WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 3, 305-330. Review Article ISSN 2277– 7105

# DUCHENNE MUSCULAR DYSTROPHY: A REVIEW

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Article Received on
24 Dec. 2021,

Revised on 14 January 2022,
Accepted on 04 Feb. 2022

DOI: 10.20959/wjpr20223-23158

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## ABSTRACT

Duchenne muscular dystrophy (DMD) is a muscle illness caused by a mutation in the dystrophin, a largest human gene that code for the cytoskeletal protein which is required for maintaining the structural and functional integrity of the muscles. It has an X-linked recessive inheritance pattern. DMD mainly affects boys (1 in every 5000), while female carriers can be symptomatic in some situations. The condition typically causes deterioration in cardiac and pulmonary functions, resulting in the patient's mortality at a young age. A multitude of medicines are being developed all the time to manage the disease complications and extend the lives of sufferers. Use of Steroids in DMD decreases the mortality rate in patients and helps in improving strength, muscle function, and overall quality of life. The cornerstone of genetically modified therapeutics is the restoration of dystrophin gene expression. Exon skipping, the use of recombinant gene associated virus to produce mini-dystrophin, and surrogate gene transfer are all possible treatments for this condition. Stem cells transplantation techniques exhibit a promising treatment option in the future. Despite the hurdles and concerns surrounding stem cell therapy, some clinical trials have shown that patients who have received such treatments have increased muscle strength. The treatment can help to fix a genetic mutation in the DMD gene and alleviate symptoms. In this review article current treatment therapies and their mechanism in reducing DMD symptoms are discussed.

**KEYWORDS:** Duchenne muscular dystrophy, Therapy, Genetic engineering, Steroids, Stem Cells.

## INTRODUCTION

Muscular dystrophies are a set of inherited, progressive muscle diseases caused by mutations in genes encoding proteins necessary for normal muscle function or clinically and genetically

heterogeneous groupings of skeletal muscle wasting diseases.[1] DMD is an X–linked hereditary neuromuscular condition caused by dystrophin gene mutations. Duchenne is one of the "dystrophinopathies," a group of muscle illnesses. Dystrophinopathies are caused by a lack of the muscle protein "dystrophin," and can range from mild to severe.[2] The DMD gene, which has 79 exons, is the biggest gene ever discovered in humans. Patients with DMD have mutations that prevent the creation of dystrophin protein.

It affects around one out of every 5000 newborn men, making it the most frequent severe neuromuscular condition in humans. The condition always affects boys, and female carriers can be symptomatic in some situations. In the United States, 15.9 instances per 100,000 live male births have been reported, while in the United Kingdom, 19.5 cases per 100 000 live male births have been reported. Muscle weakness, accompanying motor delays, loss of ambulation, respiratory dysfunction, and cardiomyopathy occur as a result of progressive muscular damage and degeneration in patients with DMD.[3] It is characterized by gradual muscle weakness that begins around the age of 3–5 years old and progresses to the loss of ambulation by the age of 10–12 years old if left untreated. Children with DMD who are diagnosed now and get the usually recommended therapies have a chance of living well into their forties. Although there is currently no effective treatment for DMD, targeted interventions and therapeutic strategies can help to improve the disease's natural history.

These therapeutic strategies include: (a) mutation-specific therapeutic approaches for repairing genetic defects or transcripts (exon skipping, nonsense codon suppression, and endonuclease-mediated gene correction); (b) gene replacement therapy, which can be used across the entire patient population; (c) cell therapy, which includes allogeneic stem cells with mutations corrected ex vivo; and (d) modulation of non-dystrophin gene expression, such as upregulation of utrophin These therapeutic techniques are being developed in animal models, and human trials have begun, raising the prospect of even more dramatic disease improvement.[4]

In addition, a precise diagnosis for DMD should include a combination of genetic testing and clinical observation of muscular strength and function after a muscle biopsy. Corticosteroids, which have been demonstrated in numerous trials to boost muscle strength, are the most used contemporary drug. Exon-skipping antisense oligonucleotides (AOs), which bind to RNA and exclude certain areas of RNA splicing, resulting in a dystrophin that is smaller but functional, and other novel developing medications are ushering in a new era of DMD treatment. In the

following part, we'll go over all of the FDA-approved medications for DMD as well as recent research trials.[5]

