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From Code to Cure : The Role of RNA- Therapeutics in Duchenne Muscular Dystrophy (DMD)

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Abstract

Duchenne muscular dystrophy (DMD)is a disorder caused by mutations in dystrophin coding gene located on the X chromosome in the human genome. Dystrophin protein is an important protein as it binds actin protein to the sarcolemma of muscle cells. Dystrophin is made up of 4 functional domains: a N terminal domain , a central rod shaped domain, cystine rich domain and a C terminal domain. In a DMD patient various pathological conditions are noticeable due to lack of functional dystrophin protein . Some of them are : membrane instability, calcium dysregulation , inflammation etc. In turns from a curative perspective RNA therapeutics plays a major role . Current treatments mainly include corticosteroids, which can control the symptoms without focusing on the real origin of the disease, which creates a large unmet medical need toward more effective interventions. Under RNA therapeutics various mechanisms are used to correct or alter the non-functional/ faulty dystrophin gene . Some are : Antisense oligonucleotide , Small interference RNA (siRNA), messenger RNA(mRNA) based etc. Clinical trials proceeding the therapy is formulated is a crucial step for ensuring safety and efficacy of the drug produced. This includes various phases of clinical trials . It is divided in various phases from 1 to 4 . Each ensures safety of patients and subjects in its own specific way depending on how phase trials are being conducted. Limitations regarding RNA therapeutics include scalability and manufacturing, immunogenicity, off target reports, stability and delivery, regulatory and testing requirements etc. Future directions regarding this RNA based approach include advances in exon skipping, gene therapy approaches , combination therapies , personalized medicine , regulatory approaches and innovation in the field etc.

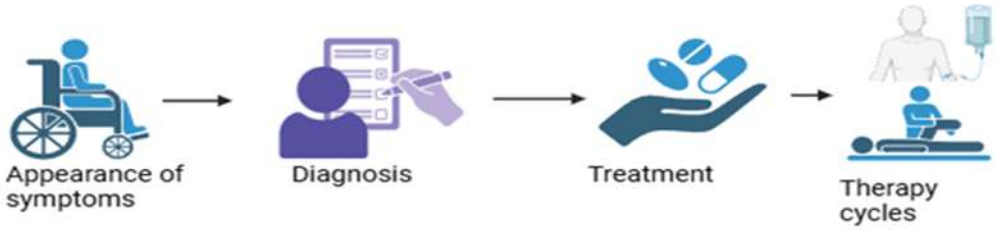
Keywords: DMD, Dystrophin, ASO, siRNA, mRNA, immunogenicity, exon skipping, clinical trial, RNA therapeutics

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, which is acute and caused by mutations in the DMD gene(Duan et al., 2021). The DMD gene encodes for dystrophin protein, which is an essential protein for maintaining sarcolemmal integrity in muscle cells.The DMD gene is the largest gene (2.4 Mb) consisting of 79 exons in the human genome. This promotes high mutation rate along with de novo mutation. It occurs in about one-third of cases and large mutations. It consists primarily of deletions

or duplications of exons. It accounts for approximately 60-70% of mutations observed in DMD patients(Liang et al., 2022),(Raghavan et al., 2025).This lack of functional dystrophin causes the degeneration of muscles fibers, its consecutive necrosis, and progressive substitution of muscle tissue with fat and fibrotic tissue, which substantially restrict the function of muscles over time(Raghavan et al., 2025). In late infancy, patients primarily exhibits symptoms like delayed muscle milestone, gait abnormalities, difficulties with gross motor tasks and muscle weakness(Mercuri et al., 2023). The main types of therapeutic measures nowadays are pharmacotherapy, and corticosteroids are the most popular administration approach in the treatment of symptoms of the DMD. The treatments are aimed to reduce inflammation and degeneration of the muscles but fail to intervene with the cause of the disease, which is absence of dystrophin[5](Raghavan et al., 2025). The corticosteroids, including prednisone, are common to use, but still they are associated with significant adverse effects and low efficacy. In recent years, another dissociative corticosteroid, namely vamorolone, was proposed, with an anticipated goal of decreasing these adverse events without compromising the therapeutic activity(RNA Therapeutics, n.d.). Among corticosteroids, other RNA therapeutics have proved to be potential approaches to DMD treatment. One of these, antisense oligonucleotide (ASO) therapies, has been noted to be able to induce the mechanism of exon skipping (which effectively results in the restoration of the open reading frame (ORF) of the dystrophin gene), and with it the possibility of producing a truncated, albeit partially functional dystrophin protein(Sun et al., 2020),(Raghavan et al., 2025). Additional RNA based strategies, including splice-switching and nonsense mutation rescue with pharmacological molecules are also being developed(Mercuri et al., 2023). These new approaches attempt to transform the extreme DMD phenotype to that of milder Becker muscular dystrophy (BMD) phenotype, changing the way patients live and fare(Saifullah et al., 2022). In spite of the therapeutic innovations, the situation with DMD is not very favourable now, and no cure yet exists, and the available remedies are minimally efficient(Raghavan et al., 2025). The on-going research concerning RNA therapeutic and other emerging gene-targeting approaches remains an important agenda in the treatment of medical needs of patients with DMD.

