Gene Therapy for Duchenne Muscular Dystrophy: A Systematic Literature Review of Emerging Therapeutic Approaches

Yeji Son

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Duchenne Muscular Dystrophy (DMD) is a genetic disorder caused by a lack of dystrophin protein, leading to progressive muscle degeneration. This systematic review evaluates the recent advancements in gene therapy as a transformative approach to treating DMD. Focusing on gene therapies like Adenine base editing-mediated exon skipping, microdystrophin gene addition, and adjunctive therapy like anti-RANKL therapy demonstrate significant potential to be used for improving the quality of life of patients diagnosed with DMD. Adenine base editing achieved up to 96% dystrophin restoration in humanized mouse models, addressing exon deletion or point mutations in the dystrophin gene. This was measured using IHC and Western blot. However, it should be noted that high efficacy in mouse models doesn't always translate to humans. Microdystrophin therapy, conducted via adeno-associated viruses (AAVs), improved muscle function and reduced fibrosis in animal studies. Anti-RANKL therapies were discovered to complement current glucocorticoid treatments by mitigating bone loss and improving muscle health. While these innovations show promise and hope, limitations remain. Challenges include immune responses to viral vectors, incomplete dystrophin restoration, and reduced efficacy in cardiac muscles – a critical aspect of DMD patients. Further research in the future should be conducted on the delivery mechanism of viral vectors to reduce immune responses. Long term safety and effectiveness in human patients are still under investigation due to the recent advancements in gene therapy, which underscores the need for further clinical trials to fully comprehend the long-term side effects of these gene therapies. This review highlights a central shift from symptom management to addressing DMD's genetic root, giving hope for changing treatment outcomes. By overcoming current barriers, gene therapy has the potential to drastically improve the quality of life and the life expectancy for individuals with DMD.

Keywords: Duchenne Muscular Dystrophy, gene therapy, dystrophin, exon skipping, microdystrophin, muscle atrophy, cardiomyopathy.

Introduction

Duchenne and Becker muscular dystrophy (DMD) is a rare disease, which affects roughly 3.6 out of 100,000 people, those diagnosed with it have a life expectancy of only 29.9 years, even with the latest advanced treatments[1]. With no cure, DMD has a 100% fatality rate. The disease is usually inherited as an X-linked recessive trait, which causes a mutation in the gene that makes dystrophin, a crucial part of the dystrophin-glycoprotein complex (DGC)[2]. There are rare cases in where, a mutation occurs randomly, however the progression and the effects of the genetic changes are the same; the mutation ultimately causes the death of muscle cells (necrosis) and the patient[2,3]. The condition mainly affects children assigned male at birth (AMAB), but females who carry one of the DMD genes can have mild symptoms. DMD causes skeletal muscle weakness that aggravates over the years. Symptoms of DMD such as muscle weakness normally appear around 2 4 years of age; however, in some cases, the disease is diagnosed as late as 6 years of age[4].

The current treatments aim to prolong the lives of the patients diagnosed with DMD include corticosteroids, physical therapy/surgery, and ACE inhibitors/beta-blockers. Corticosteroids, such as prednisolone and deflazacort, are the most common kinds of treatment that are beneficial for delaying muscle strength loss and slows the progression of cardiomyopathy (heart weakness)[2,3]. Nevertheless, this treatment has side effects including weight gain, bone fragility, and susceptibility to infections, making it not the best therapy to use for a long period of time[3]. Similarly, angiotensin-converting enzyme inhibitors and beta-blockers, when used together, are known to slow down the progression and worsening of cardiomyopathy and heart failure[1]. Physical therapy can help maintain muscle function properly, while surgeries are done to treat severe scoliosis or contractures caused by DMD. However, these treatments do nothing more than minimize the symptoms of DMD as much as possible, and they are not solutions for the underlying problem that all DMD patients have in common: the mutation in the Dystrophin gene.

Therefore, it is crucial to explore how current advancements in gene therapy for this disease targets the genetic root cause and how effective each one of them is. The review will include adjunctive therapy, such as the anti-RANKL treatment, which targets the receptor activator of nuclear factor kappa-B ligand (RANKL). This approach helps improve muscle function by addressing the underlying causes of dysfunction. This treatment proved to be most effective for DMD mice when used with deflazacort[5]. A gene therapy that is currently in development is the Microdystrophin gene addition, which uses AAV8-MD1 to strengthen muscles; this treatment proved to restore dystrophin expression and improve muscle function in mouse model[6]. Lastly, Eteplirsen (exon-skipping) is a gene therapy that aims to produce a shorter dystrophin protein by alternating the synthesis of dystrophin mRNA; it targets specific mutations within the dystrophin gene and is proven to be effective, even if it is limited to a group of patients with DMD. A limitation of these promising-looking gene therapies is the lack of long-term efficacy data[1]. Since many gene therapy approaches for DMD are in clinical trials or experimental stages and are relatively recent, there is a limitation in the availability of long-term data on the safety, applicability, and efficacy of these gene therapies.