**Pathophysiology**

The whole DAPC (Dystrophin associated protein complex) is removed from the sarcolemma in the absence of dystrophin. Muscles are unable to bear the tension of typical muscle contractions as a result. This damage causes an influx of extracellular calcium, which causes the cell's proteases to activate. Following that, a series of harmful processes occur Protease activity causes myocyte death, inflammation, and fibrosis, culminating in muscle fiber replacement with fat and connective tissues and failure of regeneration. In the lack of dystrophin, intracellular calcium levels rise, resulting in the activation of calpains, myocardial cell degeneration or apoptosis, and fibrosis. Cardiomyopathy is caused by excessive fibrosis. The renin–angiotensin system is stimulated and angiotensin II is released as cardiac function declines (AT II). Through the activation of angiotensin receptor type 1, AT II stimulates the production of transforming growth factor (TGF) in cardiac myocytes and fibroblasts. TGF- stimulates the proliferation of cardiac fibroblasts, the deposition of extracellular matrix proteins, and the development of cardiomyocyte hypertrophy. Dystrophin deficiency causes synapse integrity and interneuron transmission to be disrupted in the brain. A decrease in glucose metabolism in the cerebellum, the area associated with cognitive capacity, is suspected of causing cognitive impairment.[6]

**Overall disease management**

**Respiratory management**

In persons with DMD, respiratory problems are a leading cause of morbidity and mortality. Respiratory muscle fatigue, mucus clogging, atelectasis, pneumonia, and respiratory failure are all complications. Patients who are not treated risk developing severe dyspnea, requiring lengthy hospital stays due to atelectasis or pneumonia, and dying from respiratory arrest or respiratory-induced cardiac arrhythmias. Monitoring respiratory muscle performance and the prompt use of lung volume recruitment, helped coughing, nocturnally assisted ventilation, and following daytime ventilation are all part of an anticipatory management strategy. These essential treatments can help patients avoid respiratory issues, enhance their quality of life, and extend their lives. Before transitioning from paediatric to adult respiratory care providers, patients should be using most or all of these essential therapies by the age of 18–21 years. We propose greater pulmonary function thresholds (i.e., gentler levels of respiratory impairment)

for beginning of aided coughing and assisted ventilation in this update than in the 2010 care considerations. The new criteria are expected to lead to more proactive use of these therapies, with the prospect of treatment starting in slightly younger patients.[7]

**Ambulatory stage**

When the patient is 5–6 years old, spirometry should be started. Respiratory management necessitates regular monitoring of pulmonary function. The forced vital capacity (FVC) of an individual usually increases with age until he or she becomes non-ambulatory. FVC achieves a peak, then plateaus, before deteriorating over time. Deteriorating FVC can occur without dyspnea and go unnoticed unless pulmonary function is checked on a regular basis. The age at which ambulation was lost was predictive of the age at which peak FVC was realized, the absolute peak FVC, and the rate of subsequent decline in a large cohort study of boys who had not been treated with corticosteroids. For example, earlier loss of ambulation was linked to a lower peak FVC and a faster drop in FVC than later loss of ambulation. However, because the rate of change in FVC over time varies greatly between individuals, serial FVC measurement is required to characterize each individual's respiratory phenotype or trajectory. Sleep studies with capnography may be required during the ambulatory stage, particularly in patients who have gained weight as a result of glucocorticoid therapy and in patients who have symptoms of sleep disordered breathing. Sleep studies can also be used to monitor respiratory status in people who are unable to cooperate with pulmonary function testing. Individuals with DMD should be immunized yearly with inactivated influenza vaccine (not the live, attenuated nasal vaccine) and pneumococcal vaccines (including PCV13 and PPSV23), according to guidelines available from the US Centers for Disease Control and Prevention, 22 as well as other public health entities such as the Immunization Action Coalition, and Parent Project Muscular Dystrophy. During the ambulatory stage of DMD, patients and caregivers should be educated about respiratory complications in order to prepare them for future medical complications and therapies.[8,9]

**Early non-ambulatory stage**

The need for respiratory interventions arises primarily following the loss of ambulation. All non-ambulatory individuals should have their seated FVC (expressed as an absolute value and as a percentage predicted on the basis of arm span or ulnar length), maximum inspiratory and expiratory pressures, peak cough flow, and blood oxygen saturation by pulse oximetry (SpO2) measured at least every 6 months. Furthermore, when the necessary equipment is

available, the end-tidal or transcutaneous partial pressure of carbon dioxide in the blood (petCO2 or ptcCO2, respectively) should be measured every 6 months or whenever SpO2 is 95 percent or lower on room air.[10]

