transfer to the offspring, but in germline gene therapy, all cells contain a modified gene so they can transfer to the next generations [7].

Gene therapy based on human artificial chromosomes (HACs) and induced pluripotent stem cells (iPSCs) represents a promising approach for treating genetic disorders through cell or tissue replacement [8, 9]. HACs are engineered chromosomes that can stably maintain large transgene inserts without integrating into the host genome, thus minimizing risks associated with random insertion that could lead to oncogenesis [9]. They allow for the precise delivery of therapeutic genes while preserving the regulatory elements necessary for physiological gene expression [10]. In this context, iPSCs derived from a patient's cells can be genetically modified ex vivo using HACs to introduce desired genes [9]. This method not only ensures compatibility and reduces immune rejection but also enables the generation of patient-specific cells or tissues that can be infused back into the patient for therapeutic purposes [10]. Recent studies have demonstrated the successful use of HACs in various models, including hemophilia A, showcasing their potential to provide stable gene expression and therapeutic efficacy in regenerative medicine [9].

Today, emerging gene-editing tools have facilitated gene therapy. Among these tools, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) are more considered [11]. ZFN is an engineered DNA binding nuclease that is made from a fusion of the DNA binding domain and nuclease domain of the FokI enzyme [11]. Like ZFN, TALEN is an artificial nuclease that contains an engineered DNA binding domain or TALE-DNA binding domain and a cleavage domain [11]. In Both nucleases, the DNA binding domain attaches to the target sequence, and then the cleavage domain induces a double-strand break (DBS) [11]. CRISPR/Cas, which acts as an adaptive immune system in bacteria against phages, has become a powerful gene-editing technology for gene editing and gene therapy. In this

system, a programmable nuclease Cas9 is guided and attached to the target sequence by a short-non coding RNA or guide RNA (sgRNA) and induces a double-strand break near the protospacer adjacent motif (PAM) [12].

Naturally, after DNA cleavage, cell repair pathways are activated for DNA damage repair in two strategies: 1. error-prone pathway non-homologs end joining (NHEJ) or 2. high-fidelity pathway Homology directed repeat (HDR). In NHEJ, cleavage is re-ligated and repaired randomly in the absence of a template strand, so random insertion/deletion (Indel) is made in the cutting site, but in the HDR pathway, cleavage is repaired precisely by a template strand [12].

Nowadays, gene therapy has become a promising approach for the treatment of genetic diseases, and one option is muscular dystrophy [13]. Muscular dystrophy is a group of progressive muscle diseases caused by mutations in the gene of proteins that are responsible for muscle health So absence or production of nonfunctional muscle protein resulted in progressive muscle weakness and loss of muscle mass [14, 15]. There are about 30 kinds of muscular dystrophy that can be inherited: X-linked recessive, autosomal recessive, or autosomal dominant [14]. Signs and symptoms of diseases begin at different ages and in different muscle groups, depending on the type of muscular dystrophy (Figure 1) [15]. There is no specific cure for muscular dystrophy, and patients take supportive treatments like physical therapy to moderate symptoms of the disease [15]. Recently, gene therapy has become a promising option for treating muscular dystrophy patients (Table 1).

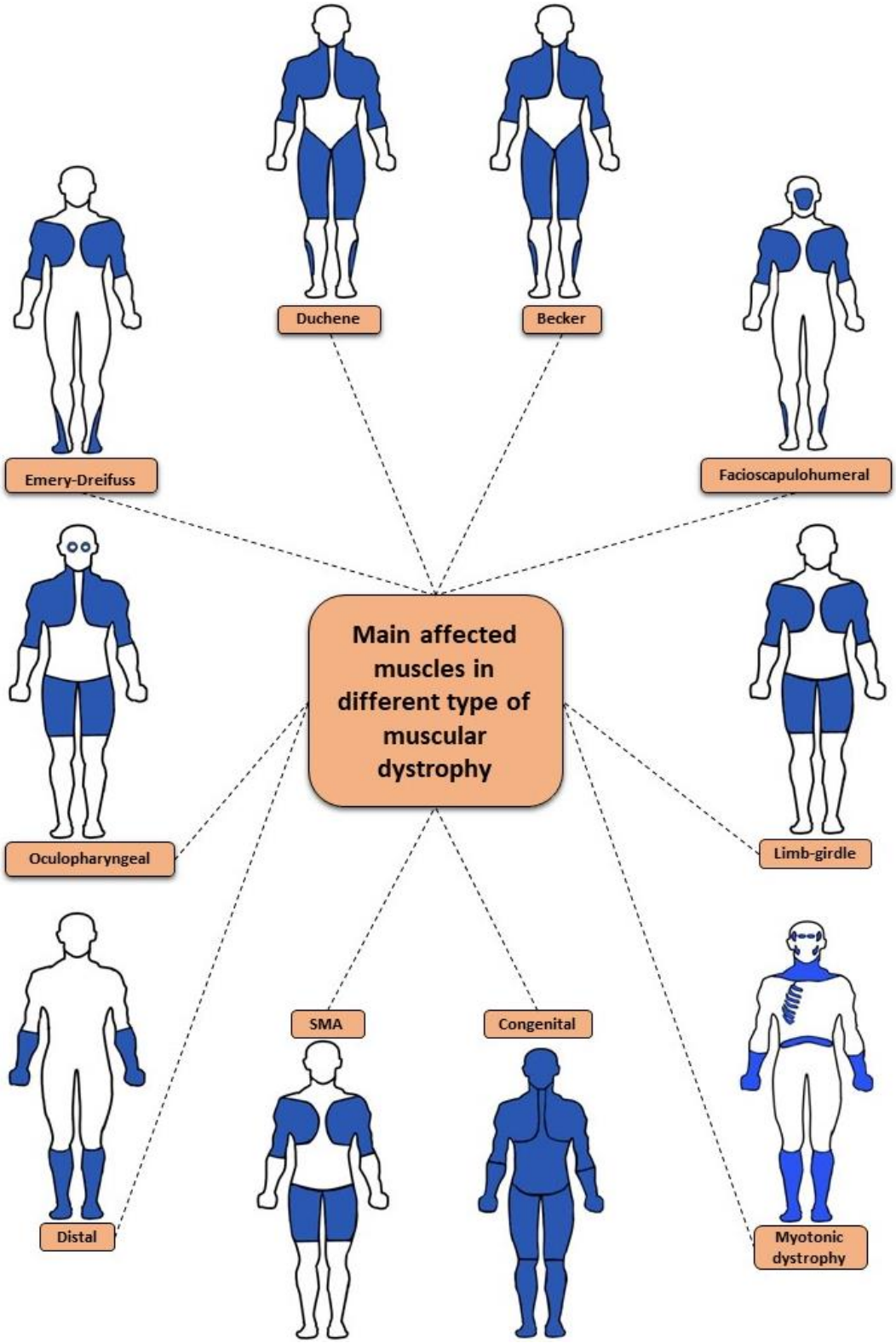

Figure 1 Main effected muscles in different types of muscular dystrophy. Each type of muscular dystrophy affects different muscle groups and exhibits a unique pattern of progression and severity. While some types primarily impact specific areas like the limbs or face, others can have systemic implications affecting the heart and respiratory muscles. Understanding the muscles involved is crucial for diagnosis, management, and therapeutic strategies.

Table 1 Gene therapy approaches for muscular dystrophies.

Disease	Gene	Gene therapy approaches	
Duchene Muscular Dystrophy (DMD)	Dystrophin (DMD)	1. Exon skipping therapy: -Eteplirsen or Exondy 51™ -Golodirsen or Vyondys 53™ -Casimersen or Amondys 45™ -DS-5141	-FDA Approved -FDA Approved -FDA Approved -Phase I and II Clinical Trials
		2. Micro-dystrophin gene therapy: -SRP-9001 -SGT-001 -PF-06939926)-	-Phase 1/2 clinical trial -Phase 3 -Phase 3
		3. Stop Codon Read-through (Translarna™ or Ataluren)	-Not Approved By FDA
		4. Gene editing (CRISPR/Cas system)	-Research Phase
Becker Muscular Dystrophy (BMD)	Dystrophin (DMD)	1.Utrophin Upregulation: -Ezutromid -TVN-102 -rhLAM111	-Phase 1 Clinical Trial Was Successful, It Failed in Phase II -No Information Is Available -Preclinical Phase
		2. Follistatin gene therapy	Phase 1/2a
Myotonic Dystrophy (MD)	Type 1: dystrophia myotonica-protein kinase (DMPK) Type 2: zinc finger 9 (ZFN9)	1. Antisense Oligonucleotide (ASO)- based therapy -ISIS 486178, -AOC1001, -FORCE-DMPK ASO, -Pip6a-PMO-CAG7 ASO -Arthex-01	Preclinical Stage
		2.Gene editing using ASO (AT466)	Preclinical Stage
		3. Gene editing using CRISPR/Cas9	Preclinical Stage
Limb-Girdle Muscular Dystrophy (LGMD)	proteins involved in muscle function and repairs like α, β, γ, and δ SARCOGLYCANS	1. The new single-dose gene therapy of ASM	Research Phase