Fig1: Rough diagrammatic representation of curative approach towards DMD



Structure And Function Of Dystrophin Protein

Dystrophin , 427kDa of mass localizes to the cytoplasmic face of the sarcolemma and is enriched at costameres in muscle fibers. A costamere is a structural and functional unit of a striated muscle. Dystrophin is an important cytoskeletal protein which is used to bind the actin filament of muscles to the sarcolemma .

The dystrophin protein is made up of 4 domains:

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1) N-terminal region which is the actin binding domain that holds the protein to the cytoskeleton;

2) Central rod like shaped domain that has 24 spectrin-like repeats and is separated by a four hinge like functioning regions;

3) Cysteine-rich domain that holds onto dystrophin to the muscle membrane via binding with a transmembrane protein;

4) C-terminal domain that binds the dystobrevin and sybtrophins which mediates sarcolemma localization .

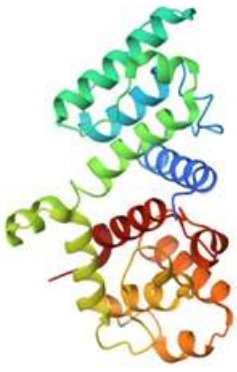
A connection is formed through dystrophin and a glycoprotein complex collectively termed as DGC. It has extracellular , transmembrane and cytoplasmic proteins providing a link between the intracellular cytoskeleton and the ECM (Angulski et al., 2023).

Fig 2 : Different motifs of Dystrophin protein. (a) N-terminal Actin-binding Domain of Human Dystrophin[https://doi.org/10.2210/pdb1DXX/pdb]. (b) Structure Of A Dystrophin Ww Domain Fragment In Complex With A Beta-Dystroglycan Peptide[https://doi.org/10.2210/pdb1EG3/pdb]. (c) Crystal Structure of N-terminal first spectrin repeat of dystrophin[https://doi.org/10.2210/pdb3UUN/pdb]. (d) Human Dystrophin tandem calponin homology actin-binding domain crystallized in a closed-state conformation[https://doi.org/10.2210/pdb9D58/pdb

(a)

(b)

(c)



(d)

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Pathological Mechanisms Of DMD

This part consists of brief account of physiological and biochemical anomalies which will take place in muscle cells of a DMD patient. It will also consists of pathways and responses which a normal cell devoid of any disorders will undergo.

Membrane Instability

In Straited muscle in which dystrophin protein holds the actin filament of muscle . Force is generated through cycles of contraction and relaxation or depolarisation and repolarisation in muscular cells as they receive the impulses from neurons which operate on a motor to sensory channel pathway . Not necessarily reflex but thats just a general pathway an impulse goes through. In healthy muscle, as the dystrophin protein is present to maintain the sarcolemmal structure integrity the contractions occur without any difficulties by connecting it to sarcolemma. In unhealthy muscle or DMD patient's the muscle , abnormal calcium efflux causes muscle injury in DMD patients in the form of sarcolemmal tears (so-called "delta" lesions)(Angulski et al., 2023). Within the lesion, there were cytoplasmic abnormalities, and in the neighboring fiber region, the myofibrils were usually highly contracted(Mokri & Engel, 1975).

