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Review

The Usage and Limitations of Gene Therapy Versus Stem Cell Therapy for Myotonic Dystrophy Type 1 (DM1): Special Focus on Immune Response

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Abstract: Muscular dystrophies include myotonic dystrophy (MD). Although there is no cure for this disorder, many treatments can manage symptoms and reduce disease development. Standard pharmacological treatments and rehabilitation methods have largely failed, prompting researchers to investigate stem cell and gene therapy options. This review will discuss gene therapy and stem cell therapy as adjuvant therapies for MD, including their benefits, drawbacks, and timeline for implementation. We searched PubMed, Scopus, Web of Science (WOS), Cochrane Library, and Embase from 1980 to 2024 for preclinical and clinical gene therapy papers for MD using keywords such as "myotonic dystrophy" and "gene therapy." A search for "myotonic dystrophy" and "Stem Cell" yielded studies on stem cell treatment for MD. The initial search found 50 gene therapy and 38 stem cell research articles. Following our exclusion and filtering criteria, we deleted 31 articles on gene therapy and 23 articles on stem cells. We found 19 gene therapy publications and 15 stem cell-based publications. Antisense oligonucleotides (ASO), CRISPR technology, and AAV vectors have become the most popular methods. Induced pluripotent stem cells (iPSCs) and muscle-derived progenitor cells (MuSCs) were important cellular sources for investigation or application. Most research focuses on myotonic dystrophy type I (DM1). It is widely established that AAV vector-induced immune responses can affect the safety and efficacy of genetic therapy. Specific stem cells may also modulate MD patients' immunological responses, improving prognosis. Using cytokines and monoclonal antibodies as adjuvants to treat this complex illness is becoming more common.

Keywords: Myotonic Dystrophy (MD); Gene Therapy; Stem Cell Therapy; Immune Response

1. Introduction

Myotonic dystrophy (MD) is an inherited disease belonging to the muscular dystrophy family and the most common adult-onset type. It is characterized by the loss of motor neurons and progressive muscle weakness. A hallmark of the condition is prolonged muscle contractions (myotonia), during which the affected muscles have dif-

ficulty relaxing after contracting, and they may stay tense for extended periods [1]. MD also presents with cataracts and cardiovascular anomalies [2, 3]. Hormonal imbalance in men can also result in early baldness and infertility amongst afflicted males [4,5]. Though these symptoms can manifest at any stage of life, they most often appear in a person's twenties or thirties. The intensity of the disorder varies greatly from person to person, even within the same family.

The two most common forms of MD are type 1 (MD1) and type 2 (MD2), caused by mutations in different genes. The general clinical features of MD2 are very similar to those of MD1, except that MD2 has a milder presentation than MD1. Muscle weakness in MD1 predominantly involves the lower legs, hands, neck, and facial muscles. MD2, on the other hand, causes weakness of the neck, shoulders, elbows, and thighs first [6]. MD is diagnosed in one out of every 8,000 people across the world, but based on type, the incidence varies by ethnicity and geographic region. Most studies show that MD1 is more prevalent than MD2 in various populations [7].

MD1, for example, is caused by expansion of CTG nucleotide repeats in the 3' UTR region downstream of the DMPK gene. In healthy individuals, these repeats usually occur from 5 to 30 times. In affected individuals, however, the number of repeats grows, resulting in chromosomal instability and abnormal expansion of this region. The severity of the disease is directly proportional to the number of repeats (congenital form ≥ 2000 copies, classic form 100–1000 copies, mild form 50–150 copies). Furthermore, 35 to 49 copies of the same trinucleotide repeat are believed to constitute a pre-mutation [8].

MD2, by contrast, is due to an expansion of CCTG repeats in intron 1 of the CNBP (ZNF9) gene. Although the specific role of these genes is not yet known, the DMPK gene is believed to produce a protein that plays a part in cellular signalling and is essential for the proper functioning of cardiac, cerebral, and skeletal muscle cells. A similar theory is that the protein produced by the CNBP gene is mostly present in skeletal muscles and the heart and is thought to modulate other gene activity [9].

Diagnosis of myotonic dystrophy can be confirmed through genetic testing to determine the number of CTG repeats in the DMPK gene [10]. The greater the repeat number, the earlier the disease onset and the more severe the clinical manifestations, with congenital cases having the highest repeat numbers [10].