Spinal Muscular Atrophy (SMA)	survival of the motor neuron gene (SMN)	1. Gene replacement therapy using Onasemnogene, abeparvovec, or Zolgensma	FDA Approved
Facio-Scapulo-Humeral Dystrophy(FSHD)	Type 1: DUX4 Type2: SMCHD1 DNMT3B LRIF1	1.Antisense Oligonucleotide (ASO)- based therapy -Phosphorodiamidate morpholinos -U7-asDUX4 2. microRNA therapy (miR-675 and ARO-DUX4) 3.Gene editing using CRISPR/Cas9 and CRISPRi	Preclinical Stag Preclinical Stag Research Phase
Congenital Muscular Dystrophy (CMD)	In each subtype of CMD, a specific gene is involved	1Spliceosome-Mediated RNA Trans-Splicing (SMaRT) for MDC1A	Research Phase
Distal Muscular Dystrophy (DD)	Eight Genes (TIA1, TTN, MYH7, MATR3, HNRNPA1, GNE, DYSF)	There is no cure or gene therapy approach for DD	
Oculopharyngeal Muscular Dystrophy (OPMD)	PABPN1	Gene replacement strategies	Research Phase
Emery-Dreifuss Muscular Dystrophy (EDMD)	genes of protein that are associated with the nuclear envelope	Gene therapy for this disease is currently in the early stages of the research phase	Research Phase

This article reviews common types of muscular dystrophies and several kinds of gene therapy approaches that are being investigated to treat them, and possibly offer the chance of a cure.

2. Duchene Muscular Dystrophy (DMD)

The most common form of muscular dystrophy is Duchene muscular dystrophy or DMD. DMD is an X-linked progressive neuromuscular disease that is caused by a mutation in the DMD gene. DMD gene is located in the short arm of the X chromosome and encodes dystrophin protein, which is a member of the protein complex involved in the maintenance of muscle cell membranes [16]. Mutation in the DMD gene resulted in the absence or production of nonfunctional dystrophin [16]. The lack of dystrophin induces a calcium signaling pathway, so the penetration of calcium into the muscle cell membrane (Sarcolemma) causes water entrance into the mitochondria and the production of stress-induced reactive oxygen species [16, 17]. Increasing oxidative stress in the cells damages sarcolemma and induces progressive muscle weakness and cell death [17]. Muscle fiber becomes necrotic and is replaced by adipose and connective tissue [17]. DMD symptoms onset in childhood between the ages of 2 and 3. As the disease progresses, the child loses the ability to walk and move. Around the age of 12, the patient becomes paralyzed and needs a wheelchair to move [17]. Later on, progressive weakness affects the heart and respiratory muscles, so premature death occurs due to heart arrest and respiratory failure in the third decade of life [17]. The prevalence of DMD is 1 in 5000 male births. There is no specific treatment for DMD. The use of corticosteroids

such as prednisolone and physical therapy delay the progress of disease and help patients improve their quality, but there is no specific cure [17]. Recently hopes have been raised for DMD treatment and results have shown promising approaches. These approaches are summarized below.

2.1 Exon Skipping Therapy

Antisense oligonucleotides (AONs) are short synthetic nucleic acids. They bind to the mutation site in pre-mRNA. This binding induces the splicing machinery to skip over the mutated exon. As a result, the reading frame is restored, producing a truncated but partially functional protein [18]. In late 2016, Eteplirsen, a morpholino type of AON, received FDA approval and is used to treat about 14% of DMD patients under the brand name Exondy51™. Eteplirsen attaches to exon 51 and skips it, which changes the downstream reading frame of dystrophin [19].

Another AON that received FDA approval in 2019 for DMD treatment is Golodirsen or SRP-4053, which is sold under the brand Vyondys 53™. Golodirsen is used in 8% of DMD cases with a mutation in exon 53 [20].

Amondys 45™ as a brand name for Casimersen or SRP-4045, received the FDA's conditional approval in 2020 [21]. Amondys 45™ skips exon 45 and so corrects the reading frame in the mutated DMD gene. All of these Exon skipping therapy developed by Sarepta Therapeutics (USA) [21]. This recently approved treatment targets exon 45 and is applicable to approximately 8% of DMD patients. In April 2017, a Japanese company announced the results of Phase I and II clinical trials of DS-5141 [22]. DS-5141 skips exon 45 and is offered as a new treatment option for patients with DMD [22].

2.2 Micro-Dystrophin Gene Therapy

Gene transfer is possible for DMD patients. However, the transfer of full-length dystrophin is not possible. Micro-dystrophin is a shorter but functional form of dystrophin delivered to the DMD cells by AAV vectors to reduce muscle cell damage [23].

SRP-9001, also known as delandistrogene moxeparvovec, is a groundbreaking gene therapy developed by Sarepta Therapeutics to treat DMD [24]. It is specifically designed for ambulatory pediatric patients aged 4 to 5 years with a confirmed DMD gene mutation. This therapy utilizes an AAV vector to deliver a micro-dystrophin gene, which aims to restore dystrophin production, a crucial protein for muscle function deficient in DMD patients. In June 2023, the FDA approved SRP-9001 under the brand name ELEVIDYS, marking it as the first gene therapy approved for DMD. The approval was based on data from several clinical trials, including the phase 1/2 SRP-9001-101 study and the phase 2 SRP-9001-102 study, which demonstrated significant expression of micro-dystrophin and some functional improvements in treated patients. The therapy is contraindicated for patients with specific deletions in exons 8 and/or 9 of the DMD gene due to the risk of severe immune-mediated myositis observed in clinical trials [23, 25]. In these studies, patients with such deletions experienced significant muscle weakness, including symptoms like dysphagia (difficulty swallowing), dyspnea (difficulty breathing), and hypophonia (reduced voice volume), approximately one month after receiving the therapy [25]. This reaction is believed to result from a T-cell-mediated immune response triggered by the micro-dystrophin protein produced by the treatment, which includes regions corresponding to exons 1-17 of the DMD gene. Patients lacking self-tolerance to these regions due to their specific genetic deletions may be more susceptible to this adverse reaction [25].

To mitigate this risk, ELEVIDYS is contraindicated in individuals with deletions in exon 8 and/or exon 9 of the DMD gene. Additionally, limited data are available for patients with mutations in other regions, such as exons 1 to 17 and 59 to 71, who may also be at risk for severe immune-mediated myositis reactions [25]. Therefore, thorough genetic screening is essential before considering SRP-9001 therapy to ensure patient safety and treatment efficacy.

PF-06939926, also known as fordadistrogene movaparvovec, is another investigational gene therapy developed by Pfizer for the treatment of DMD [26]. This therapy employs a recombinant adeno-associated virus serotype 9 (rAAV9) vector to deliver a mini-dystrophin gene, which is a shortened version of the dystrophin protein essential for muscle integrity.

2.3 Stop Codon Read-Through

Stop codon read-through is an investigational approach that aims to bypass premature stop codons caused by nonsense mutations in the DMD gene, allowing for the production of a full-length dystrophin protein. This strategy holds promise for treating DMD, as nonsense mutations account for approximately 10-15% of cases [27]. Aminoglycoside antibiotics like gentamicin have been studied for their ability to induce read-through of premature stop codons. Gentamicin was shown to increase dystrophin levels and reduce creatine kinase (a marker of muscle damage) in some DMD patients. Still, it did not demonstrate apparent clinical efficacy at the doses tested [28].

Ataluren, marketed as Translarna, is an oral medication used to treat DMD caused by reducing ribosome sensitivity to the premature stop codon and suppressing nonsense translation termination allowing muscle cells to produce full-length and functional dystrophin in patients aged 2 years and older [29]. Administered three times daily at a total dose of 40 mg/kg body weight, ataluren aims to enable the production of functional dystrophin by allowing ribosomes to bypass the genetic mutation. While it received conditional marketing authorization in the European Union in 2014, subsequent evaluations raised concerns about its long-term efficacy, leading to a recommendation against renewing its authorization in 2024. In the United States, the FDA initially rejected its application in 2017 due to insufficient evidence of effectiveness, although resubmission plans are underway. Common side effects include vomiting, diarrhea, and nausea [27, 29].

2.4 Human Artificial Chromosome (HAC)

The human artificial chromosome (HAC) approach for gene therapy in DMD presents a novel strategy to deliver the full-length dystrophin gene, essential for muscle integrity. Traditional viral vectors encounter challenges due to the large size of this gene, which comprises 79 exons. HACs can accommodate extensive genomic sequences without integrating into the host genome, thereby minimizing risks associated with insertional mutagenesis and facilitating stable episomal maintenance [30].

Recent studies have demonstrated the successful creation of HACs that carry the full-length human dystrophin gene [8]. For instance, a chimeric mouse model was developed with a 2.4-Mb human dystrophin gene loaded onto a HAC, resulting in dystrophin's regular expression in skeletal and cardiac muscles [8]. This expression led to significant improvements in muscle function and histological characteristics in dystrophin-deficient mice. The HACs facilitate not only the delivery of the dystrophin gene but also its physiological regulation, as they retain necessary regulatory elements that support proper gene expression [30].