Calcium Dysregulation

In DMD muscle cells , an increase of calcium leak resulted in leading to calcium leak from Sarcoplasmic Reticulum which reduced Sarcoplasmic reticulum ATPase function. This leads to an increase in cytosolic calcium levels. Loss of dystophin protein causes destabilization or damage in dystrophin associated glycoprotien complex (DAGC). It leads to membrane tear and activation of store operated calcium channels (SOCCs) . This results in abnormal Calcium ion entry(Mareedu et al., 2021). Due to the absence of dystrophin protein micro lesions in sarcolemma which leads to more influx of calcium ions through them. The increased calcium ion concentration leads to activation of calcium dependant proteases aka calpains. This protease degrade the sarcolemma and hence cause muscle degeneration. Calcium-dependent proteolysis is also involved in the control of cell cycle. In muscle tissue, in particular, calpains intervene in the regeneration process(Dargelos et al., 2007). Experiments and works have shown calcium ion's concentration negatively impacting the calcium cycling and muscle repair. The dysregulation of calcium handling protein which operate in response to calcium concentration showed pathogenesis of myonecrosis(Pertille et al., 2009).

Inflammation

Skeletal muscle tissue consist of heterogeneous combination of endothelial , stem cell, immune cells including macrophages , natural killer (NK) cells, T and B cells and neutrophils. Immune cells in skeletal muscle play a crucial role by controlling repair, homeostasis and regeneration. Due to absence of MCH expression cells induce less antigenic response. MCH stands for Major histocompatibility complex. This in turn cause less tissue necrosis and leads to lower capability to make abscesses. The regenerative capability of skeletal muscular cells rely majorly on the presence of a population of mononucleated , myogenic cells called satellite cells(Tidball & Villalta, 2010). They retain their ability to proliferate and then differentiate into either fusing with existing fibers or with other myogenic cells to generate new fibers. In healthy muscle cells , membrane damage caused by intense exercise causes leakage of cytoplasmic content into extracellular area. This damage is associated with muscle protein like fatty acid binding protein and muscle specific creatine kinase which are used as biomarkers for such muscle damage as creatine kinase is used to breakdown creatine phosphate which act as a phosphate donor to ATP in times of excess energy utilization. In DMD muscle cells , due to loss or absence of dystrophin protien , damaged myofibers release DAMPs (danger associated molecular patterns). DAMPs are molecules that are released from a damaged or dying cell to activate the innate immune system by interacting with PRRs pattern recognition receptors(Roh & Sohn, 2018). The increase of oxidative stress and defective calcium handling triggers the activation of innate immune cells as neutrophils and macrophages etc enter the skeletal muscle cells. The increased concentration of calcium in cytoplasm causes overactivation of calpain which induces NF -kB dependent pro inflammatory pathway. This pro-inflammatory pathway causes cytokines to induce the expression of MHC 1 and 2 which causes the immune privilege to be lost.

Role Of RNA Theraputics In DMD

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RNA therapy approach in Duchenne muscular dystrophy (DMD) is a prospective one. Frontier on the management of this X linked recessive disease which is a severe condition as a result of loss of dystrophin, which is a key protein of muscle mechanical stability. The DMD is brought on by DMD gene mutations as the loss of muscle mass over time, and serious morbidity, which demands new approaches to treatment. Current treatments mainly include corticosteroids, which can control the symptoms without focusing on the real origin of the disease, which creates a large unmet medical need toward more effective interventions(Liang et al., 2022),(Raghavan et al., 2025). New developments within the field of RNA-based therapies, and especially antisense oligonucleotides. The mechanism of action of nucleotides (ASOs), has received attention due to its ability to alter the splicing of the DMD (for Dystrophy Muscle dystrophy) gene, which allows producing a shortened, yet functional dystrophin protein. The purpose of these treatments is to turn the severe DMD phenotype into the less severe one, the phenotype of Becker muscular dystrophy (BMD), thus enhancing the quality of patient. functional outcomes and life(Sun et al., 2020),(Saifullah et al., 2022). Small interfering RNA methods were the other RNA strategies. Small interfering RNA (siRNA) and messenger RNA (mRNA) technologies, captured under investigation are also being evaluated, Configured and moreover, each having distinct mechanisms that can modify the encoding of genes, thus to reduce the consequences of deficiency of dystrophin(Sun et al., 2020),(Haque & Yokota, 2025). Immunogenicity in contrast with RNA therapeutics hold promise even though other issues surround them including translation efficiency and delivery efficiency continue to be major challenges to their shortage and spread application. There is also regulatory environment of these innovative therapies is complicated, and a lot of clinical trials are necessary to certify its safety and efficacy. The landscape of RNA therapeutics is optimized with constant research work, tried to deal with these problems as well as also aimed to develop safer and more effective therapies of DMD patients[16](Damase et al., 2021b). Entirely, the research to use RNA therapeutics in DMD is demonstrative of essential changes in the direction of specific gene therapies of rare genetic illnesses. Due to its expansion in the field of radiation therapy, personalised medicine approaches, mainframe medicine, combination therapies, which combine new treatments, low-cost sequencing, high resolution display screens and over-riding counseling interventions, are synergized RNA-based modalities with current-therapeutics, are expected to improve therapeutics and offer prospects of better control of DMD(Braun, 2025b).