Methods

Peer-reviewed paper was used to conduct this review. These papers were found using PubMed, MEDLINE, and Google Scholar. The main search terms were "Duchenne Muscular Dystrophy", "gene therapy", "exon skipping", and "microdystrophin" when finding case studies. The inclusion criteria for the case studies were the publication timeline (within the last ten years). Both in vivo and in vitro studies were looked at. The in vitro studies were categories based on human or animal models. Most of the papers and case studies focused on the current gene therapy trials with documented clinical outcomes and challenges. The information extracted ranged from study designs, therapeutic outcomes, and challenges in gene therapy application in the real world as well as trends in therapeutic outcomes of the different types of gene therapy, their success rates, and their biological challenges.

ABE mediated exon skipping

Exon skipping therapy restores the dystrophin by selectively excluding specific exons during mRNA splicing, allowing the production of a shorter yet functional dystrophin protein. Eteplirsen and viltolarsen are two FDA-approved exon-skipping agents, target exon 51 and exon 53 respectively which is applicable to specific subsets of DMD patients. A study has introduced a genetically humanized mouse model of DMD to evaluate the therapeutic potential of adenine base editing for restoring dystrophin expression and muscle function. The researchers established the humanized mouse model with DMD by replacing mouse exons 50 and 51 with the human DMD exon 50. The model phenotypically mirrors DMD patients, such as having dystrophin deficiency and muscle dysfunction. Adenine Base Editor (ABE) was used to target splicing areas around the human exon 50 to induce exon skipping. This successfully restores the dystrophin expression in the muscles of the heart and diaphragm. However, this method doesn't create double strand breaks that are common in other CRISPR methods, which is another advantage of this technique[7].

The systemic administration of ABE through adeno-associated virus (AAV) in the male mice led to significant improvements in muscle function, with similar level as to the control (almost at the same level of mouse without DMD. This bar chart shows the immense difference of dystrophin synthesis between the untreated DMD mice, and the mice treated with either ABE1 or ABE2, with ABE2 having a more significant effect

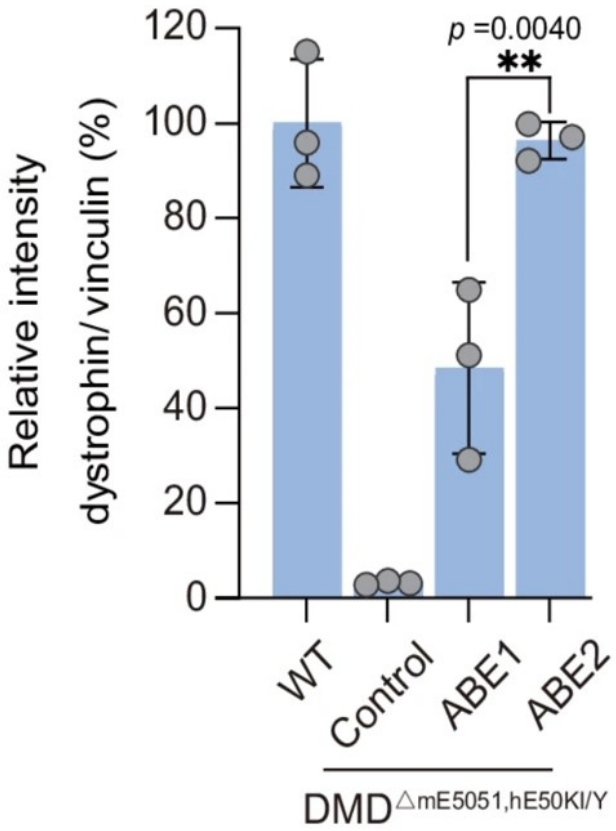

Fig. 1 Shows the different relative intensities of dystrophin, or the abundance of dystrophin, based on the mice groups[7].