MD is a sex-linked genetic disorder of somber impact that primarily affects men, caused by a mutation in a sex-linked gene that blocks the body's ability to produce an important protein(dystrophin), which is required for muscle building [11]. In the absence of dystrophin, the muscles slowly weaken and waste away as adolescence advances. By the age of 12, most people with the disorder cannot walk, and most die of heart or respiratory failure in their 30s. However, the availability of ventilators has enabled some patients to live longer. Adaptive agents in therapeutic gene applications through engineered modified AAV vectors in animal models of MD have been developed to replace dystrophin [1], which should reduce the severity of this characteristic. Of course, for those of you who are not aware, utroseq is simply a synthetic version of utrophin (a naturally occurring protein) that is both safe and efficacious. This strategy maintains muscle in genetically modified animal models mimicking similar MD mutations and even in large deletions relative to human MD mutants [12]. Here we demonstrate both understanding and a modified aircraft version of the gene, known as the creeping of dystrophin-associated, which can be used to inhibit anaesthesia and safely maintain function on long-term animal models.

That is a problem, because the body mounts an intense immune response to foreign molecules, making it difficult to restore dystrophin levels through gene therapy or other means. Because MD patients do not have dystrophin, this therapy faces the danger of their immune systems attacking the replacement proteins as if they were foreign invaders. However, utrophin is a family member of dystrophin, and it is also present in other Fat of the body, so potentially, a hypothesis may be proposed that the immune system does not recognize it as the enemy. Cytokine-independent immunomodulatory activity may be mediated through monocytes and Tregs [13], which have been previously established [7]. It has been reported that adipose-derived mesenchymal stem cells (AMSCs) support stronger immunomodulatory effects [13,14] than those obtained from the well-known type of stem cell, bone-marrow-derived mesenchymal stem cell (BMSC), thus suggesting that AMSCs represent the ideal therapeutic cell for immunotherapy. HUC-MSCs represent one such promising therapy, as MSCs are immunogenic *in vivo* immune challenge [15]. Other studies may suggest: Human-originating MSCs optimize more effective therapeutic effects for mouse tumours [15], sheep tibial defects, pig bone healing [16], and canine cartilage regeneration. MSCs have been shown, in an extensive number of preclinical studies, to play an important role in both innate/adaptive immune response through their interaction with T cells, B cells, neutrophils, monocytes,

macrophages, natural killer (NK) cells, and dendritic cells (DCs) [17].

Instead of specificity markers, their functional and morphological characteristics define MSCs, since they do not express hematopoietic cell markers such as CD14, CD7, CD8, and CD45 or co-stimulatory molecules such as CD40, CD86, and CD80. However, MSCs express other markers and even the expression of these markers is very variable, depending on the isolation source and lab conditions [18]. Moreover, MSCs also perform immunomodulatory functions that modulate both innate and acquired immune systems 12. They function in the inhibition and modulation of immune response in various immune cells, including T lymphocytes, B lymphocytes, NK cells, and dendritic cells, as well as downregulation of pro-inflammatory TH1 cytokines, including TNF-α and IL-12 [19,20].

There are many immunotherapy strategies that scientists hope might augment the effects of gene or stem cell therapies. An alternative promising application is chimeric antigen receptor T-cell (CAR T-cell) therapy [4], which has risen to prominence as an immunotherapeutic for diverse and challenging diseases, particularly malignancies [21]. Several versions of cellular immunotherapy for cancer target specific antigens, permitting the treatment of types of malignancies [22].

The use of mRNA is another emerging method of immunotherapy. In the past several decades, mRNA vaccines moved from being an abstract idea to a reality in the clinic. These vaccines have many advantages over conventional vaccines owing to their high potency, fast development, lightweight manufacturing, and safe delivery [23]. However, the instability and the inefficient distribution of mRNA in the body had deterred their broad use before. However, recent advances in technology have dramatically reduced these challenges, enabling the discovery and manufacture of mRNA vaccines against many infectious diseases and cancer types [24].

Because of the pathophysiological link between muscular dystrophy (MD) and the immune system, and the immunological effects of the various treatment options currently available, gene therapy and/or stem cell therapy offer great promise as a potential therapeutic option for MD.

There is no currently known cure for muscular dystrophy (MD), but there are treatments that can help to relieve symptoms and slow the course of the disease. Symptom-specific treatments include corticosteroids, pulmonary rehabilitation methods, medications for heart diseases and cardiovascular conditions, as well as surgeries that target muscle disorders [25]. Studies using gene therapy with antisense oligonucleotide (ASO) technology for MD are underway, which may provide new potential for treating MD [26]. Other recent research has investigated stem cells for the treatment of MD [27], with findings suggesting that stem cells can promote muscle recovery and delay disease progression. Clinical experience has shown potential benefits after stem cell treatment in some case studies with remarkably favorable results of muscle strength and daily activity ability [28].

However, drug treatment and rehabilitation do not work for MD, prompting researchers to turn to stem cell and gene therapies. It has been shown that combining these two approaches delivers better results [29]. Although there has been some progress in this area, full genetic correction and a cure for the disease have yet to be achieved. The focus of this study is to examine the potential, benefits, limitations, and safety of gene therapy and stem cell therapy for MD and autoimmunity. Additionally, the review discusses gene therapy prospects for immunodeficient patients from multiple perspectives.