Furthermore, HACs can be transferred into various cell types through methods such as microcell-mediated chromosome transfer, ensuring that they are stably maintained both in vitro and in vivo [30]. This approach holds promise for developing effective therapies for DMD by enabling precise gene replacement while reducing potential side effects associated with other gene delivery methods [30]. Overall, the HAC strategy represents a significant advancement in the field of gene therapy for DMD, paving the way for future clinical applications.

2.5 Gene Editing

The emergence of gene-editing technology paved the way for gene therapy for diseases like DMD. DMD gene editing approaches utilize various technologies, including ZFNs, TALENs, and CRISPR-Cas9, each with unique mechanisms and applications [31]. ZFNs are engineered proteins that bind to specific DNA sequences and create double-strand breaks. This method has been used to target the dystrophin gene, particularly exon 51, to induce small deletions or insertions that restore the reading frame of dystrophin mRNA [32]. In studies, ZFNs have successfully corrected mutations in DMD patient-derived myoblasts and demonstrated dystrophin expression in animal models, although challenges include the complexity of designing effective ZFNs and potential off-target effects [33]. TALENs operate similarly to ZFNs but utilize a different DNA-binding mechanism, allowing for a more straightforward design and targeting of specific genomic sequences. TALENs have induced exon skipping in the dystrophin gene, providing a potential therapeutic avenue for DMD. Their ability to create targeted genomic alterations has shown promise in preclinical models. However, like ZFNs, TALENs also face challenges regarding delivery and specificity [32] CRISPR-Cas9 is a versatile and widely used genome editing tool that allows for precise modifications of the DNA sequence [33]. In the context of DMD, CRISPR has been used to correct various mutations across the dystrophin gene, including large deletions involving multiple exons. For instance, CRISPR has enabled the targeted deletion of exons 45-55, which addresses a significant proportion of DMD mutations [34]. Additionally, CRISPR can facilitate base editing and prime editing, allowing for more refined corrections, such as converting stop codons into coding sequences or skipping problematic exons during mRNA processing. While CRISPR shows excellent potential for one-time treatments, challenges remain in optimizing delivery methods and minimizing off-target effects. Base editing, a refinement of CRISPR technology, allows converting specific nucleotides without causing double-strand breaks. This has been effectively applied to induce exon skipping or correct nonsense mutations in DMD models, leading to improved dystrophin expression. Prime editing, which offers even greater precision, has been demonstrated to correct frame shifts and point mutations in the dystrophin gene, showing promise for future therapies [35]. Currently, no clinical trials explicitly using CRISPR-based therapies for DMD that have been translated to human subjects. Although significant preclinical progress has been made, including successful applications of CRISPR-Cas9 in animal models and human cells, challenges such as effective delivery methods, safety concerns, and immune responses remain to be addressed before clinical trials commence. Research is ongoing to optimize these factors, and while there are promising developments, the transition to clinical trials is still in the planning stages [36].

3. Becker Muscular Dystrophy

Becker Muscular Dystrophy (BMD) is another type of inherited muscle-wasting disease. BMD is between 1 in 18,000 and 1 in 30,000 male births. BMD is an X-linked recessive disorder caused by an in-frame mutation in the dystrophin gene so, unlike DMD, mutated dystrophin is produced in this disease [37]. BMD is a milder form of DMD with a later onset around 5 to 6 age. Patients show a wide variety of symptoms and slowly progressive muscle weakness initially in the legs and pelvis due to loss of muscle mass and may need a wheelchair to move [37]. Gradually heart and respiratory muscles are affected. The life expectancy of BMD patients varies from 50 to 70 years [37]. There is no known treatment for BMD, so patients take supportive care and physical therapy to improve their quality of life [37]. The use of corticosteroid drugs such as prednisolone can decrease some symptoms. In the following, the therapeutic achievement for the treatment of BMD will be reviewed.

3.1 Utrophin Upregulation

Utrophin protein is an autosomal homolog of dystrophin produced during fetal development. In adults, Utrophin production is limited to neuromuscular junctions [38]. The upregulation of Utrophin compensates for the lack of dystrophin in DMD patients, thereby increasing the strength of muscle fibers and preventing muscle weakness and atrophy. Studies on the upregulation of Utrophin yield promising results for DMD and BMD treatment. Based on this strategy, three therapies were developed: Ezutromid, TVN-102, and rhLAM111. Ezutromid, or SMT C1100, is a small molecule that acts as a Utrophin modulator and enhances Utrophin levels. Although using Ezutromid in animal models and phase 1 clinical trials was successful, it failed in phase II [38]. TVN-102 is a recombinant biglycan used as an experimental treatment for DMD and BMD; it is injected into the bloodstream and increases Utrophin expression to prevent and delay muscle weakness. Recently, clinical trials for TVN-102 have been planned [38]. Laminin is a structural protein typically found in embryonic muscle tissue but absent in adult muscles. Recombinant human laminin-111, or rhLAM-111, increases the production of Utrophin and alpha7beta1 integrin to prevent the progression of muscle weakness in DMD. Additionally, rhLAM-111 stimulates satellite cells to differentiate and regenerate muscle atrophy, which could promote muscle healing in DMD patients. Currently, rhLAM-111 is in the preclinical phase [38].

3.2 Follistatin Gene Therapy

Follistatin is an autocrine glycoprotein encoded by humans' FST gene [39]. This secretory protein is expressed in nearly all tissues at variable concentrations. Follistatin binds and neutralizes members of the TGF-β superfamily, focusing on activin, a paracrine hormone [39]. One of the TGF-β superfamilies is Myostatin. Myostatin is a muscle-specific secretory protein that negatively regulates muscle growth, which down-regulates the expression of muscle transcriptional factors that act in the differentiation and development of muscles, such as MyoD and Pax-3, resulting in suppression of myogenic cell differentiation and muscle fiber growth [39]. Studies in animal models show follistatin interferes with myostatin binding to the receptors in skeletal muscles, so overexpression of follistatin in myostatin null mice significantly increases muscle mass and muscle growth following myostatin inhibition [39]. According to the effect of follistatin in muscle growth enhancement, it’s become a favorite candidate in treating muscular dystrophy with potential

advantages over other myostatin-binding proteins [39]. Phase 1/2a follistatin gene therapy trial for Becker muscular dystrophy was done. In this study, AAV was used to deliver follistatin in the muscle of BMD patients. The results demonstrated an increase in muscle strength and a decrease in muscle hypertrophy [39].

4. Myotonic Dystrophy

Myotonic dystrophy (MD) is a neuromuscular disease and the most common form of muscular dystrophy that begins in adulthood. It affects multiple organ systems and is characterized primarily by the inability to relax muscles after contraction and progressive muscle loss and weakness [40]. MD affects multiple organ systems and patients' manifestations may include cataracts, heart conduction problems, intellectual disability, early balding in men, and infertility [40]. While myotonic dystrophy can occur at any age, including in congenital, infantile, juvenile, and adult (classic), onset is typically in the 20s and 30s. MD affects about 1 in 8,000 people worldwide and that is inherited in an autosomal dominant pattern [40]. MD type1 (MD1) is caused by CTG trinucleotide repeat in the dystrophia myotonica-protein kinase (DMPK) gene [41]. CTG trinucleotide repeat expansion becomes pathogenic when DMPK transcripts contain 50 or more repetitions resulting in the sequestration of the muscle blind-like (MBNL) family of proteins and causing the symptoms of the disease [41]. Abnormally expanded CCTG causes MD type 2 (MD2) repeats in a gene ZNF9. Although MD2 is usually milder than MD1. The muscle weakness associated with MD1 mainly affects distal muscles, such as those of the lower legs, hands, neck, and face. Muscle weakness in MD2 primarily involves proximal muscles, such as neck, shoulders, elbows, and hips. There is currently no cure or specific treatment for MD [41].

4.1 Antisense Oligonucleotide (ASO)-Based Therapy

The promise of oligonucleotide-based drugs is starting to be realized with the increasing rate at which these drugs reach approval, with at least ten already approved for clinical use. Although ASO drugs are highly specific, they are challenging to deliver to muscle cell tissues in MD cases. More than 5 types of these drugs have progressed to the preclinical stage, which we will discuss below [42].

Ionis Pharmaceuticals company recently published preclinical animal efficacy studies of ISIS 486178. ISIS 486178 is an ethyl-modified ASO that targeted the 3'- UTR of the DMPK gene consequently leading to a 70% reduction in CUGexp RNA abundance and foci in different mouse skeletal muscles and a 30% reduction in the heart. The reduction of CUGexp RNA led to eliminating toxic RNAs and improving muscle strength in DM1 [42].