Mechanisms Of RNA Theraputics

RNA therapeutics adopted a number of approaches to regulate gene expression, and treat heritable diseases especially in cases of Duchenne Muscular Dystrophy (DMD).

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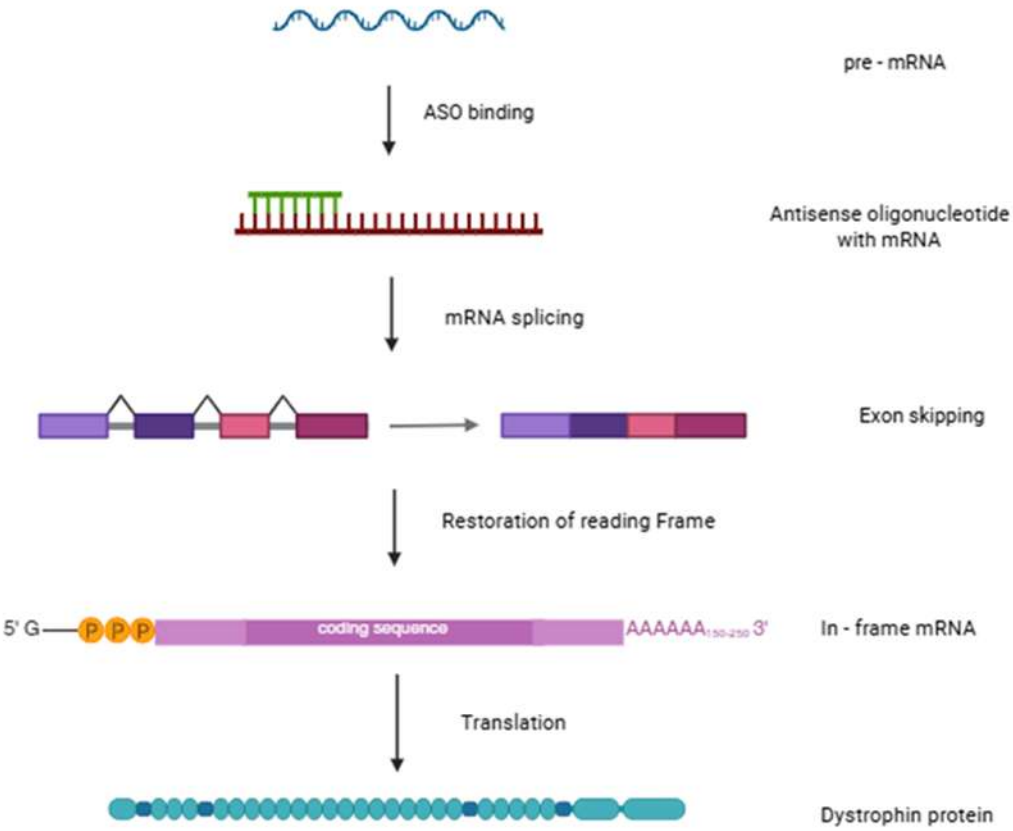
Fig 3 : Mechanism of RNA Therapeutic to regulate gene expression.



Its main strategies involve antisense oligonucleotides use (ASOs), small interfering RNA (siRNA) and messenger RNA (mRNA) technologies(Sun et al., 2020).

Antisense Oligonucleotides (ASOs)

ASOs are small, altered RNA components that are developed to specifically bind to an mRNA, thus altering pre-mRNA splicing and blocking translation of harmful proteins. These oligonucleotides with an average length of 15-20 nucleotides which are complementary to the target mRNA can be used to initiate exon skipping, whereby particular exons can be omitted in mature mRNA transcript.