Stem cells, especially mesenchymal stem cells (MSCs), have attracted increasing attention in recent years owing to their potential application for treating diverse diseases. The immune regulatory functions and suppressive properties of MSCs make them a candidate for use as an immune system regulatory tool in the treatment of autoimmune diseases. MSCs play a role in immune system regulation, and that is the topic of this article, as well as their clinical application in it.

2. Methods

2.1. Gene Therapy for Muscular Dystrophy

Gene therapy is the delivery of a functional gene into a cell to have a therapeutic effect. This is accomplished by restoring the healthy copies of the gene into the patient to reverse the characteristics of the mutated phenotype [30]. It is to be noted here that genetic diseases can also be dealt with in many approaches (more than just the mutated gene) and newer techniques such as rDNA technology. This technology enables intervention at the most fundamental level—the gene. Gene therapy is designed to treat the patient by restoring the mutation phenotype [31].

Somatic (non-germ) cells need to be targeted for gene therapy to work. There are generally three aims of introducing a gene into somatic cells, as follows. First, a mutated gene that acts with a loss-of-function mutation, for example, in the autosomal recessive disorder phenylketonuria, can be compensated for by gene therapy. Second, it can substitute or knock out an invasive mutant gene, making an irregular protein, causing maladies to develop, for instance, Huntington's disease [32]. Gene therapy can be used on a much larger scale to produce a pharmacological effect in a patient by counteracting the effect of a mutated gene or to block the course of a disease (most relevant to patients with acquired diseases like cancer) [33].

Gene therapy for muscular dystrophy has aimed to deliver the parts, or instructions, that make up dystrophin to muscle cells. Because dystrophin is a big protein, vector viruses are used to deliver smaller versions of it into cells. Viral delivery methods usually use adeno-associated viruses (AAV), which deliver genetic materials directly into the cell nucleus. This also allows the modified genetic code to be deposited into the nuclei of muscle cells. As such, this viral "infection" basically rewrites the muscle cells' programming such that they can produce a smaller, albeit functional, version of the dystrophin protein.

2.2. Searching Strategy

An extensive search process was performed in PubMed, Scopus, Web of Science (WOS), Cochrane Library, and Embase ranging from 1980 to 2024, using the keywords "myotonic dystrophy" AND "Gene therapy" to find preclinical and clinical studies on gene therapy for myotonic dystrophy. In addition, we searched the same databases using the search terms "myotonic dystrophy" AND "Stem Cell" to identify all clinical and preclinical trials of stem cell therapy for myotonic dystrophy available in the literature, without any language restriction. This search excluded letters, commentaries, notes, and any type of review article.

3. Results

This search provided 50 articles on gene therapy and 38 papers on stem cell therapy (**Figure 1**). Our predefined exclusion criteria led to the removal of 31 for gene therapy and 23 for stem cell therapy. "Ultimately, we found 19 articles reporting on gene therapy and 15 on stem cell therapy.