Conjugation of therapeutic ASOs to peptides or antibodies enhances their target cell uptake. Avidity Biosciences company develops a small interfering (si) RNA conjugated with a proprietary monoclonal antibody against the transferrin receptor 1 (TfR1) protein, AOC1001 [43]. AOC1001, delivered to muscle cells, boosts the reduction in DMPK mRNA levels in a durable and dose-dependent manner [43].

Dyne Therapeutics is developing the FORCE-DMPK ASO candidate using a similar approach. FORCE-DMPK ASO is the result of conjugating a proprietary ASO with a TfR1-binding Fab for effective delivery to muscles [40].

Another strategy to improve muscle and heart uptake of ASOs is conjugation with cell-penetrating peptides (CPPs). The Pip6a-PMO-CAG7 is a conjugate that combines a CPP moiety (Pip6a) and an ASO with morpholino chemistry-targeting repeats that target the toxic CUG repeat [44]. In vivo, CPP-conjugated CAG7 PMO displayed significant improvement in oligonucleotide delivery into the striated muscles of HSALR mice following systemic administration, which promoted the reversion of myotonia and muscle weakness [44].

Although endogenous MBNL genes remain normal in patients, CTG trinucleotide repeat in the DMPK gene causes MBNL proteins sequestration and depletion inside the cells and resulting in alterations in splicing patterns in transcripts that contribute to clinical symptoms. miR-23b and miR-218 as endogenous translational repressors of MBNL1/2 genes. ARTHEx Biotech is working on the preclinical evaluation of the anti-miR Arthex-01 [40]. Arthex-01 is a class of chemically engineered ASOs complementary to their cognate target micro-RNA and significantly upregulated MBNL1/2 protein levels so improved molecular defects and muscle function mouse models [40].

4.2 Gene Editing Approaches

To overcome the problem of bio-distribution of AONs, AAVs were used to transfer them into the target cells [40].

AT466 is a vectorized ASO that targets the reduction of toxic DMPK RNA levels in cells from patients with DM1 by RNA degradation or exon skipping [40].

Both approaches prevent the accumulation of toxic DMPK RNA, restoring normal cellular function. Preclinical studies have begun to determine the optimal construct for AT466 [40].

4.3 CRISPR/Cas9

Emerging of CRISPR/Cas9 technology as an innovative approach to gene editing is paving the way for gene therapy. For MD1 treatment, CRISPR/Cas9 can effectively target the underlying cause of DM1 in two ways; 1. Intramuscular injection of recombinant AAV vectors expressing CRISPR-SaCas9 components to excision of long CTG repeats and reduced pathological RNA foci within tibialis anterior muscle in the DMSXL mouse model (DMSXL stands for Dystrophia Myotonica Protein Kinase (DMPK) with expanded CTG repeats. The DMSXL mice carry a mutated human DMPK transgene that contains more than 1,000 CTG repeats) [45]. 2. targeting the toxic RNA molecules by dCas9 [46]. dCas9 is a modified Cas9 enzyme with distinct regulatory functions that enable the CRISPR-Cas9 system to target without cleavage activity [46]. Based on this feature, Locanabio is developing AAV vectors encoding PIN-dCas9 (a dCas9 fused to the PIN RNA endonuclease) and a single-gRNA targeting CUG repeats, which, in intramuscular and systemic administration in adult and neonatal mice model. Two approaches are moving through preclinical stages [45, 46].

CRISPR technology is being adapted for DM1 through approaches like CRISPRi, which silences the mutated DMPK gene. This method has demonstrated over 80% reduction in DMPK expression in cultured muscle cells from DM1 patients, effectively correcting cellular abnormalities associated with the disease. CRISPRi, short for CRISPR interference, is a gene regulation technique that utilizes the CRISPR-Cas9 system to silence specific genes without altering the DNA sequence. It employs a catalytically inactive version of the Cas9 protein (dCas9), which can bind to DNA but does not cut it. This allows researchers to inhibit gene expression by preventing the transcription machinery from accessing the target gene [47].

CRISPR interference (CRISPRi) is particularly valuable in functional genomics, allowing scientists to study gene function and regulatory networks more controlled. It can be used to create gene knockdowns, making it a powerful tool for investigating the roles of specific genes in various biological processes and diseases. The technology is considered a safer alternative to traditional CRISPR gene editing, as it does not introduce permanent changes to the genome, thus minimizing potential off-target effects and ethical concerns associated with permanent genetic modifications [47].

5. Limb-Girdle Muscular Dystrophy

After the dystrophinopathies and myotonic dystrophy, Limb-girdle muscular dystrophy (LGMD) is the fourth most common muscular dystrophy with prevalence estimates ranging from 1 in 14,500 to 1 in 123,000 individuals [48].

LGMD is a group of genetically heterogeneous muscular dystrophy With an autosomal inheritance pattern. LGMD has two types autosomal dominant (LGMD D) and autosomal recessive (LGMD R) and more than 30 subtypes [48]. LGMD is caused by genetic mutations of proteins involved in muscle function and repairs like α, β, γ, and δ sarcoglycans [48]. The disease, accompanied by progressive weakness of the proximal muscles, first involves the shoulders and hip and then progresses to the girdle, thigh, shoulder girdle, and/or upper arm muscles. Muscle weakness typically begins in childhood or adulthood, depending on the type of disease [48].

Although LGMD is not fatal, in case of involvement and weakness of the heart and respiratory muscles, it can lead to the death of the patient due to secondary factors [48]. Currently, there is no known cure or treatment for LGMD. In some subtypes, the use of corticosteroids is a moderate sign of the disease. Gene therapy has been proposed as a promising treatment for this disease [48].

5.1 The New Single-Dose Gene Therapy

A mutation in dysferlin causes LGMD2B. This subtype has an autosomal recessive inheritance pattern and adult-onset muscular dystrophy that causes progressive muscle weakness and wasting [49]. Dysferlin deficiency is related to a lack of repair of muscle membrane damage after exercise-induced injury [49]. Dysferlin is a membrane protein that facilitates lysosomal vesicle fusion with the sarcolemma [49]. Acid sphingomyelinase (ASM), used to convert membrane sphingomyelin to ceramide, helps in a process that leads to membrane repair [49]. The process involves lysosomal exocytosis and membrane repair mechanisms initiated in response to cellular injury. When the sarcolemma (muscle cell membrane) is damaged, lysosomal vesicles fuse with it, releasing ASM [50]. This enzyme hydrolyzes sphingomyelin, a key membrane component, converting it into ceramide. The generation of ceramide is crucial as it facilitates the clustering of membrane rafts, promoting the stabilization and sealing of the damaged area. Additionally, this process is triggered by an influx of calcium ions (Ca^{2+}), which initiates vesicle fusion and activates various proteins involved in membrane dynamics [50]. Overall, this sophisticated response highlights the role of lysosomes in maintaining cellular integrity and promoting recovery following injury.

The dysferlin gene is too large so gene therapy by normal gene replacement therapeutically has been a challenge. A promising study was performed on a mouse model of LGMD2B [49]. In this study, the ASM gene (a downstream target of dysferlin) is delivered by the AAV to the liver, so the liver produces and provides long-lasting muscle repair [49].

Their findings demonstrated that a single dose of human ASM delivered to the liver by AAV has the potential to improve functional muscle function in limb-girdle muscular dystrophy type 2B [49].

6. Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder characterized by loss of motor neurons and progressive muscle weakness and wasting [51]. The weakness tends to be more severe in the proximal muscles, with the arm, leg, and respiratory muscles being affected first. Associated problems may include poor head control, scoliosis, difficulties swallowing, and joint contractures [51].

SMA is caused by a mutation in the survival of the motor neuron gene (SMN), which is one of a group of proteins called the SMN complex and plays a crucial role in the survival of motor neurons [51]. SMN on chromosome 5 has two identical copies, one in the telomere region (SMN1) and the other in the centromere region (SMN2) [51]. All types of spinal muscular atrophy are caused by mutations in the SMN1 gene [51]. The severity of SMA symptoms is broadly related to how well the remaining SMN2 genes can make up for the loss of function of SMN1 so having multiple copies of the SMN2 gene typically leads to mild and moderate forms of SMA symptoms [51]. The most common genetic cause of infant death is SMA. Five types of SMAs differ in age of onset and severity of muscle weakness, with some symptoms overlapping [51].

SMA type 0: Spinal muscular atrophy type 0 is the most severe form and the usual age of onset is in the prenatal period. Their respiratory muscles are very weak and they usually survive only a few weeks, even with 24-hour respiratory support. This type accounts for about 2% of cases [51].

SMA type I: Spinal muscular atrophy type 1, also called Werdnig-Hoffman disease, is About 60% of SMA cases. Muscle weakness appears at birth or within an infant's first six months. These children have swallowing and breathing problems. Most children with type 1 SMA die due to respiratory failure [51].

SMA type II: Spinal muscular atrophy type II, also called Dubovitz disease, is diagnosed in about 20% of patients and occurs in children between 6 and 18 months of age. Children of this type can sit without support but never learn to walk unsupported. Life expectancy in SMA II varies but live into their twenties or thirties [51].