**Late non-ambulatory stage**

Individuals with DMD acquire inadequate cough efforts as they advance through the non-ambulatory stage, putting them at risk for atelectasis, pneumonia, ventilation–perfusion mismatch, and progression to respiratory failure, especially during respiratory tract infections. Manual and mechanically aided coughing are used to treat patients whose FVC is less than 50% expected, peak cough flow is less than 270 L/min, or maximum expiratory pressure is less than 60 cm H2O. Individuals who are treated with assisted coughing during respiratory illnesses should have a home pulse oximeter. To prevent and treat mucus clogging, atelectasis, and pneumonia, the frequency of aided coughing should be increased when SpO2 is less than 95% on room air. When people have three of the five indications of pneumonia: fever, high white blood count or C-reactive protein concentration, sputum production, a pulmonary infiltrate on a chest radiograph, or hypoxemia or respiratory distress, we urge starting antibiotic therapy right away. Individuals with DMD who are non-ambulatory in the late stages of the disease require assisted ventilation to survive. To avoid apnea, ventilation equipment should have a backup rate of breathing.[11]

Symptoms of hypoventilation or sleep-disordered breathing, regardless of pulmonary function, are indications for nocturnally assisted ventilation; relevant symptoms include fatigue, dyspnoea, morning or continuous headaches, frequent nocturnal awakenings or difficult arousal, hypersomnolence, difficulty concentrating, awakenings with dyspnea and tachycardia, and frequent nightmares. When a patient's FVC is less than 50% expected, or when the absolute value of maximum inspiratory pressure is less than 60 cm H2O, nocturnally assisted ventilation should be commenced. It should also be started when the person is awake and any of the following applies due to daytime hypoventilation: petCO2 or ptcCO2 is greater than 45 mm Hg; pCO2 in arterial, venous, or capillary blood is greater than 45 mm Hg; or baseline SpO2 on room air is less than 95 percent. A working committee of experts from the National Heart, Lung, and Blood Institute (NHLBI) recently presented updated comprehensive DMD cardiac care considerations, which included critical areas for future research.[12]

**Methods for treatment**

**Corticosteroids**

The first line of treatment for DMD was corticosteroids, which were originally utilised by following promising positive outcomes in their trial after using prednisone (anti-inflammatory glucocorticosteroid). Many researches have been conducted since then to investigate the therapeutic efficacy of such treatment, which has been found to increase muscle performance. An Italian group and other groups employed Deflazacort (DFZ), an oxazolidine derivative of prednisone, and the medicine showed efficacy in disease treatment and retained lung function. The exact mechanism of DFZ is unknown, but it is thought to regulate some signaling pathways. It has been discovered that it activates the calcineurin/NF-AT pathway. DFZ may also reduce the degree of muscle degeneration by reducing necrosis and muscular inflammation.[13] In addition to having favorable effects on muscle tissue mass, it can function via regulating dystrophin expression and stimulating myogenesis. Despite the benefits of using steroids, they had side effects such as weight gain, decreased bone mineral density, which resulted in vertebral fractures, and behavioral changes. Furthermore, substantial doses are needed to provide the desired effect and remain active at the site of inflammation. In addition, the medication can build up in non-targeted locations.[14] Their findings indicated that when supplied intravenously, such a structure targeted the diaphragm selectively in vivo (using the mdx mice model), and that the treatment reduced macrophage infiltration and blood levels of transforming growth factor beta. Most crucially, the study found that long-term use of this composition results in greater muscle strength and mobility.

**Exon skipping**

One of the mutation-based therapies for Duchenne muscular dystrophy is exon skipping. Some exon losses in DMD cause the reading frame of the dystrophin protein to be disrupted, resulting in the generation of a truncated product lacking a significant portion of the protein (usually missing the rod domain and C-terminal domain). However, deleting additional exons can sometimes restore the reading frame and result in the production of dystrophin protein that is missing only a portion of the central rod domain while the C-terminal domain is intact, resulting in a protein product that is semi-functional and can cause Becker-like symptoms rather than complete muscular function loss. Exon skipping is a technique that involves utilizing "antisense oligonucleotide" molecules to cause the skipping of a specific exon (other than the one that has already been altered) and preventing it from being translated in order to restore the reading frame.