The mechanism of ASOs can be of two types :

1) Cleaving the RNA

These are further classified into two sub parts each. Under RNA cleavage it can either stop translation by RNase H cleavage or RNA interference. They promote translation blocking by either steric blocking of translation or by destruction of bound mRNA via RNase H enzyme. Similarly RNA blockage can be achieved by either steric hindrance or splice modulation.

a) RNase H1 mediated degradation

ASOs target RNA , forming hetroduplexes . These act as substrates for RNase enzyme present in cytoplasm. Such hetroduplexes formed by ASO binding also aid in RNase H1 activity and increase its binding affinity. These RNases degrade then RNA. Most of the drugs that have been FDA approved have antisense effect via RNases(Dhuri et al., 2020).

b) RNA interference (RNAi)

Exogenous small interfering RNAs (also called siRNAs) are some of the double stranded nucleotide RNA sequences with a 2-nucleotide overhang on the 3' end of any of the two stranded molecule. This sequence interacts with Argonaute 2 (also called Ago 2) enzyme to form the RNA induced silencing complex (also called RISC) where the passenger strand is degraded and released . This step is crucial as the releasing of passenger strand allows guide strand

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to guide the strand towards RISC .This then guides siRNA to mRNA. Then it binds to complementary mRNA region. Here Ago 2 enzyme cleaves the mRNA. Thereby exerting the gene silencing effect(Dhuri et al., 2020).

2) Blocking the RNA

a) Translation stopping due to steric hindrance

There exists a class of Anti-sense oligonucleotides (ASOs) that binds to the targeted RNA sequences and cause translational stoppage by inhibiting its interaction with the 40s ribosomal subunit or prevents their ordered queuing on 40s or 60s subunit of 80s ribosome. Important thing to notice here is that such steric hindrance based ASOs do not activate RNase H1 mediated cleavage. Due to this the pre-mRNA structure remains intact. The affinity of such ASOs determines the steric hindrance. The more the binding affinity of the ASO with target RNA results in high probability of translational arrest .

Furthermore some synthetic oligonucleotides are designed to bind to the miRNA (micro RNA) which is a small class of ncRNA (non coding RNA) . miRNA is believed to modulate upto 60% of protein coding genes in the human genome. These oligo nucleotides bind with miRNA and prevent their interaction with mRNA by steric hindrance mechanism and hence control the gene expression(Catalanotto et al., 2016).

b) Splice modulation or splice based switching mechanism

There are two types of splice modulation :

Exon skipping and , exon inclusions.

In exon skipping , ASO binds with transcripts of pre-mRNA (Havens et al., 2013)(Matsuo, 2025).They correct the disrupted reading frame and produce a short, functional protein. Whereas in exon inclusion, ASO binds with sites of pre-mRNA and prevents the spliceosome and splicing factors from interacting with the splicing site(Bauman et al., 2009).

This is most pertinent to DMD, where mutations tend to take place within the pathway of dystrophin gene reading frame(*Clinical Trials*, n.d.)(*ClinicalTrials.gov*, n.d.). Currently FDA-approved ASOs for DMD are designed to target precise exons in order to reinstate the synthesis of an efficient dystrophin protein, although not very effective in producing full-length protein(*Clinical Trials*, n.d.).

Small Interfering RNA (siRNA)

siRNA devices are important constituents of the RNA interference (RNAi) pathway, which hits the RNAs and breaks down as mRNA, and blocking the expression of undesirable genes. Originally introduced in 1990, siRNAs are under investigation as a possible treatment in a wide variety of systemic diseases through their capability to bring out modulation of gene expression post-transcriptionally(Haque & Yokota, 2025). siRNAs have a special potential to attack the diseases related to this overexpression of some genes, offering a line of therapy for DMD-associated conditions.

a) Target gene identification

Scientists compare expressions of genes in DMD related patients in order to identify all genes that accelerate the progression of the disease, not related to immune functions. They apply bioinformatics tools (e.g. GO and KEGG pathway analysis and protein-protein interaction networks) to identifying hub genes such as COL1A2, FBN1 and FN1, which are elevated in patient DMD muscle tissue(Li et al., 2024).

b) siRNA design

After the confirmation of the hub genes in animal and cell models (e.g. mice with Down syndrome syndrome, known as mdx), researchers incorporate siRNAs that hit the messenger RNA (mRNA) of these genes. The siRNAs can silences the target gene sequences and each siRNA is complementary to a sequence of the mRNA within the gene(Li et al., 2024).

c) siRNA Delivery

Various researchers rely on delivery agents to introduce siRNAs into muscle cells, whether it is lipid nanoparticles of chemical modification to facilitate the delivery of siRNA into the cells. The optimization of delivery systems is necessary, since effective siRNA therapies demand local delivery (tissue (muscle) targeting or protection against biological degradation)(Li et al., 2024).