SMA type III: Spinal muscular atrophy type III or Kugelberg-Welander disease is diagnosed in around 30% of patients and appears after a child's first 18 months of life. The disease progresses slowly and Individuals can stand and walk unaided. over time, symptoms can affect the ability to walk or stand. Type 3 SMA usually have a normal life expectancy [51].

SMA type IV: Spinal muscular atrophy type IV or the rare adult form of SMA occurs in approx. 5% of patients typically appear until the mid-30 s. the symptoms progress slowly, so most people with type 4 have a normal life expectancy [51].

6.1 Gene Replacement Therapy

The FDA first approved onasemnogene, abeparvovec, or Zolgensma in May 2019 for the treatment of children under age 2 with SMA [52].

The basis of Zolgensma treatment is inserting the SMN1 transgene gene into the cell nucleus transfer using the scAAV-9 variant. Zolgenzma makes up for the nonworking or missing SMN1 gene, which helps motor neurons work correctly [52].

7. Facioscapulohumeral Dystrophy (FSHD)

Facioscapulohumeral muscular dystrophy (FSHD) is a type of familial muscular dystrophy with an estimated prevalence of 1/8,000 to 1/20000 [53]. The signs and symptoms of facioscapulohumeral muscular dystrophy usually manifest at ages 15-30 years. FSHD is characterized by degeneration of muscle and progressive weakness of the face (facial-), around the shoulder blades (scapula-), and in the upper arms (humeral) [53].

Facial muscle weakness causes difficulty drinking from a straw and turning up the corners of the mouth when smiling or whistling. Usually, weakness may be more severe on one side of the face. Muscle weakness around the eyes can prevent fully closing eyes while asleep, leading to dry eyes and other eye problems [53]. Scapular winging and foot drops are considered known symptoms of FSHD. There is no available cure for FSHD. Two types of facioscapulohumeral muscular dystrophy with the same signs and symptoms and different genetic causes have been described [53]: FSHD1 (95% of cases) and FSHD2 (5% of cases). FSHD1 and FSHD2 are inherited in an autosomal dominant pattern [53]. FSHD is caused by hypo methylation of the DUX4 gene (double homeobox 4 gene). Commonly, DUX4 is expressed during embryogenesis and then repressed by hypermethylation in all tissues except the testes [53].

FSHD1 is caused by deletion or contraction of the D4Z4 region (a microsatellite repeat array in the subtelomeric region of the DUX4 gene), which results in the prevention of hypermethylation of DUX4. Production of DUX4 protein is toxic to muscles [53]—mutations in other genes involved in the methylation of DUX4 cause FSHD2 [53]. The importance of these three genes includes the following, respectively: SMCHD1 (structural maintenance of chromosomes flexible hinge domain containing 1), DNMT3B (DNA methyltransferase 3B), and LRIF1 (ligand-dependent nuclear receptor-interacting factor 1) [53]. For the treatment of FSHD, multiple approaches to gene therapy are in the preclinical stage of development. Using ASO that binds to DUX4 mRNA induces degradation and prevents protein production. Recently, phosphonoimidate morpholinos and U7-asDUX4 have been two approaches for treating FSHD in the preclinical stage [54]. Developing an RNAi treatment against DUX4, such as miR-675 and ARO-DUX4, is a promising approach to treating FSHD [55]. Another potential is the use of the CRISPR/Cas system, which is followed by two methods: 1. Knock out the polyadenylation signal in DUX4 using CRISPR/Cas9, 2—use of CRISPR inhibition or CRISPRi that induces DUX4 silencing without altering the genomic sequences [56].

8. Congenital Muscular Dystrophy

Congenital muscular dystrophies (CMD)are a larger group of heterogeneous muscular disorders with many subtypes that are characterized by muscle weakness, hypotonia, and degeneration of various voluntary muscles.CMD can be either autosomal dominant or autosomal recessive and have different ages of onset and severity (Table 2). In each subtype of CMD, a specific gene is involved and a broader picture of these diseases has emerged. The following table shows the subtype of CMD [57].

Table 2 Subtype of Congenital Muscular Dystrophy.

Category of CMD	Subtype of Diseases
Defects in structural proteins of the basal membrane or extracellular matrix of muscle fiber	1. Congenital Muscular Dystrophy Type 1A (**MDC1A**; Merosin-Deficient CMD; CMD with Laminin Alpha 2 Deficiency) 2. Collagen Type VI-Related Disorders: **Bethlem** myopathy and **Ullrich** congenital muscular dystrophy. 3. Congenital Muscular Dystrophy with **Integrin 7 Alpha Deficiency** 4. Congenital Muscular Dystrophy with **Integrin 9 Alpha Deficiency**
Dystroglycanopathies. (Caused By Glycosylation Defects Of A-Dystroglycan (A-DG))	1. Congenital Muscular Dystrophy Type 1C (**MDC1C**; CMD with secondary merosin deficiency type 2) 2. **Muscle-Eye-Brain (MEB**) Disease Spectrum 3. **Fukuyama** Type Congenital Muscular Dystrophy 4. **Walker-Warburg** Syndrome 5. Congenital Muscular Dystrophy Type 1D **(MDC1D**)
Defects in the Selenoprotein1 (*SEPN1*) gene.	1. **SEPN1-**Related Myopathy
Defects in proteins of the nuclear envelope.	1. **LMNA**-Related CMD 2. **SYNE1**-Related CMD
Without a known genetic defect.	1. Congenital Muscular Dystrophy Type 1B (**MDC1B**; CMD with secondary merosin deficiency type 1) 2. **CHKB**-Related Muscle Disease

8.1 Spliceosome-Mediated RNA Trans-Splicing

Approximately one-third of all CMD cases are caused by mutations in the LAMA2 that lead to Congenital Muscular Dystrophy Type 1A (MDC1A or CMD with Laminin Alpha 2 Deficiency or Merosin-Deficient CMD), which has an onset in the first year of life and is characterized by progressive axial weakness and wasting, limited motor movement, spinal rigidity, and frequent respiratory failure [58].

Some studies have been performed on cell lines and animal models based on spliceosome-mediated RNA trans-splicing (SMaRT) technology for gene therapy of MDC1A [58].

SMmRT is an emerging technology in which RNA pre-therapeutic molecules (PTMs) are designed to recode a specific pre-mRNA by suppressing cis-splicing while enhancing trans-splicing between the PTM and its pre-mRNA target. This gene therapy strategy reduces mutated transcript expression. The LMNA-related congenital muscular dystrophy (L-CMD) mouse model partially rescued the mutant phenotype [58].

9. Distal Muscular Dystrophy

Distal muscular dystrophy (DD) is a group of rare diseases affecting the legs and lower arms muscles and can progress and involve other muscles. DD usually appears in adults after the age of 30 years and has several forms with different inheritance patterns (Table 3). There is no cure or

gene therapy approach for DD; supportive care like Physical therapy and Occupational therapy care can help with ways to adapt to activities [59].

Table 3 Subtype of Distal Muscular Dystrophy.

Type Of DD	Onset	Inheritance Pattern	Frist Affected Muscles	Couse
Welander Distal Myopathy	greater than 40 years of age	Autosomal Dominant	Hands And Feet and Certain Muscles of The Fingers and Toes	TIA1 gene (Cytotoxic granule associated RNA binding protein)
Udd Distal Myopathy or Tibial Distal Myopathy	usually after 35 years	Autosomal Dominant	Long Extensors of The Toes	TTN gene (Titin)
Laing Distal Myopathy or Laing Early-Onset Distal Myopathy or Distal Myopathy 1 (MPD1)	before the age of 5	Autosomal Dominant	The Ankles and Great Toes Are Affected	MYH7 gene (myosin heavy chain beta)
Distal Myopathy with Vocal Cord and Pharyngeal Signs or Distal Myopathy 2 (MPD2)	between about 35 and 60 years	Autosomal Dominant	Vocal Cord Muscles and Pharyngeal Muscle	MATR3 gene (nuclear matrix protein)
Distal Myopathy 3 (MPD3)	from 32-45 years of age.	Autosomal Dominant	Distal Muscles of The Arms or Legs	HNRNPA1 gene (Heterogeneous nuclear ribonucleoprotein A1)
Inclusion Body Myopathy Type 2 (IBM2) or Distal Myopathy with Rimmed Vacuoles (DMRV) or Nonaka Myopathy	from 10 to 40 years of age	Autosomal Recessive	Distal Muscles of The Legs	GNE gene (UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase)
Miyoshi Myopathy	usually between 15-30 years	Autosomal Recessive	Calf, The Gastrocnemius and Soleus	DYSF gene (Dysferlin)

10. Oculopharyngeal Muscular Dystrophy (OPMD)

Oculopharyngeal muscular dystrophy (OPMD) is a slowly progressive rare genetic muscle disorder with an autosomal dominant inheritance. It is characterized by weakness in the eyelids and pharynx muscles [60].