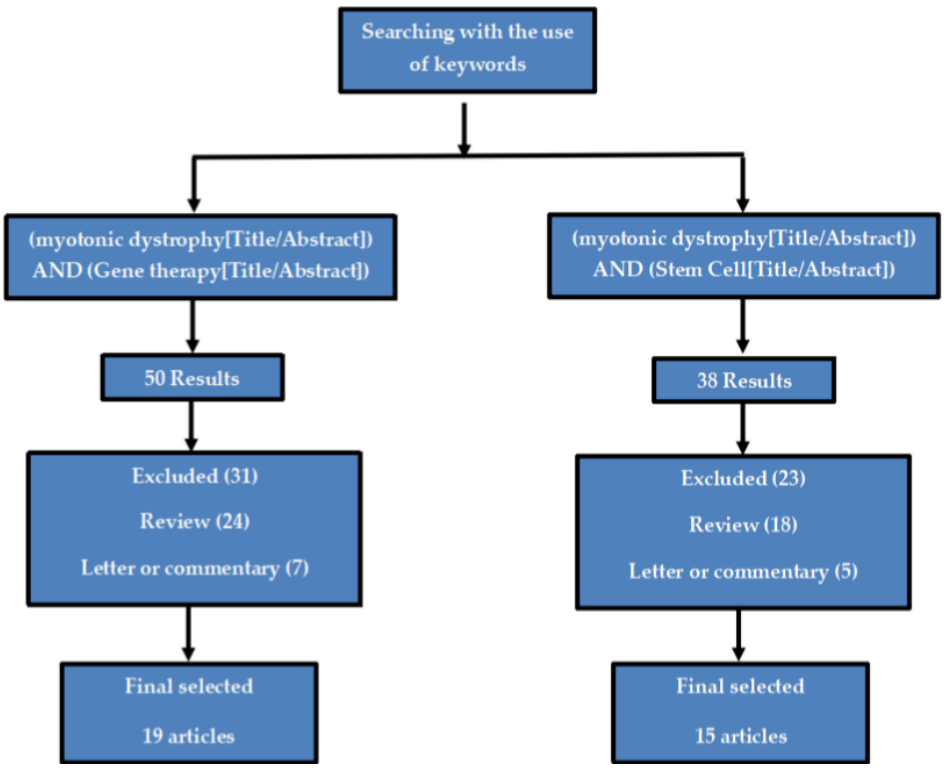

Figure 1. Flow diagram of search and selection committed in the study.

CRISPR and antisense oligonucleotides (ASO) were the most common gene therapy methods used, with adeno-associated virus (AAV) being the most common vector used (**Table 1**). In stem-cell therapy, iPSC and MuSC were the most commonly used (**Table 2**). All but one study dealt exclusively with DM1.

Table 1. Gene therapy for myotonic dystrophy.

Study ID, Reference	Target Population	Gene Therapy Technique	Disease	Vector	Limitations	Final Findings
Poukalov et al., 2024 [34]	C57BL/6 mice	CRISPR/Cas9-based gene editing	MD1/2	AAV vs. AAV-Cas9-AI14	low rates of myo-nuclear transduction	Effective with higher rates of gene editing
Almeida et al., 2023 [35]	Cell line	ASOs to knock down *DMPK* expression	MD1	AAV8, AAV.U7snRNAs	Limited AAV volume	Effective with Spliceopathy correction of genes implicated in DM1
Porquet et al., 2023 [36]	Cell line	*DMPK*-promoter silencing strategy by CRISPRi	MD1	rAAV	Limited AAV capacity	Effective with *DMPK*-promoter silencing
Rogalska and Sobczak, 2022 [37]	Cell line	Auto-regulated MBNL1 overexpression system	MD1	AAV	Uncontrolled overexpression of MBNLs	Beneficial effects on alternative splicing patterns in DM1
Kelley et al., 2022 [38]	Cell line	CRISPR-Cas13 -based gene editing	MD1	AAV6	Not demonstrated efficacy in preclinical animal models	Illustrates advantages of compact regulatory systems
Benichou et al., 2022 [39]	DMSXL mice	ASOs to knock down *DMPK* expression	MD1	AAV8	-	A significant improvement in muscle strength
Cardinali et al., 2022 [40]	DMSXL mice	CRISPR-Cas9 -based gene editing	MD1	AAV9	-	Efficient and inducible improvement
André et al., 2019 [41]	MT null mice	Isogenic cDM myoblasts obtained by gene editing	MD1	AAV	Repeat Excision Limited and Little Congruence	Effective
Lo Scrudato et al., 2019 [42]	DMSXL mice	CRISPR-Cas9 -based gene editing	MD1	AAV9	The age of treatment	Feasible in muscle improvement
Dong et al., 2019 [43]	DMSXL mice	CUG-expansion and Celf1 overexpression C2C12 cell	MD1/2	Mir-206	the role of miR-206 needs to be tested further in vivo	Not effective and incompletely reverses myoblast differentiation inhibition
Wang et al., 2018 [44]	DMSXL mice	CRISPR-Cas9 -based gene editing	M MD11	AAV2-MCS2	Did not observe the antisense transcription	Viable approach to develop therapeutic genome editing
Mosbach et al., 2018 [45]	Cell line	$TALEN_{CTG}$ expanded CTG triplet	MD1/2	pCMha182KNR1 and pCMha188KNL1	-	TALEN efficacy is very low
Provenzano et al., 2017 [46]	DMSXL mice	CRISPR-Cas9 -based gene editing	MD1	rAAV	limiting possible off-target effects	Affected cells can be permanently reverted to a normal phenotype
Batra et al., 2017 [47]	Cell line	RCas9 including elimination of RNA foci	MD1/2	AAV	limited packaging capacity	The potential for human therapeutics.
Jauvin et al., 2017 [48]	DMSXL mice	ASOs to knock down *DMPK* expression	MD1	VECTASHIELD H-1000	Needed to determine the long-term effects and toxicity	Muscle weakness may improve following elimination of toxic RNAs
Bisset et al., 2015 [49]	HSA^{LR} mice	RNAi therapy	MD1	rAAV and AAV6	-	Potential to provide a long-term therapy