Patients with exon 45 deletions, for example, could be treated by skipping one exon. 3 Duchenne Muscular Dystrophy (DMD) Treatment: Perspectives from the Past and Present Exon 44 is an extra exon. Eteplirsen (Exondys51TM), an FDA-approved antisense treatment based on phosphonodiamidite morpholino oligomer (PMD), skips exon 51 for patients with mutations 49-50.[15] Dispersion (based on 2′-O-methyl phosphonothioate; 2′-OMePS-modified AOs) is another AO meant to treat DMD patients with mutations that can be alleviated by exon 51 skipping, although it has yet to be approved by the FDA. The sugar of the oligonucleotide or the backbone of the oligo can be altered in a variety of ways. Phosphonodiamidite morpholino, locked nucleic acid (LNA), or peptide-conjugated oligo are examples of this. In the case of morpholinos, the oligonucleotide backbone is substituted with the morpholino backbone, resulting in a benign oligonucleotide with a high affinity for RNA molecules. The locked nucleic acids are oligonucleotides having a modified ribose sugar, in which the 2′ oxygen is linked to the 4′ carbon atom, forming a locked ribose ring. The LNAs are also nontoxic and have a high affinity for complementary RNA sequences. The main problem with developing such treatments based on exon skipping is that it will only fit a small group of patients (a mutation-specific AO should be developed for each group of patients, and it will not be suitable for other patients); additionally, some patients have deletions in critical parts of the protein, and thus skipping of other exons will not have a therapeutic impact; and finally, some patients have deletions in critical parts of the protein, and thus skipping of other exons will not have a therapeutic.[16]

**Induced pluripotent**

Stem cells are in combination with genome editing. The existence of a mutation that affects the DMD gene is the sole cause of DMD. Patients could be given muscle cells containing the normal copy of the DMD gene to permanently correct such mutations and treat this ailment. Because it is difficult to obtain mature muscle fibers from a healthy individual to be used as a source of healthy muscle cells with a normal DMD gene, the availability of such a source of cells does not guarantee the success of the grafting process in the patient's muscles, as it may be rejected by the body and trigger an aggressive immune response. Techniques such as cell reprogramming and genome editing are effective in resolving this perplexing problem.[17]

By starting with patient-specific adult cells and inducing the production of induced pluripotent stem cells (iPSCs) (using the Nobel Prize-winning technology of reprogramming using specific transcription factors like Oct4, Sox2, Klf4, and L-Myc), the path to developing

normal muscle fibers was paved. Also, some microRNAs (such as miR-302b and miR-372) have the ability to effectively reprogram adult cells.[18] Gene editing methods should be utilized to fix the gene mutation after the reprogramming and generation of stem cells. The RNA-guided DNA endonuclease system allows for the correction of the DMD segment, which is required for dystrophin restoration. CRISPR/Cas 9 is now a leading technology that is being explored as a therapy option for DMD.[19]

To carry out a gene editing experiment with the CRISPR/Cas9 system, two essential components must be present: a guide RNA (gRNA) specific for the target gene and a Cas9 nuclease (Sp. Cas9 (from Streptococcus pyogenes; 4.10 kb) or Sp. Cas9 (from Staphylococcus aureus; 3.16 kb). Cas9 can cause a double-strand break when it binds to the gRNA, which is then repaired by the cell using nonhomologous end joining. This triggers a repair mechanism in which nucleotides are added or deleted at the cleaved site, restoring the DMD gene's reading frame to its normal state.

After the gene editing process is completed, the modified cells will be treated with myogenic factors to turn them back into myoblasts for myogenic differentiation.[20]

**Gene therapy**

One of the most intriguing ways for delivering a normal copy of the DMD gene to express the fully functional dystrophin protein is gene therapy. This procedure entails injecting plasmids with normal dystrophin cDNA (12 kb) into the patients. The first phase 1 trial of DMD gene therapy employing full-length dystrophin was conducted in 2002.[21] Then, for gene therapy, adeno-associated viral vectors carrying mini forms of dystrophin cDNA were utilized, which was better in terms of plasmid packaging size and much easier to transfer/deliver mini forms of DMD gene.[22]