The bar graph above represents the different relative inten-

sities of dystrophin, or the abundance of dystrophin, based on the differently treated mice groups. Both ABE treated groups showed a significant increase in dystrophin presence compared to the untreated control group, and the ABE2 treated mice had very similar dystrophin intensity as wild type mice.

This underscores the potential of ABE in treating DMD caused by exon deletion or point mutations. To be more specific, the exon-skipping strategy restored at most 96% of dystrophin levels in treated muscles of the mouse model. This was measured using IHC and Western blot. However, it should be noted that high efficacy in mouse models doesn't always translate to humans. In conclusion, making the adenine base editing-mediated exon skipping promising for future therapeutic applications[7].

Although dystrophin was restored, the levels did not reach those of wild type, meaning that the treatment could not completely cure DMD. Potential immune response to AAV vectors and the ABE machinery were also a concern, although no severe immune responses were reported in this study. While muscle targeting was successful, restoration of the cardiac muscle lagged skeletal muscle, raising concerns about the efficiency, especially in terms of DMD-related cardiomyopathy.

Additionally, though this method is promising in humanized mouse models, translating this technique to human patient's present challenges, such as vector delivery efficiency, immune rejection, and the safety concern for long term usage of ABE in humans.10

Microdystrophin Gene

Microdystrophin gene addition therapy uses adeno-associated viruses (AVVs) to deliver a shortened, yet functional, version of the dystrophin gene into the muscle cells. This helps restore partial dystrophin expression, improving muscle stability and reducing damage caused by contractions. The 2023 study by Cernisova et al. demonstrated significant improvements in the diaphragm of juvenile DMD diagnosed mice, such as increased muscle mass and decreased fibrosis. However, challenges remain, such as the immune response to viral vectors and the limited size of genes that can be carried by AAVs. Additionally, long-term efficacy and safety in human patients still need further research, as current treatments are very experimental and may not completely predict disease progression[6].

Anti-RANKL Therapy

Anti-RANKL therapy, although not a gene therapy, has been explored for its potential to counteract bone loss in DMD patients treated with corticosteroids. By inhibiting RANKL, the therapy prevents bone resorption and reduces bone loss. This treatment has shown promise in improving dystrophic skeletal muscle function in Duchenne Muscular Dystrophy. Boys with DMD typically require glucocorticoids (ex. deflazacort) to reduce muscle inflammation, but an adverse side effect of these drugs is bone loss[5].

Anti-RANKL therapy could be an alternative or simultaneous treatment to prevent bone damage caused by glucocorticoids. A study was conducted on MDX mice, a common animal model used for studying DMD. Mice were treated with anti-RANKL, Deflazacort (DFZ), or both for 8 weeks. Based on the graph below, the grip force of both anti-RANKL and DFZ treated mice groups improved, especially when both treatments were combined. Compared to the mdx-lgG, the treatment group that was given DFZ only and another group of mice that received DFZ and Anti-RANKL therapy had significantly closer grip force to the wild type mice[7].

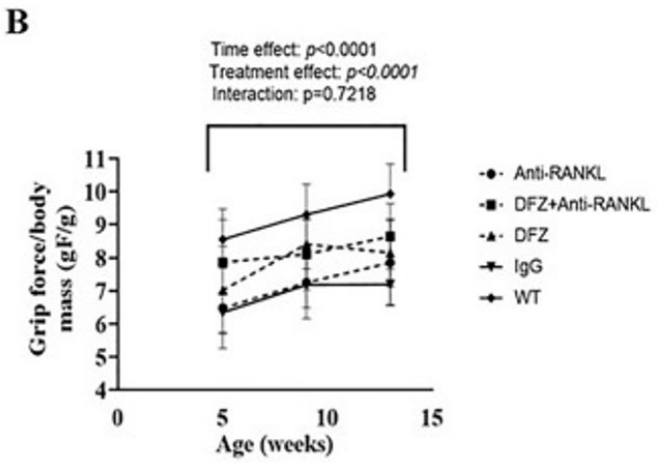

Fig. 2 Shows grip force of mice groups based on treatments.

The line graph above showcases the grip force of mice groups based on their treatments. The group that received both Anti-RANKL therapy and DFZ treatment showed the highest grip force after 15 weeks of observation. Anti-RANKL therapy improved ex vivo muscle contractility, which is an important indicator of muscle health. Anti-RANKL also significantly reduced muscle damage, fibrosis, and the number of inflammatory cells, indicating a protective effect on muscle tissues.