Affected individuals may develop droopy eyelids (ptosis) and Difficulty swallowing (dysphagia). The prevalence of oculopharyngeal muscular dystrophy is less than 1 in 100,000 people with onset during adulthood most often between 40 and 60 years of age [60]. OPMD is caused by a 3 nucleotide expansion (GCN repeat) mutation in the poly adenylate binding protein nuclear 1 (PABPN1) gene, resulting in PABPN1 protein having extra alanine. So the extra alanines in the PABPN1 protein formed nonfunctional clumps within muscle cells and impaired muscles' normal function [60].

10.1 Gene Replacement Strategies

According to this approach, which is based on the "silence and replace" strategy, both mutant and wild-type PABPN1 are completely knockdown using selective microRNAs (miRNAs) [60]. However, the miRNA affects the expression of wild-type PABPN1 with potentially negative consequences for the treated muscles so an optimized-codon wild-type PABPN1 (opt-PABPN1) designed that is cleavage resistant by miRNAs and hammerhead ribozymes (hhRzs) [60]. This dual gene therapy approach package in an AAV vector has also been developed and tested in the A17 mouse model of OPMD and C. elegans OPMD models [60]. Results of this strategy in animal models show restoration of muscle strength, muscle weight and a reduction of fibrosis. This is currently the only preclinical gene therapy approach for OPMD treatment. BB-301 is a single AAV vector that delivers a unique single sequence with a bi-functional construct under the control of the muscle-specific Spc5-12 promoter for the co-expression of both two small inhibitory RNAs (siRNAs) against PABPN1 modeled into microRNA (miRNA) backbones and the codon-optimized PABPN1 protein [60].

11. Emery-Dreifuss Muscular Dystrophy

Emery-Dreifuss muscular dystrophy (EDMD) is a rare type of muscular dystrophy with slow progressive impairment of muscles that affects the arms and lower legs (humero peroneal regions), face, neck, spine, and heart [61]. Most people with EDMD develop heart problems, such as conduction block and arrhythmia that lead to heart failure, or sudden cardiac death. Seven types of EDMD with different gene disorders are known [61]. EDMD is caused by mutations in the genes of protein that are associated with the nuclear envelope and can be inherited in an X-linked (EDMD1), autosomal dominant (EDMD2 and EDMD7), or autosomal recessive pattern (EDMD3-6). The prevalence of EDMD is less than 1 in 100,000 people of all ages. Seven types of EDMD with different gene disorders are known. Mutation of the EMD, emerin (EDMD1) or LMNA, A-type lamins (EDMD2) gene is the most common cause in 40% of cases, and the disease course of EDMD 2 is more severe than others. There are no treatments for EDMD [61].

Gene therapy for this disease is currently in the early stages of research. CRISPR technology is hoped to pave the way for gene therapy for this disease.

12. Discussion

Gene therapy has become a promising approach for treating genetic diseases [1]. This new approach uses gene manipulation instead of surgery or drugs to treat disease, using four strategies: gene repair, gene replacement, gene deletion, and gene modulation [3].

Among the candidate diseases for gene therapy are muscular dystrophies that result from mutations in genes that encode proteins important for the health of muscles [13].

There are about 30 types of muscular dystrophy, but most gene therapy studies have been conducted on Duchene muscular dystrophy due to its higher prevalence than other types of muscular dystrophy [2, 16].

In this article, we have done a complete and comprehensive review of all types of gene therapy methods performed on muscular dystrophy diseases.

Some gene therapy approaches for DMD are in the clinical trial stages, while studies for other types of muscular dystrophies are still in the research phase due to their rarity. However, the results of these studies have been promising [14].

Most clinical trials on muscular dystrophy use antisense oligonucleotides (AOS), but the emergence of gene editing systems such as ZFN, TALEN, and especially CRISPR has made tremendous progress in gene therapy [11, 12, 23, 62]. Gene therapy has advantages and disadvantages like any other treatment method [63]. The benefit of gene therapy is that it gives affected people the ability to live "normal" lives and moderates or cures the symptoms of the disease [63].

Gene therapy presents many ethical, social, and regulatory challenges that require careful consideration to ensure its responsible application [64]. Ethically, the complexity of gene therapies complicates the informed consent process, as patients may struggle to fully grasp the risks and benefits involved, particularly with long-term effects remaining uncertain [64]. The distinction between somatic and germline therapies raises significant moral dilemmas; while somatic therapies target non-reproductive cells, germline modifications could have unforeseen consequences for future generations, prompting debates about the ethics of altering human heredity. Additionally, the high costs associated with gene therapies create concerns about equitable access, risking a scenario where only affluent individuals benefit from these advancements, thereby exacerbating existing healthcare inequalities [64]. Socially, public perception plays a crucial role; transparent communication is essential to combat misinformation and foster acceptance of genetic modifications [65]. There is also a risk of stigmatization for individuals with disabilities or genetic disorders if society begins to view certain traits as undesirable. From a regulatory perspective, the lack of comprehensive frameworks in many countries poses significant risks, particularly in regions where emerging therapies could greatly impact public health but remain unregulated [65]. Furthermore, the rapid development of gene therapy technologies necessitates international collaboration to establish consistent regulatory standards that ensure patient safety while promoting innovation [65]. Addressing these multifaceted challenges through ongoing dialogue among scientists, ethicists, policymakers, and the public is essential for navigating the complexities of gene therapy responsibly and effectively.

To drive progress in gene therapy for muscular dystrophies, particularly Duchenne muscular dystrophy (DMD), it is essential to establish clear research priorities and explore potential clinical applications [66]. First and foremost, understanding the underlying molecular mechanisms of muscular dystrophies is critical, as this knowledge will inform targeted therapeutic strategies. Developing more efficient and safer viral vectors for gene delivery is another priority, as current methods face challenges related to immune responses and the size limitations of the dystrophin gene [66]. Innovative approaches such as exon-skipping techniques and CRISPR-based gene editing hold promise for correcting mutations at the genetic level, potentially producing functional dystrophin or microdystrophin to restore muscle function [67]. Additionally, conducting patient-centric clinical trials that prioritize the preferences and values of patients and caregivers is vital for

designing effective therapies. Longitudinal studies are also necessary to monitor the long-term effects of gene therapies on efficacy and safety. In terms of clinical applications, developing one-time gene therapies that provide lasting benefits could revolutionize care for DMD patients by halting disease progression and improving quality of life [67]. Exploring combination therapies that integrate gene therapy with existing treatments may enhance overall effectiveness by addressing multiple pathways involved in muscle degeneration [35]. Furthermore, personalized medicine approaches tailored to individual genetic profiles could optimize treatment outcomes. Investigating regenerative strategies that promote muscle repair through stem cell integration or enhanced satellite cell activity could lead to innovative therapies that not only stop degeneration but also facilitate tissue repair [68]. Finally, engaging with the community to ensure patients and families are well-informed about emerging therapies will foster better decision-making regarding clinical trial participation and acceptance of new treatments [69]. Collectively, these efforts can significantly advance the field of gene therapy for muscular dystrophies, offering hope for improved outcomes for affected individuals.

On the other hand, the ethical implications of gene therapy, particularly for Duchenne muscular dystrophy (DMD), involve critical considerations regarding informed consent, equity, and the responsible use of emerging technologies [70, 71]. Informed consent is paramount, especially in germline gene therapy, where modifications affect future generations who cannot consent for themselves, raising questions about autonomy and parental rights. Additionally, the high costs associated with gene therapies may restrict access to wealthier individuals, exacerbating health disparities and raising concerns about justice and fairness in healthcare [70]. This inequity could lead to a society where genetic enhancements create new classes of individuals based on genetic makeup, reminiscent of eugenics [70, 71]. Furthermore, the distinction between therapeutic interventions for curing diseases and enhancements for non-therapeutic traits blurs ethical lines, posing moral dilemmas about "neo-eugenics" [72]. Thus, ongoing public discourse and regulatory frameworks are essential to guide the ethical use of these technologies, ensuring they align with societal values while safeguarding individual rights and promoting equitable access to life-saving treatments.

13. Limitations

Gene therapy holds great promise for treating genetic disorders but faces several significant challenges that impact its efficacy and safety [73]. Key issues include immunogenicity, off-target effects, and scalability. Immunogenicity is a critical concern in gene therapy, particularly with AAV-based vectors [73]. The immune response can lead to adverse events, diminishing the therapy's effectiveness and posing safety risks. Factors influencing immunogenicity include: Many individuals have neutralizing antibodies (NAbs) against AAV due to prior exposure, which can hinder the vector's ability to deliver therapeutic genes effectively [73, 74]. This pre-existing immunity can block the transduction of target cells, thereby reducing therapeutic gene expression. Administration of AAV vectors can also stimulate new immune responses against the vector and the transgene, potentially leading to severe adverse events [74]. Strategies to mitigate these responses include optimizing vector design, using immunosuppressive therapies before treatment, and selecting appropriate AAV serotypes. Both humoral and cellular immune responses can limit the durability of therapeutic effects and cause inflammation. Understanding these mechanisms is vital for

developing effective therapies and minimizing immunotoxicities. Off-target effects are a significant challenge in gene editing technologies like CRISPR [74]. These unintended modifications can lead to Non-specific targeting and may result in mutations at unintended sites, disrupting normal gene function or regulatory mechanisms [73]. This raises concerns about the long-term safety of such therapies. Introducing foreign components (like Cas9 protein) may trigger immune reactions, complicating treatment outcomes further. Ongoing research aims to refine these techniques to enhance specificity and reduce off-target effects.