Table 1. *Cont.*

Study ID, Reference	Target Population	Gene Therapy Technique	Disease	Vector	Limitations	Final Findings
Zhang et al., 2014 [50]	HSA^{LR} and $Mbnl1^{\Delta E3/\Delta E3}$ mice	Artificial site-specific RNA endonucleases (ASREs) bind and cleave $(CUG)_n$ repeats RNA	MD1	AAV	limited its off-target effect toward low-repetitive substrate	Provides a new route of gene therapy
Langlois et al., 2003 [51]	Cell line	Accessible ribozyme target sites in the 3'UTR of the DMPK mRNA	MD1	tRNAmet-RBZ	limitations for a ribozyme cleavage site	Providing a potential gene therapy agent
Furling et al., 2003 [52]	Cell line	A retrovirus expresses a 149-bp antisense RNA complementary to the (CUG)13 repeats	MD1	MMLV	Wider use of antisense technology	Can ameliorate dystrophic muscle pathology

Note: AAV: adeno-associated virus; MD1: myotonic dystrophy type 1; ASOs: antisense oligonucleotides; CRISPRi: CRISPR interference; rAAV: recombinant AAV; MBNLs; muscleblind-like proteins; RCas9: RNA-targeting Cas9; mRNA containing an expanded CUG repeat [CUG(exp)]; moloney murine leukemia virus (MMLV).

Table 2. Stem cell therapy for myotonic dystrophy.

Study ID, Reference	Target Population	Stem Cell Type	Disease	Limitations	Final Findings
Raaijmakers et al., 2024 [53]	Six patients and two unaffected controls	stem cell-like (Human pericytes)	MD1	No evidence for inter-nuclear transport of expanded RNA in cell	Promise for the development of cell transplantation strategies
De Serres-Bérard et al., 2024 [54]	Two patients	CDM into iPSC	MD1	-	An important resource and potential treatments
Pierre et al., 2024 [55]	One patient	iPSC lines derived from lymphoblastoid cell	MD1	Lacked residual viral vector	Support the robustness and reliability of iPSC
De Serres-Bérard et al., 2023 [56]	Two pediatric CDM patients and two age-matched controls	Dermal fibroblasts derived from CDM into iPSC	MD1	Smaller than the minimal resolution	Their reliability as therapeutic strategies
Conte et al., 2023 [57]	One hundred and three participants	MuSC/myoblasts	MD1	-	Opens a therapeutic avenue
Kawada et al., 2023 [58]	Three patients	iMuSC	MD1	CTG repeats was relatively long	Promising for drug discovery against disease
Li et al., 2022 [59]	One patient	iPSC	MD1	-	Potential therapeutic targets
Yanovsky-Dagan et al., 2022 [60]	Four patients	hESCs	MD1	A very small number of individuals	Promising for drug discovery against disease
Ausems et al., 2019 [61]	Six patients and two unaffected controls	stem cell-like (Human pericytes)	MD1	No major variations in myogenic capacity	A potential of pericytes to ameliorate muscle features
Mondragon-Gonzalez et al., 2019 [62]	DMSXL mice	iPSC	MD1	RNA foci in donor-derived engrafted myonuclei, is pathogenic	Effective but not safe
Maury et al., 2019 [63]	Six patients and two unaffected controls	iPSC	MD1	Effect is calcium dependent and can be synergic to the inhibition of the ERK signaling pathway	Underscore the value of stem-cell-based assays for drug discovery

Table 2. *Cont.*

Study ID, Reference	Target Population	Stem Cell Type	Disease	Limitations	Final Findings
Wang et al., 2018 [64]	One patient	iPSC	MD1	-	A robust platform for pathogenesis as well as drug testing and gene therapy
Dinarelli et al., 2018 [65]	Two healthy subjects and 2 patients	hiPSC-CMs	MD1	Issue remains regarding the maturity of hiPSC-CMs	Lead to cellular response to the applied mechanical stimulus
Spitalieri et al., 2018 [66]	Two healthy subjects and 2 patients	hiPSC-CMs	MD1	Issue remains regarding the maturity of hiPSC-CMs	Identifying novel biomarkers effective in patient-tailored therapies
Ueki et al., 2017 [67]	Three patients	iPSC	MD1	Closed chromatin status	The role of instability in the CTG repeat

Note: Congenital myotonic dystrophy (CDM); Induced pluripotent stem cells (iPSC); iPSCs-derived muscle stem cell (iMuSC); Muscle stem cells (MuSC); Human embryonic stem cell (hESCs); Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).

3.1. Gene Therapy

Having cracked the code of the human genome, researchers entered a second phase aimed at correcting faulty genes. This field of science is commonly known as genetic engineering. Gene therapy is the manipulation of genes to prevent or cure genetic diseases [67]. Gene therapy is used in three main ways [68].

The first method is to replace a defective gene with a dominant, healthy gene. The second approach reformats and repairs an errant gene to work properly. The third approach is to turn off an active gene, or vice versa, turn on an inactive gene. So far, the most common strategy has been the first approach: inserting a healthy gene in place of the broken one.