However, such a therapeutic approach ran into a problem with plasmid distribution across all affected muscular tissue throughout the body, which is why micro dystrophin plasmids and systemic AAV delivery were developed and improved to address this issue. Many animal studies have shown that gene therapy can result in the long-term production of functional protein. In 2017, they investigated the effect of delivering a canine micro dystrophin (cMD1)-expressing rAAV2/8 vector to golden retriever muscular dystrophy (GRMD) dogs in the absence of immunosuppression.[23] The decrease of muscle function was slowed by this treatment, and gene expression was maintained for a long time. Genthon and Sarepta recently

hired Yposkesi to manufacture the AAV micro dystrophin vector on a big scale in 2020.[24] Chimeric cells producing dystrophin as previously stated, dystrophin deficiency is the primary cause of DMD disease and its severe symptoms, which include muscular weakening and degeneration.[25, 26, 27]

However, such technology might cause off-target mutations, which can have negative consequences, which is why new therapies were needed to address the drawbacks of such procedures. Chimeric cell therapy is a type of alternative medicine that involves fusing normal myoblasts with dystrophin-deficient myoblasts using polyethylene glycol (PEG).[28,29] Immunophenotyping (flow cytometry) and dystrophin immunostaining could be used to assess the success of this procedure. Following the fusing, chimeric cells will be transplanted into the defective muscle. Chimeric cells usually behave like donor cells in terms of dystrophin expression and myogenic differentiation, which increases muscle function considerably following transplantation.[30]

**Cardio sphere-derived cells (CDCs)**

Cardio sphere-derived cells are cells that can be cultivated in vitro after being extracted from cardiac explants. Anti-inflammatory, antioxidant, and antibacterial properties are all present in these cells. CDCs were investigated in several experiments to see if they may change the pathophysiology of DMD after being injected directly into the heart muscle.[31,32] It was recently discovered that utilizing CDCs improved the phenotypic status of cardiac and skeletal muscles significantly. The production of specialized exosomes conveying specific genetic material to distal cells to exert its biological function is commonly attributed to the therapeutic benefits of CDCs. These CDCs, together with their secreted exosomes, can be injected intravenously, and it has been discovered that they can significantly improve skeletal and cardiac muscle functioning, as well as promote muscle creation.[33,34]

**Stop codon read-through therapy**

A premature stop codon is formed in some DMD gene mutations, which can drastically disrupt the reading frame and result in a shortened aberrant protein that cannot retain the structural and functional features of muscle fibers. Aminoglycoside antibiotics link to rRNA at their decoding sites, preventing stop codons from being read by binding to the A site (acceptor site) in ribosomes and causing the cell to cease reading the stop codon, resulting in the synthesis of fully functional proteins.[35,36] PTC124 (ataluren; commercial name, Translarna TM) is one of the medications used to treat DMD that has readthrough

capabilities. In clinical trials, this medication induced the expression of the dystrophin protein when taken orally. This medication, however, can only be administered in 15% of cases where the stop signal is caused by a point mutation in the DMD gene. It must also be given in escalating amounts, and it has numerous negative effects, including kidney damage. As a result, other solutions with different architectures are needed to make chronic consumption safer.[37]

**Utrophin modulation**

Recently, DMD symptoms were shown to be managed after utrophin protein expression enhancers (a dystrophin homolog with a 395 KDa size) were given to DMD patients. This delayed the need for a wheelchair and effectively replaced non-functional dystrophin. Advances in Cellular and Molecular Basis, Diagnosis, and Therapeutics for Muscular Dystrophy... 10 Utrophin, like dystrophin, is present in the sarcolemma throughout the early stages of development before being replaced by dystrophin during muscular maturity.[38] SMTC-1100 is one of the chemical substances that have shown a lot of promise in terms of increasing DMD transcript and protein expression. If it was shown to be safe and well tolerated in volunteers, this medicine can be used orally. However, more research is needed to determine whether or not high doses of this medicine are safe.[39] ASA, or adenyl-succinic acid, was recently found to improve the condition of the TA muscles of mdx mice after being given this substance in their drinking water. This molecule regulated the utrophin protein's expression, resulting in a significant reduction in the injured area.[40] In adulthood, however, utrophin was discovered in the myotendinous junction. Surprisingly, utrophin expression rises as a natural repair mechanism to compensate for the lack of functional dystrophin in regenerated muscles. SMTC-1100 is one of the chemical substances that have shown a lot of promise in terms of increasing DMD transcript and protein expression.[41]