Scalability remains a significant hurdle in the widespread application of gene therapies [74]. Key considerations include producing AAV vectors at scale while maintaining quality and consistency, which is complex. Variability in vector preparation can affect immunogenicity and therapeutic efficacy [73].

The need for extensive preclinical and clinical testing to ensure safety and efficacy adds time and cost to the development process. Regulatory bodies require thorough characterization of gene therapy products, including assessments of immunogenicity and off-target effects [73].

14. Conclusion

In conclusion, gene therapy represents a groundbreaking advancement in the treatment of genetic diseases, particularly muscular dystrophies such as DMD. Through innovative strategies like gene repair, replacement, deletion, and modulation, gene therapy has the potential to significantly improve the quality of life for individuals affected by these debilitating conditions. While many promising clinical trials are underway, particularly utilizing antisense oligonucleotides and emerging gene editing technologies such as CRISPR, addressing the ethical, social, and regulatory challenges accompanying these advancements is crucial. Ensuring informed consent, equitable access, and public acceptance are essential for the responsible application of gene therapies. Furthermore, establishing clear research priorities and exploring personalized medicine approaches will enhance the effectiveness of treatments. By fostering collaboration among scientists, ethicists, policymakers, and the community, we can navigate the complexities of gene therapy and ultimately drive progress toward effective therapies that offer hope for a brighter future for those living with muscular dystrophies.

Acknowledgments

The authors would like to express their gratitude to Ms. Firooze Dara, the Language editor, for her valuable comments on editing and improving the English in this manuscript.

Author Contributions

All of the authors contributed substantially to the concept and design of the study. Data collection, and analysis were performed by M.Da., M.Di., N.A, and R.T. The primary draft of the manuscript was written by M.Da. All authors read, and approved the final manuscript.

Funding

This work is supported by Shiraz University of Medical science.

Competing Interests

The authors declare that they have no conflict of interest.

Data Availability Statement

All data are available in manuscript.

References

1. Bulaklak K, Gersbach CA. The once and future gene therapy. Nat Commun. 2020; 11: 5820.
2. Shahryari A, Saghaeian Jazi M, Mohammadi S, Razavi Nikoo H, Nazari Z, Hosseini ES, et al. Development and clinical translation of approved gene therapy products for genetic disorders. Front Genet. 2019; 10: 868.
3. Shim G, Kim D, Le QV, Park GT, Kwon T, Oh YK. Nonviral delivery systems for cancer gene therapy: Strategies and challenges. Curr Gene Ther. 2018; 18: 3-20.
4. Young LS, Searle PF, Onion D, Mautner V. Viral gene therapy strategies: From basic science to clinical application. J Pathol. 2006; 208: 299-318.
5. Lundstrom K, Boulikas T. Viral and non-viral vectors in gene therapy: Technology development and clinical trials. Technol Cancer Res Treat. 2003; 2: 471-485.
6. Ramamoorth M, Narvekar A. Non viral vectors in gene therapy-an overview. J Clin Diagn Res. 2015; 9: GE01-GE06.
7. McDonough PG. The ethics of somatic and germline gene therapy. Ann N Y Acad Sci. 1997; 816: 378-382.
8. Hiramuki Y, Abe S, Uno N, Kazuki K, Takata S, Miyamoto H, et al. Full-length human dystrophin on human artificial chromosome compensates for mouse dystrophin deficiency in a Duchenne muscular dystrophy mouse model. Sci Rep. 2023; 13: 4360.
9. Sinenko SA, Ponomartsev SV, Tomilin AN. Human artificial chromosomes for pluripotent stem cell-based tissue replacement therapy. Exp Cell Res. 2020; 389: 111882.
10. Sun C, Shen L, Zhang Z, Xie X. Therapeutic strategies for Duchenne muscular dystrophy: An update. Genes. 2020; 11: 837.
11. Shan Q, Baltes NJ, Atkins P, Kirkland ER, Zhang Y, Baller JA, et al. ZFN, TALEN and CRISPR-Cas9 mediated homology directed gene insertion in Arabidopsis: A disconnect between somatic and germinal cells. J Genet Genomics. 2018; 45: 681-684.
12. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science. 2018; 361: 866-869.
13. Fletcher S, Wilton SD, Howell JM. Gene therapy and molecular approaches to the treatment of hereditary muscular disorders. Curr Opin Neurol. 2000; 13: 553-560.
14. Mendell JR, Rodino-Klapac L, Sahenk Z, Malik V, Kaspar BK, Walker CM, et al. Gene therapy for muscular dystrophy: Lessons learned and path forward. Neurosci Lett. 2012; 527: 90-99.
15. Asher DR, Thapa K, Dharia SD, Khan N, Potter RA, Rodino-Klapac LR, et al. Clinical development on the frontier: Gene therapy for Duchenne muscular dystrophy. Expert Opin Biol Ther. 2020; 20: 263-274.
16. Duan D, Goemans N, Takeda S, Mercuri E, Aartsma-Rus A. Duchenne muscular dystrophy. Nat Rev Dis Primers. 2021; 7: 13.

17. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: Diagnosis, and pharmacological and psychosocial management. Lancet Neurol. 2010; 9: 77-93.
18. Guncay A, Yokota T. Antisense oligonucleotide drugs for Duchenne muscular dystrophy: How far have we come and what does the future hold? Future Med Chem. 2015; 7: 1631-1635.
19. Lim KR, Maruyama R, Yokota T. Eteplirsen in the treatment of Duchenne muscular dystrophy. Drug Des Devel Ther. 2017; 11: 533-545.
20. Frank DE, Schnell FJ, Akana C, El-Husayni SH, Desjardins CA, Morgan J, et al. Increased dystrophin production with golodirsen in patients with Duchenne muscular dystrophy. Neurology. 2020; 94: e2270-e2282.
21. Wagner KR, Kuntz NL, Koenig E, East L, Upadhyay S, Han B, et al. Safety, tolerability, and pharmacokinetics of casimersen in patients with Duchenne muscular dystrophy amenable to exon 45 skipping: A randomized, double-blind, placebo-controlled, dose-titration trial. Muscle Nerve. 2021; 64: 285-292.
22. Igarashi J, Niwa Y, Sugiyama D. Research and development of oligonucleotide therapeutics in Japan for rare diseases. Future Rare Dis. 2022; 2: FRD19.
23. Mendell JR, Shieh PB, Sahenk Z, Lehman KJ, Lowes LP, Reash NF, et al. A phase 2 clinical trial evaluating the safety and efficacy of SRP-9001 for treating patients with Duchenne muscular dystrophy. Proceedings of the 27th International Annual Congress of the World Muscle Society (WMS); 2022 October 11-15; Halifax, Canada. Cambridge, MA: Sarepta Therapeutics Headquarters.
24. Tang A, Yokota T. Duchenne muscular dystrophy: Promising early-stage clinical trials to watch. Expert Opin Investig Drugs. 2024; 33: 201-217.
25. Iannaccone ST, Cai C, Rhem B, Batley K, Rajaram V, Greenberg BM, et al. Immune-mediated myositis following gene therapy for Duchenne muscular dystrophy: A case report. J Neurol. 2024; 271: 5659-5664.
26. Philippidis A. Boy dosed with Pfizer's Duchenne muscular dystrophy gene therapy dies a year after phase II trial. Hum Gene Ther. 2024. doi: 10.1089/hum.2024.53426.bfs.
27. Ortez C, Medina J, Vigo M, Moya O, Padros N, De Benito DN, et al. DMD CLINICAL THERAPIES I: P. 113 long-term effect of ataluren in fourteen patients with nonsense mutation Duchenne muscular dystrophy. Neuromuscul Disord. 2018; 28: S63.
28. Politano L. Read-through approach for stop mutations in Duchenne muscular dystrophy. An update. Acta Myol. 2021; 40: 43-50.
29. Dori A, Scutifero M, Passamano L, Zoppi D, Ruggiero L, Trabacca A, et al. Treatment with ataluren in four symptomatic Duchenne carriers. A pilot study. Acta Myol. 2024; 43: 8-15.
30. Ramos J, Chamberlain JS. Gene therapy for Duchenne muscular dystrophy. Expert Opin Orphan Drugs. 2015; 3: 1255-1266.
31. Choi E, Koo T. CRISPR technologies for the treatment of Duchenne muscular dystrophy. Mol Ther. 2021; 29: 3179-3191.
32. Liu Q, Sun Q, Yu J. Gene editing's sharp edge: Understanding zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR). Trans Mater Biotechnol Life Sci. 2024; 3: 170-179.