Nonetheless, one of the biggest hurdles in gene therapy is getting the gene into the cell, which is often done with a vector. Viruses can carry and transfer genetic materials, a common use of vectors. In the laboratory, these types of viruses are modified to become non-pathogenic, so that they can deliver genetic fragments without causing any disease [69].

Gene therapy can be used to treat genetic diseases, cancers, and some systemic diseases that have genetic roots [70] (**Figure 2**). However, the key point is that gene therapy is a very young science, despite its many and varied capabilities, and has brought with it various issues, considerations, and cancers [71]. Gene therapy is carried out in two ways: gene therapy on somatic cells and gene therapy on germ-line cells [72]. In the first method, gene changes are carried out in somatic cells, such as liver or muscle cells, and in the second method, gene therapy is carried out on sperm and eggs. In somatic gene therapy, the changes resulting from the treatment are limited to the person who has undergone the treatment. However, in germ cell gene therapy, these changes are also transmitted to subsequent generations because they have been carried out in sperm and eggs. Today, researchers are focusing more on gene therapy of somatic cells to treat genetic, cancer, and cardiovascular diseases [73]. It should be noted that there are many obstacles in the way of gene therapy. One of the most important obstacles is controlling the virus carrying the healthy gene. This is important because the virus must accurately deliver the gene to the target cell. Sometimes, due to abnormalities that occur, these viruses cause severe illness or a dangerous immune response in the recipient of the gene.

3.2. Stem Cell Therapy

Stem cell therapy is a method that utilizes stem cells to regenerate damaged muscle cells. These stem cells can be delivered in two ways: through intravenous and intramuscular injections [74]. While intravenous injection is more convenient and less expensive, its success depends on the migration of the injected cells to the muscle. In contrast, a more effective delivery approach, such as intramuscular injection, increases the chances of hitting only tissues of interest, but it is more expensive and time-consuming. The selection of the right administration technique depends on the specific type of muscular dystrophy (MD), the stage of disease progression, and the type of stem cells used [75].

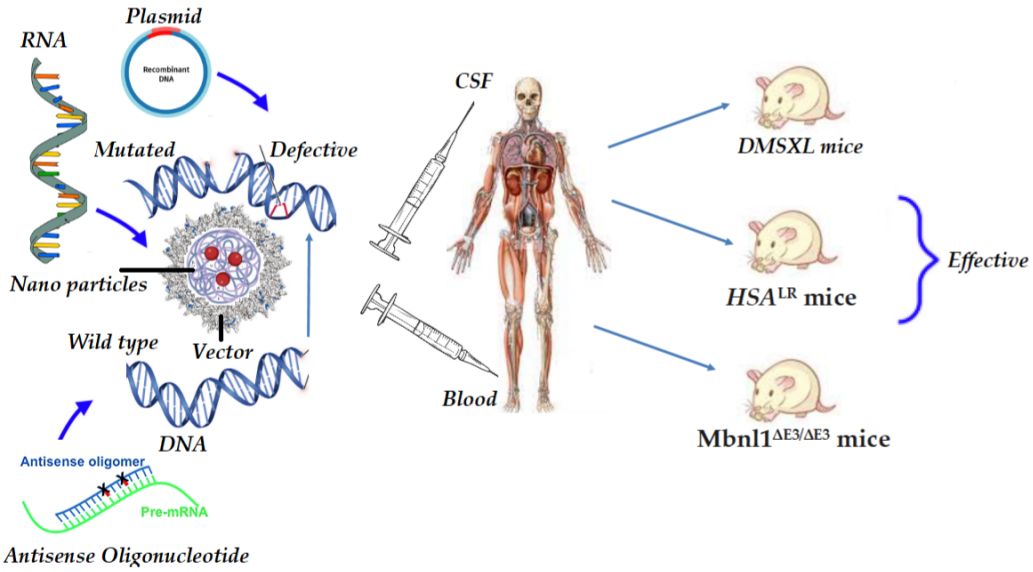

Figure 2. The mechanism of gene therapy and available animal models reported so far.

There are different types of stem cells that could be used for muscular dystrophy (MD) treatment, one of which is the embryonic stem cells [76]. These cells, obtained from early-stage embryos, possess a high differentiation potential. We will now discuss another key form of stem cell, which are mesenchymal stem cells (MSCs), found in other tissues, notably fat and bone marrow. These cells can turn into muscle cells and help patch tissue.

MD treatment with stem cells comes through several methods. Directly injecting stem cells into injured muscle is one way scientists are working to advance this field. This approach involves directly injecting stem cells into the damaged muscle, which would enable the cells to be delivered directly to the site of injury. A related technique—muscle tissue engineering—involves using stem cells and other biological materials to create new muscle tissue.