The bar chart above is a representation of the percentage of muscle damage in different treatment and control groups. All three treatment groups (Anti-RANKL, DFZ, and both) showed a significant reduction in muscle damage compared to the mdx-lgG. The combination of Anti-RANKL and DFZ possibly reduces muscle damage more significantly than when used separately, as it is closer to the muscle damage of wild type mice[5].

As for the bones, the mouse group treated with Anti-RANKL showed improved trabecular bone structure, both in the presence and absence of DFZ. This suggests that the therapy effectively

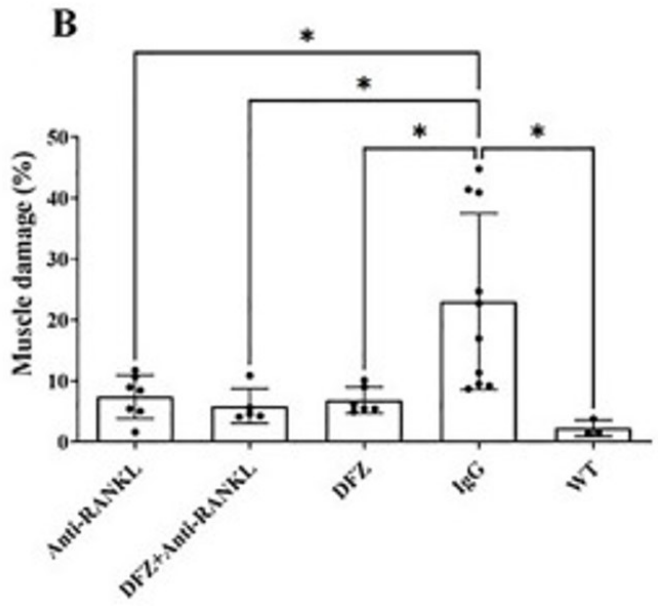

Fig. 3 Shows the percentage of muscle damage in different treatment and control groups

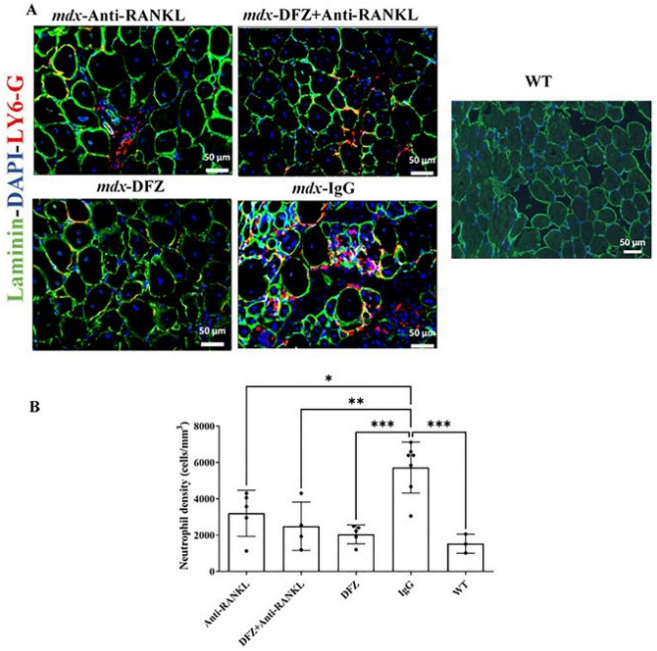

Fig. 4 Shows the effect of Anti-RANKL treatment and DFZ

mitigates the bone loss caused by glucocorticoids[5].

The diagram below shows the effect of Anti-RANKL treatment and DFZ. The bar graph indicates that both treatments significantly reduced neutrophil cell infiltration in the mice when compared with the neutrophil density of mdx-lgC, since all three treatment groups showed a significant decrease in neutrophils, like the level in wild type mice. However, it is visible that there were no additive benefits when the two treatments were combined, since DFZ alone reduced the neutrophil density the most out of all treatment groups. This suggests that the two therapies do not seem to work better together[5].

There is uncertainty about the effects of stopping anti-RANKL treatment, especially in the context of glucocorticoids-induced bone loss. More research is needed to determine if the benefits of anti-RANKL therapy are long-lasting even after discontinued usage. There might be follow-up therapy such as drugs with zoledronate needed to maintain bone health after stopping Anti-RANKL treatment, which may add complications to patient treatment. Also, while results in mdx mice have a promising outlook, there are still many unknown facts about how this therapy will conduct in human patients[5].