33. Alizadeh F, Abraghan YJ, Farrokhi S, Yousefi Y, Mirahmadi Y, Eslahi A, et al. Production of Duchenne muscular dystrophy cellular model using Crispr-Cas9 exon deletion strategy. Mol Cell Biochem. 2024; 479: 1027-1040.
34. Dara M, Razban V, Mazloomrezaei M, Ranjbar M, Nourigorji M, Dianatpour M. Dystrophin gene editing by CRISPR/Cas9 system in human skeletal muscle cell line (HSkMC). Iran J Basic Med Sci. 2021; 24: 1153-1158.
35. Ferrie J. The advantages of base and prime editing over traditional CRISPR in the treatment of monogenic diseases. Int J High Sch Res. 2024; 6: 45-51.
36. Duan D. Full-length dystrophin gene therapy for Duchenne muscular dystrophy. Mol Ther. 2024; 32: 2817-2818.
37. Bushby KM, Thambyayah M, Gardner-Medwin D. Prevalence and incidence of Becker muscular dystrophy. Lancet. 1991; 337: 1022-1024.
38. Szwec S, Kapłucha Z, Chamberlain JS, Konieczny P. Dystrophin-and utrophin-based therapeutic approaches for treatment of Duchenne muscular dystrophy: A comparative review. BioDrugs. 2024; 38: 95-119.
39. Mendell JR, Sahenk Z, Malik V, Gomez AM, Flanigan KM, Lowes LP, et al. A phase 1/2a follistatin gene therapy trial for Becker muscular dystrophy. Mol Ther. 2015; 23: 192-201.
40. Pascual-Gilabert M, López-Castel A, Artero R. Myotonic dystrophy type 1 drug development: A pipeline toward the market. Drug Discov Today. 2021; 26: 1765-1772.
41. Wheeler TM. Myotonic dystrophy: Therapeutic strategies for the future. Neurotherapeutics. 2008; 5: 592-600.
42. Yadava RS, Yu Q, Mandal M, Rigo F, Bennett CF, Mahadevan MS. Systemic therapy in an RNA toxicity mouse model with an antisense oligonucleotide therapy targeting a non-CUG sequence within the DMPK 3' UTR RNA. Hum Mol Genet. 2020; 29: 1440-1453.
43. Philippidis A. RNA in its DNA: Avidity biosciences combines mAb selectivity, oligonucleotide precision. Gen Edge. 2021; 3: 100-105.
44. Klein AF, Varela MA, Arandel L, Holland A, Naouar N, Arzumanov A, et al. Peptide-conjugated oligonucleotides evoke long-lasting myotonic dystrophy correction in patient-derived cells and mice. J Clin Invest. 2019; 129: 4739-4744.
45. Van Agtmaal EL, André LM, Willemse M, Cumming SA, Van Kessel ID, Van Den Broek WJ, et al. CRISPR/Cas9-induced (CTG CAG) n repeat instability in the myotonic dystrophy type 1 locus: Implications for therapeutic genome editing. Mol Ther. 2017; 25: 24-43.
46. Furling D. Cas9 targeting of toxic foci of RNA repeats. Nat Biomed Eng. 2021; 5: 130-131.
47. Porquet F, Weidong L, Jehasse K, Gazon H, Kondili M, Blacher S, et al. Specific DMPK-promoter targeting by CRISPRi reverses myotonic dystrophy type 1-associated defects in patient muscle cells. Mol Ther Nucleic Acids. 2023; 32: 857-871.
48. Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: The 2014 update. Acta Myol. 2014; 33: 1-12.
49. Mendell JR, Rodino-Klapac LR, Rosales-Quintero X, Kota J, Coley BD, Galloway G, et al. Limb-girdle muscular dystrophy type 2D gene therapy restores α-sarcoglycan and associated proteins. Ann Neurol. 2009; 66: 290-297.
50. Han WQ, Xia M, Xu M, Boini KM, Ritter JK, Li NJ, et al. Lysosome fusion to the cell membrane is mediated by the dysferlin C2A domain in coronary arterial endothelial cells. J Cell Sci. 2012; 125: 1225-1234.

51. Kolb SJ, Kissel JT. Spinal muscular atrophy. Neurol Clin. 2015; 33: 831-846.
52. Hoy SM. Onasemnogene abeparvovec: First global approval. Drugs. 2019; 79: 1255-1262.
53. Tawil R, Van Der Maarel SM, Tapscott SJ. Facioscapulohumeral dystrophy: The path to consensus on pathophysiology. Skelet Muscle. 2014; 4: 12.
54. Rashnonejad A, Amini-Chermahini G, Taylor NK, Wein N, Harper SQ. Designed U7 snRNAs inhibit DUX4 expression and improve FSHD-associated outcomes in DUX4 overexpressing cells and FSHD patient myotubes. Mol Ther Nucleic Acids. 2021; 23: 476-486.
55. Wallace LM, Liu J, Domire JS, Garwick-Coppens SE, Guckes SM, Mendell JR, et al. RNA interference inhibits DUX4-induced muscle toxicity in vivo: Implications for a targeted FSHD therapy. Mol Ther. 2012; 20: 1417-1423.
56. Zhang Y, Nishiyama T, Olson EN, Bassel-Duby R. CRISPR/Cas correction of muscular dystrophies. Exp Cell Res. 2021; 408: 112844.
57. Zambon AA, Muntoni F. Congenital muscular dystrophies: What is new? Neuromuscul Disord. 2021; 31: 931-942.
58. Azibani F, Brull A, Arandel L, Beuvin M, Nelson I, Jollet A, et al. Gene therapy via trans-splicing for LMNA-related congenital muscular dystrophy. Mol Ther Nucleic Acids. 2018; 10: 376-386.
59. Udd B. Molecular biology of distal muscular dystrophies-sarcomeric proteins on top. Biochim Biophys Acta Mol Basis Dis. 2007; 1772: 145-158.
60. Malerba A, Klein P, Bachtarzi H, Jarmin SA, Cordova G, Ferry A, et al. PABPN1 gene therapy for oculopharyngeal muscular dystrophy. Nat Commun. 2017; 8: 14848.
61. Muchir A, Worman HJ. Emery-Dreifuss muscular dystrophy: Focal point nuclear envelope. Curr Opin Neurol. 2019; 32: 728-734.
62. Moorehead T. Pfizer PF-06939926: Gene therapy safety and tolerability study in Duchenne muscular dystrophy (DMD). Proceedings of the Parent Project Muscular Dystrophy Annual Conference; 2018 June 28-30; Scottsdale, Arizona. Washington, D.C.: Parent Project Muscular Dystrophy.
63. Jafarlou M, Baradaran B, Saedi TA, Jafarlou V, Shanehbandi D, Maralani M, et al. An overview of the history, applications, advantages, disadvantages and prospects of gene therapy. J Biol Regul Homeost Agents. 2016; 30: 315-321.
64. Karunarathna I, Hapuarachchi T, Ekanayake U, Gunathilake S. The ethics of genetic editing: Navigating the future of science. Proceedings of the Uva Clinical Research; 2024; Teaching Hospital Badulla, Sri Lanka. Berlin, Germany: ResearchGate. Available from: https://www.researchgate.net/publication/383181517_The_Ethics_of_Genetic_Editing_Navigating_the_Future_of_Science.
65. Kohn DB, Chen YY, Spencer MJ. Successes and challenges in clinical gene therapy. Gene Ther. 2023; 30: 738-746.
66. Duan D. Duchenne muscular dystrophy gene therapy in 2023: Status, perspective, and beyond. Hum Gene Ther. 2023; 34: 345-349.
67. Abid H, Shah S, Ahmed A, Habib N, Bibi M, Ibrahim M. Role of physical therapy intervention and gene therapy on muscular dystrophies, current status and future perspectives: A narrative review: Role of physical therapy intervention and gene therapy. Pak J Health Sci. 2024; 5: 9-14.
68. Roberts TC, Wood MJ, Davies KE. Therapeutic approaches for Duchenne muscular dystrophy. Nat Rev Drug Discov. 2023; 22: 917-934.

69. Aartsma-Rus A. The future of exon skipping for Duchenne muscular dystrophy. Hum Gene Ther. 2023; 34: 372-378.
70. Ryan MM. Gene therapy for neuromuscular disorders: Prospects and ethics. Arch Dis Child. 2022; 107: 421-426.
71. Landrum Peay H, Fischer R, Tzeng JP, Hesterlee SE, Morris C, Strong Martin A, et al. Gene therapy as a potential therapeutic option for Duchenne muscular dystrophy: A qualitative preference study of patients and parents. PLoS One. 2019; 14: e0213649.
72. Leroi AM. The future of neo-eugenics: Now that many people approve the elimination of certain genetically defective fetuses, is society closer to screening all fetuses for all known mutations? EMBO Rep. 2006; 7: 1184-1187.
73. Gonçalves GA, Paiva RD. Gene therapy: Advances, challenges and perspectives. Einstein. 2017; 15: 369-375.
74. Shahryari A, Burtscher I, Nazari Z, Lickert H. Engineering gene therapy: Advances and barriers. Adv Ther. 2021; 4: 2100040.