Many cell types have been characterized for cell therapy of muscle disorders, including (i) myoblasts, (ii) induced pluripotent stem cells, (iii) muscle stem cells, and (iv) mesenchymal cells for muscle degeneration and regeneration [77]. Myoblasts have demonstrated muscle repair properties, but their short life span and heterogeneous distribution lead to a lack of long-term efficacy [78]. In contrast, mesenchymal stem cells (MSCs) from bone marrow, adipose tissue (AT), and umbilical cord blood have proven effective in preclinical MD, and through their ability to mediate immune suppression, integrate into dystrophic muscle, and secrete a variety of growth factors, they show great potential as therapies for muscle disease [79].

This has been shown to increase dystrophin expression when myotubes from patient muscle are co-cultured with these cells. On the other hand, transplant of adipose-derived mesenchymal stem cells dystrophic animal models results in improved muscle strength, reduced fatigue, new muscle fiber formation, and decreased inflammation [80].

Umbilical cord blood stem cells are a novel method for MD treatment [81]. This approach has garnered interest due to its non-invasive nature and because these cells are easily obtained. Studies have indicated that cord blood stem cell transplant might enhance muscle function in patients with MD. Transplantation is usually carried out through intravenous or intramuscular injections, with both methods having their advantages and disadvantages [27].

However, several issues remain to be resolved for the implementation of MD cell therapy. Technological limitations are a significant challenge because many stem cell therapies are still in the experimental stage, and further studies are needed. There are also ethical considerations, especially relating to the source of embryonic stem cells, which can be at odds with some ethical and moral viewpoints.

3.3. Challenges in Clinical Translation

While preclinical models of gene and stem cell therapy for DM1 show promising results, significant hurdles remain before widespread clinical adoption. These include safety concerns, vector delivery efficiency, immune re-

sponses, and patient-specific factors such as repeat expansion variability. Current human trials are limited in size, and long-term efficacy and safety data are still pending.

4. Discussion

We provide a systematic review of the available evidence on the use and efficacy, safety, and limitations of gene therapy and stem cell therapy approaches aimed at the treatment of Myotonic Dystrophy (MD). Genetic Modifications for MD1: Insight from the Findings Gene therapy, most represented in recent anti-dystrophy applications by CRISPR/Cas9 and Antisense Oligonucleotides (ASOs), does appear to show promise in correcting the genetic defects specific to MD1. Adeno-associated viruses (AAVs) are potent gene transduction vectors [82]. Nevertheless, issues like immune responses and poor transduction rates are still hurdles.

Glial cell transplantation and stem cell therapy, particularly with iPSCs and MuSCs, as well as their application to the injured or diseased muscle, have shown promise in ameliorating muscle function and delaying disease progression [83]. However, potential issues remain with the survival of donor cells and the risk of tumorigenesis [84]. Interestingly, only MD1 has been the subject of all active preclinical and clinical studies, pointing to an obvious lack of research on the MD2 component [85]. This highlights the importance of additional studies focused on therapeutic approaches for MD2. Taken together, both therapies have enormous promise. Gene therapy has the potential to directly address the pathogenic process causing the disease, while stem cell therapy targets symptom mitigation and muscle regeneration. A potential future direction will be to combine both approaches to improve therapeutic efficacy. Due to their non-pathogenicity, low immunogenicity, stability, and gene induction ability, AAVs have become a promising gene therapy vector [86]. AAV vectors have not been associated with any diseases in humans or animals and thus represent a promising candidate for gene transfer. Adenoviruses have been used in gene therapy for their ability to incorporate genes into dividing as well as terminally differentiated cells, hence allowing for long-term expression of transgenes in organs such as the brain, skeletal muscle, and liver, making them a good vector for gene therapy, including MD [87]. Nevertheless, AAV vectors have certain disadvantages. They have a maximum transfer capacity of 5 kilo-base pairs of DNA, and titering of viral recombinants is limited. In addition, co-infection with a helper virus, such as adenovirus or herpes simplex virus, in some situations is also essential. AAV-based gene therapy also raises the issue of immune reactions, which is a great concern [88]. The immunogenic properties of AAV and relevant toxicities need to be well understood to overcome immune-related barriers [89].

These observations are in agreement with recent reports, which demonstrated that stem cells (SCs) possess potential immunomodulatory properties and can influence both the innate and adaptive pathways of the immune system. This can lead to suppression and regulation of immune response by inhibiting key immune cells such as B lymphocytes, T lymphocytes, and dendritic cells [90]. These cells then upregulate surface markers, including CD11c, CD83, and MHC class II, on monocytes and induce downregulation of pro-inflammatory cytokines (i.e., TNF-α, IL-12) [91]. SCs, on the other hand, promote IL-10 production in monocytes [92] that inhibits the activation of dendritic cells, as IL-10 is an anti-inflammatory cytokine.