d) Cell uptake and RICS factor

When the siRNA enters the cell, it is taken in as part of a type of complex in which it is incorporated and

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act as an RNA-induced silencing complex (RISC). The RISC idea relies on the siRNA acting as a guide to seek, and bind to the corresponding mRNA transcript that the disease-promoting gene produces(Li et al., 2024).

e) mRNA cleavage

Targeting the siRNA-loaded RISC complex to its target mRNA results into the selective cleavage and degradation of the target mRNA. This inhibits transcription into the disease-proving protein and in effect silences the gene(Li et al., 2024).

f) Therapeutic effect

Interfering with the circulating amount of proteins like COL1A2, FBN1, FN1, the siRNA therapy can decrease the extent of muscle fibrosis and other pathological outcomes of DMD, which is a beneficial compilatory approach to improve the outcome of a disease, irrespective of the specifics of dystrophin mutations(Li et al., 2024).

This enzymatic cascade demonstrates the rational approach and design of siRNA therapeutics to silence major disease-linked genes in Duchenne muscular dystrophy, which operate subsequent to the disease-causing mutations.

Messenger RNA (mRNA)

mRNA therapy is going to be a revolutionary novelty, with the use of artificial mRNA to program cells to synthesize definite proteins. Contrary to DNA-based therapies, mRNA requires no entry into the nucleus and once it is received can be translated instantly it is a better alternative source of transient protein expression in the cytoplasm. The capacity of mRNA to avoid genomic insertion decreases the danger of insertional mutagenesis because it is safer to use in such an application like cancer vaccine and prevention of infectious diseases(*Clinical Trials*, n.d.),(Haque & Yokota, 2025). In the existence of DMD, mRNA might be designed so that it encodes dystrophin or some other proteins that enhance muscle health and healing.

a) Therapeutic mRNA design

To aid synthesize synthetic mRNAs that encode a full-length dystrophin or functional fragment using proprietary 5’ and 3’ ends UTR domains to enhance stability and protein expression(*LB: Development of mRNA Therapeutic Delivering Full-length Dystrophin for Duchenne Muscular Dystrophy - MDA Clinical &Amp; Scientific Conference 2026*, 2024).

b) Formulation and delivery

These mRNAs are packaged in delivery vehicles including lipid nanoparticles (LNPs), which ensure that the mRNA is not degraded and allow it to target entry into muscle tissues upon injection locally or into the bloodstream(Sasaki et al., 2025).

c) Cellular uptake

The nanoparticles get into the cells of the muscles through endocytosis. After the release into the cell, the mRNA is translated to ribosomes(*LB: Development of mRNA Therapeutic Delivering Full-length Dystrophin for Duchenne Muscular Dystrophy - MDA Clinical &Amp; Scientific Conference 2026*, 2024).

d) Translation of dystrophin protein

Therapeutic mRNA is read by cellular ribosomes and translated into dystrophin protein (or its analog), lacking or defective in the DMD patients. This reestablishes the necessary structural and signaling of dystrophin in muscle fibers(Drug Target Review, 2024).

e) Restoration of muscle function

Recently orchestrated dystrophin inserted into muscle cell membranes enhances muscle stability, degradation is lowered and increases strength and endurance in the preclinical and early human trials(Drug Target Review, 2024).

So, mRNA based therapies aims to restore the production and function of dystrophin, either by the synthesis of protein or by the correction of mRNA in DMD.

Clinical Trials

Clinical trials are also important in development of RNA therapeutics in Duchenne Muscular Dystrophy (DMD), which is the main tool in reviewing the safety, efficacy, and pharmacokinetics of experimental medicines. These trials are planned and transformed into the particular stages that aimed to gather distinct data about the studied treatment. The stages normally involve initial researches that center on safety and then there are efficacy and best dose trials(*What Is the Mechanism of Action of HG-302?*, n.d.).