Limitations

There are some major biological challenges in implementing these three gene therapies in diagnosed human patients. One example is the variability in genetic mutations for the disease. Since different mutations in the dystrophin gene require personalized approaches, treatments such as exon skipping are specific to certain mutations, restricting their use to certain groups of patients[3]. This limits the broader application of these therapies, presenting a challenge to develop a treatment that can address the needs of the entire population of DMD patients.

Furthermore, the use of viral vectors, such as adeno-associated viruses (AAVs), can lead to immune responses in patients, limiting the ability to redose and shortening the effectiveness of the treatment[8]. This happens when pre-existing neutralizing antibodies can inhibit vector transduction, while adaptive immune responses against the transgene product may limit long-term expression. Strategies such as transient immunosuppression, capsid engineering, and the development of non-viral delivery systems are under active investigation to mitigate these challenges[9]. Even worse, some patients may develop antibodies that are against some viral vectors, which can prevent further usage and diminish the effectiveness of these gene therapies[8].

Finally, there is heterogeneity in patient outcomes. Patient-specific factors—including mutation type, age at treatment initiation, disease stage, and pre-existing immunity—contribute to variability in therapeutic outcomes. While younger patients with less advanced disease may benefit most from early intervention, the effectiveness of gene therapy in older or more advanced-stage patients remains uncertain.

Discussion

This review highlights the promise of gene therapy as a potentially groundbreaking approach in the treatment of Duchenne Muscular Dystrophy (DMD). The findings demonstrate the capacity of gene therapies, particularly exon skipping, microdystrophin gene addition, and anti-RANKL therapy, to address the root cause of DMD: the dystrophin gene mutation. (Table 1)

These therapies represent a significant departure from the current symptomatic treatments, offering a chance to directly modify the disease progression instead of just elongating the lifetime of patients. By contributing to the understanding of gene therapy's role in managing DMD, this review fills a critical gap and underscores the potential shift from managing symptoms to addressing the genetic root of the disease.

The objectives of this review were to evaluate the current advancements in gene therapy and identify biological challenges that could affect their efficacy. These objectives were largely met, as the finding outlines the success of therapies like exon skipping and microdystrophin gene addition in animal models and some human trials. However, certain challenges, such as immune responses to viral vectors and incomplete dystrophin restoration, indicate that while progress has been made, these therapies are not yet a definitive cure for DMD. The immune responses suggest that the gene therapy has side effects that will likely diminish the patient's health and, therefore, also lower the quality of life. Unexpected outcomes such as the lag in cardiac muscle restoration, proves the need for continued refinement in these therapies, particularly for addressing cardiomyopathy, a critical concern in DMD patients.

Several factors contribute to this tissue-specific difference such as:

- Vascularization and Vector Delivery: Cardiac muscle's dense extracellular matrix and unique vascularization can make it hard to efficiently deliver viral vectors, such as adeno-associated viruses (AAVs), compared to skeletal muscle.
- Promoter Activity: The promoters used in gene therapy constructs may have varying activity levels in different tissues, potentially leading to reduced transgene expression in cardiac cells.
- Immune Environment: The heart's immune milieu may differ from that of skeletal muscle, influencing the uptake and expression of therapeutic genes[9–11].

These challenges are important since they demonstrate that further advancements and research is needed in these imperfect gene therapies to improve the quality of DMD patients' lives without possible harm.

Future research should focus on overcoming the current limitations of gene therapy for DMD, specifically in enhancing the delivery efficiency of viral vectors and reducing immune responses. Investigating alternative delivery mechanisms could help broaden the application of the treatments. Additionally, further research is needed to determine the long-term safety and efficacy of these therapies in human patients, especially as current studies primarily involve animal models or early-phase clinical trials, exploring more precise gene-editing techniques, like adenine base editors, might also provide more definite solutions, reducing the risks of off-target effects. Based on the data collected about the effects.

These authors acknowledge several limitations, the lack of long-term data on the efficacy and safety of emerging gene therapies remains a significant barrier. Most of the current evidence is drawn from animal studies or limited human trials, which may not fully represent how these therapies will work in a broader patient population. Furthermore, the immune responses to viral vectors and the limited impact on cardiac muscle function are ongoing concerns that must be fixed before these gene therapies can become widely used for DMD.

While gene therapy for DMD is still in its developmental stages, its potential to fundamentally alter the disease's trajectory gives us hope for a future where DMD may no longer be a terminal disease. Continued advancements in gene therapy and a deeper understanding of how to overcome current challenges could eventually transform DMD treatment, providing patients with a significantly improved quality of life and maybe even an extended life expectancy.

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