Moreover, SCs down-regulated the expression of NKP44, NKG2D, and NKP30 in natural killer (NK) cells, inhibiting their cytotoxic activity and ultimately leading to suppression of proliferation and inhibition of IFN-γ production by NK cells [93]. Some SCs can suppress neutrophil activation by causing less hydrogen peroxide generation, thus relieving inflammatory response in both the adaptive and innate immune system [94]. Given that T lymphocytes play pivotal roles in immune function, the effects of SCs on T cell activity are especially of interest. SCs are able to inhibit T cell proliferation in response to polyclonal mitogens, allogeneic cells, or specific antigens [95].

Additionally, mesenchymal stem cells (MSCs) may induce T lymphocyte differentiation by the secretion of cytokines, decreasing the pro-inflammatory cytokines (IL-17, IL-6, TNF-α, IFN-γ), and increasing anti-inflammatory cytokines (IL-10 and IL-4) [96–98]. Goodwin et al. [99] and Sun et al. [100] showed that MSCs derived from bone marrow suppress Th2 cell-mediated allergic airway inflammation by inducing IFN-γ production.

5. Ethical and Regulatory Considerations

Both gene and stem cell therapies raise important ethical questions, especially concerning germline editing, long-term monitoring, and informed consent. Regulatory frameworks vary by region; the FDA and EMA have developed stringent protocols for gene-editing interventions. Ethical approval and public trust are essential for clinical

advancement, particularly when using embryonic stem cells.

6. Conclusion and Clinical Implications

The results of this study underscore the potential of gene and stem cell therapies for the treatment of MD. Gene therapy holds an attractive promise of enabling a direct correction of genetic defects; however, the ultimate success will depend on the possibility of *in vivo* gene transfer evasion of immune responses and longevity of gene expression. It is a non-curative treatment, but it could be combined with muscle regenerative and disease progression processes for patients with advanced MD stages. The combination of gene therapy and stem cell therapy represents an innovative path to the greatest synergy of benefits associated with these treatments, including gene modification for genetic correction and stem cell therapy for muscle repair, that will complement each other in the treatment of muscle disease. On top of that, immunotherapy associated with gene and stem cell therapy could turn out to be an even more powerful therapeutic strategy. Immune rejection is a known limiting factor for many gene and stem cell therapies, and different immunomodulatory approaches have been described, including those based on the admixture of cytokines, monoclonal antibodies, or immunizing adaptive immune tolerance pathways. The immune tolerance/hypo-responsivity of the adaptive immune response plays a critical role in the therapeutic success of gene therapy, motivating further exploration of the immunomodulatory effects that could promote these therapies. However, some stem cells are thought to remain in the body for weeks after being injected, requiring further study to fully understand their lingering effects on the immune system and the risk of chronic restimulation. Furthermore, the influence of combo therapy with immunomodulatory drugs on stem cell injections must be defined so that the safety of such a treatment can be assured, and the maximal effects of therapy can be achieved. Similarly, the immunogenicity of the stem cells themselves, if altered post-treatment, would also need to be characterized. Further studies are needed to explore the optimal clinical scenario for co-administration of stem cells plus immunosuppressive drugs to modulate immune responses in MD. Comprehensively, gene and stem cell therapies seem promising candidates in MD treatment and combining them with immunotherapy and further investigation into immunomodulatory strategies will be critical to improving efficacy and potential safety in MD patients. Early diagnosis and genetic testing to inform the treatment of these patients is a high priority for both clinicians and healthcare systems. This underscores the importance of interdisciplinary care, longer-term follow-up, and close monitoring of patients, all of which will be critical regarding the successful translation of these therapies into the clinic. However, combining these therapies introduces complexities such as the need for synchronized timing, individualized dosing, and significantly increased costs. Multimodal clinical trials are scarce, and the logistics of co-administering gene vectors, stem cells, and immunomodulators remain a practical challenge. Clinicians should closely monitor patients for immune-related side effects and consider pre-screening for immune predispositions. Researchers should explore novel delivery vectors and immunosuppressive protocols. Policymakers should facilitate supportive legislation for clinical trials involving combination therapies.

Author Contributions

Conceptualization, B.H.J., Z.A.A., S.H.I., A.M.H., S.S., and N.K.N.; validation, S.H.I. and A.M.H.; formal analysis, B.H.J., Z.A.A., S.S., and N.K.N.; data curation, B.H.J., Z.A.A., S.S., and N.K.N.; writing—original draft preparation, B.H.J. and Z.A.A.; writing—review and editing, S.H.I. and A.M.H. All authors have read and agreed to the published version of the manuscript.

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The article is comprehensive in its consideration of ethical concepts. The ethics committee gave the study the all-clear.

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Conflicts of Interest

The authors declare no conflict of interest.

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