Structure Of Clinical Trials

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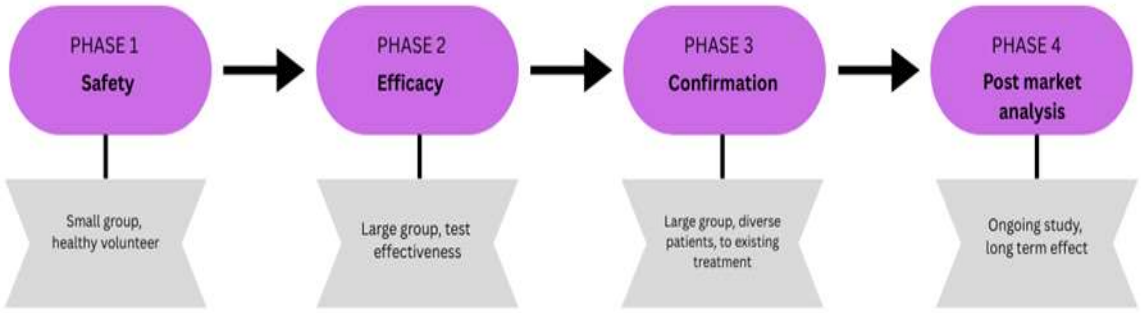
Each clinical trial has a comprehensive protocol, which explains the research, procedures and inclusion criteria. These standards can consist of such things including age, sex and how bad the disease is. Interested persons are advised to visit their healthcare professionals to whether a certain trial is suitable to them or not. After an application has been made, trial personnel carries out a screening process to pick potential participants, since not everyone of the applicants might become eligible(*What Is the Mechanism of Action of HG-302?*, n.d.).

Phases Of Clinical Trials

Generally, clinical trial process is split into four phases:

Fig 4 : Phases of Clinical Trial.



Phase I: This is a phase that mainly evaluates the safety of a drug and, in most cases, it is performed using a small number of healthy volunteers. This is aimed at establishing the safety profile and any side effects that the treatment may have.

Phase II: The second phase has a greater number of patients, and it aims at evaluating the effectiveness of the therapy, as well as, ongoing safety testing.

Phase III: In this phase, larger population is used to test the treatment to determine its performance, observe its side effects and compare it with standard or equivalent treatments.

Phase IV: Post marketing studies that are conducted after the treatment is approved, exploring long-term efficacy and quality-of-life effect.

In clinical trials associated with rare disease, such as DMD, the size of the sample is often smaller, and it is critical to collect relevant information in the most efficient way(*What Is the Mechanism of Action of HG-302?*, n.d.).

Endpoints and Evaluation

Every clinical trial comes up with specific endpoints-criteria that can be used to measure the efficiency of the experimental drug. Some of the common endpoints are objective health outcomes, including biomarker changes of the disease or functional skills. After a trial is done, the data received is examined, results are reported to regulatory bodies to guide the decision making on the treatment, approval and subsequent development(*What Is the Mechanism of Action of HG-302?*, n.d.).

Importance of Volunteer Participation

The volunteers are an essential part of clinical trial, particularly in the in the context of rare diseases, in which the patient population is small. Their involvement gives necessary scientific data to determine whether an investigational drug is safe and effective(*What Is the Mechanism of Action of HG-302?*, n.d.),(Forman & Forman, 2025). Monitoring organizations, including Data Monitoring Commit Data Safety and Monitoring Boards (DSMB) or Data Safety and Monitoring Committees (DMC) are responsible to monitor trials and assure safety of the participants and may advise to cancel a trial in case of

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a need(*What Is the Mechanism of Action of HG-302?*, n.d.).

Challenges And Limitations

RNA therapeutics, especially in the Duchenne Message Muscular Dystrophy (DMD) encounter a lot of problems that may affect their successful implementation and development.

Limitations	Description
Scalability and Manufacturing	Scalability is one of the main chellenges in implementation of RNA therapies. Production of AVV(adeno-associated virus vector), which are generally use for gene delivery at a scale that can support widespread clinical use with maintained quality and consistency is very difficult. Preparation of vectors is subject to variability that can have a direct impact on immunogenicity and therapeutic efficacy making the production challenging(Saw & Song, 2024b).
Immunogenicity	Immune reaction can result in ad verse events, which reduce the performance of the therapy and present safety hazards. In the case of AAV vectors, a lot of people have neutralizing antibodies (NAbs) with AAV because of prior exposure, which may inhibit the performance of the vector. Moreover, AAV vectors administration may induce novel immune responses both against the vector and against the transgene, which can cause serious undesirable events(Saw & Song, 2024b). To reduce these risks, patient selection and monitoring might be necessary for immunological parameters in the course of treatment(Corey et al., 2021).
Off-Target Effects	Another major limitation that is linked to RNA therapeutics is the off target effects. These accidental contacts may affect the safety and effectiveness of treatment. which requires the perfecting of the means of delivery and the specificity of medicines(Damase et al., 2021b). Variations in methodologies and assumptions that were applied in order to make evaluations safety and efficacy may present different statistical outcomes, which makes interpretation more difficult.
Stability and Delivery	RNA molecules are unstable in nature, so an efficient delivery is required. Transporting RNA therapeutics to the organs that are problematic to treat, including the brain, adds further hazards because of obstacles such as blood-brain barrier (BBB), restrict the diffusion of the large macromolecules. New lipid-based nano particles LNPs are under investigation as a way to increase tissue and therapeutic specificity, also the design of such systems is a complicated endeavor(Damase et al., 2021b).

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Regulatory and Testing Requirements	The long process of preclinical and clinical trials that needs to be done to ascertain the safety and RNA therapeutics efficacy also makes the development process longer and more expensive. Regulatory authorities require in-depth characterization of these products, such asimmunogenicity and off-target evaluations that can extend a drug to market timeline and their practical use(Saw & Song, 2024b).

Future Direcrtions

RNA therapeutics has a great future in Duchenne muscular dystrophy (DMD), motivated by the new developments in manipulation of RNAs and improved understanding of the background of various genetic processes. As the research carries on, a number of important findings can be mentioned.

Advances in Exon Skipping Technologies

Exon skipping where an ASO (antisense oligonucleotides) is used to skip a mutated exon in dystrophin gene regions, where it has been centred, is still a target of therapeutic innovation. Existing research is geared toward increasing the effectiveness and provision of ASOs, which might be possible through the integration of bioactive molecules as carriers to enhance cellular uptake and exon skipping efficacy(Liang et al., 2022),(Sun et al., 2020). Also, new methods such as pseudoexon skipping would contribute to broaden the therapeutic arms, which would enable future adjustment of the dystrophin gene and re-establishment of viable protein synthesis(*Defining Meaningful Outcomes in Gene Therapy Trials for Duchenne Muscular Dystrophy*, n.d.).

Gene Therapy Approaches

Gene therapy, especially the adeno-associated viruses (AAV) driven gene therapy which is used to deliver microdystrophin is another important area of development. Microdystrophins, are shorten forms of the protein dystrophin, have proved to be profitable in preclinical trials through acting similar to the roles of the full-length dystrophin and being less difficult to deliver(*Research - Duchenne Muscular Dystrophy (DMD) - Diseases | Muscular Dystrophy Association*, 2025),(Saw & Song, 2024). Future studies will aim at the optimization of the AAV vectors to make them safe and efficient for delivery of muscle tissues to different populations of patients.

Combination Therapies

The future of combination therapy is to combine RNA-based strategies with ex existing therapies can possibly improve the general treatment effects of DMD patients. As an example, the exon skipping can be combined with other modalities, including the anti-inflammatory drug or muscle regeneratory drug could be solve several aspects of disease pathology side by side. Also, the mRNA-based therapies combined with cell-based products can also contribute to the effectiveness of therapy because of high delivery of the targeted proteins into the damaged tissues(*Clinical Trials*, n.d.).

Personalized Medicine

With the maturing of the field of RNA therapeutics, there is a trend towards a more personalized medicine is expected. By modifying RNA-based-based treatments depending on the mutations that are present in an individual patient, clinicians might be capable of maximizing the therapeutic effects or reducing the possible side effects. Such a strategy is in line with the increasing trend in biomedicine to use genetic information for the development of specific therapies(*Clinical Trials*, n.d.),(Braun, 2025b).

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Innovations

Future of RNA therapeutics in DMD concentrated on increasing the efficacy and specificity of such methods. New technologies have been developed like ligand conjugated antisense near infrared is presented by oligonucleotides (LICA) in order to enhance the targeting and delivery of the ASO, thereby increasing their therapeutic potential(*Clinical Trials*, n.d.),(Dara et al., 2025). Besides, the development of chemical modifications, which include peptide nucleic acid (PNAs) and locked nucleic acid (LNAs) is under research in order to promote stability and uptake of RNA therapies to treat a few of the existing restrictions connected to cellular transfer and effectiveness(*Clinical Trials*, n.d.),(*ClinicalTrials.gov*, n.d.).

Regulatory Considerations

As multiple RNA-based therapies progress through clinical trials, discussions on the possibility and the need of such therapies continue to be held. Regulatory agencies will also be important for the approval process of these innovative treatments. A clear effective way of evaluating the assessment of RNA therapeutics will make sure that potential therapies can be delivered to patients within sufficient timeliness(Tominari & Aoki, 2022).

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