**Induced pluripotent stem cells along with genome editing technique**

The existence of a mutation that affects the DMD gene is the sole cause of DMD. Patients could be given muscle cells containing the normal copy of the DMD gene to permanently correct such mutations and treat this ailment. Because it is difficult to obtain mature muscle fibers from a healthy individual to be used as a source of healthy muscle cells with a normal DMD gene, the availability of such a source of cells does not guarantee the success of the grafting process in the patient's muscles, as it may be rejected by the body and trigger an aggressive immune response.[42] Techniques such as cell reprogramming and genome editing

are effective in resolving this perplexing problem. By starting with patient-specific adult cells and inducing the production of induced pluripotent stem cells (iPSCs) (using the Nobel Prize-winning technology of reprogramming using specific transcription factors like Oct4, Sox2, Klf4, and L-Myc), the path to developing normal muscle fibers was paved. Also, some micro-RNAs (such as miR-302b and miR-372) have the ability to effectively reprogram adult cells.[43] Gene editing methods should be utilized to fix the gene mutation after the reprogramming and generation of stem cells. The RNA-guided DNA endonuclease system allows for the correction of the DMD segment, which is required for dystrophin restoration. CRISPR/Cas 9 is now a leading technology that is being explored as a therapy option for DMD.[44] To carry out a gene editing experiment with the CRISPR/Cas9 system, two essential components must be present: a guide RNA (gRNA) specific for the target gene and a Cas9 nuclease (Sp. Cas9 (from Streptococcus pyogenes; 4.10 kb) or Sp. Cas9 (from Staphylococcus aureus; 3.16 kb)) or CJ. Cas9 (Cas9 can cause a double-strand break when it binds to the gRNA, which is then repaired by the cell by nonhomologous end joining. This triggers a repair mechanism in which nucleotides are inserted or deleted at the cleaved spot, restoring the DMD gene's reading frame to normal ORF. In some circumstances, a single (or several) gRNA molecule could be constructed to target splicing sites, causing specific exons to be skipped and functional proteins to be produced. Furthermore, base editing mediated by CRISPR/Cas9 could be achieved using Cas9 enzymes that lack nuclease activity, resulting in a single-strand break. Base editing (A: T -> G:C) can be catalyzed by enzymes with cytidine deaminase activity.[45] In their investigation, they deleted exon 51 from the transcript from patient-derived myoblasts using a different editing process (zinc finger nuclease). Young et al. also conducted a CRISPR/Cas9 experiment in iPSCs using a single pair of guide RNAs to delete exons 45–55, which resulted in the development of stable dystrophin protein with increased membrane stability in skeletal myotubes and cardiomyocytes. Duchene et al. used a single guide RNA to create a hybrid exon, which resulted in the synthesis of a fully functioning dystrophin protein with a normal structure. The key benefit of this reprogramming process is that it allows for autologous muscle cell grafting in patients.[46,47,48] Adeno-associated virus (AAV) vectors will be employed to express the appropriate gRNA molecules inside the muscle cells. Because of improper interaction with another identical DNA sequence inside the host cell, gRNA production can sometimes result in off-target effects. AAV vectors expressing numerous gRNA molecules could be employed to avoid this harmful effect. After the gene editing process is completed, the modified cells will be treated with myogenic factors to turn them back into myoblasts for myogenic differentiation.[49,50]

**Diagnosis**

A quick and accurate diagnosis of DMD is an important element of treatment. Since 2010, the approach for diagnosing DMD has remained largely unchanged. The diagnostic process usually starts in early childhood when suggestive signs and symptoms, such as weakness, clumsiness, a Gowers' sign, stair climbing difficulty, or toe walking, are noted. Referral to a neuromuscular expert as soon as possible, with the help of a geneticist or genetic counsellor, can help avoid diagnostic delays. Developmental delay or elevated blood enzyme values such as alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, or creatine kinase are less usually used to make the diagnosis. Increased alanine aminotransferase, aspartate aminotransferase, or lactate dehydrogenase levels might sometimes lead to an overemphasis on hepatic dysfunction, delaying DMD diagnosis.[51]

Dystrophin gene deletion and duplication testing is frequently the first confirmatory test because around 70% of people with DMD have a single-exon or multi-exon deletion or duplication in the dystrophin gene. Multiplex ligation-dependent probe amplification (MLPA) or comparative genomic hybridization array are the best options for testing because multiplex PCR can only detect deletions. The use of MLPA or a comparative genomic hybridization array to determine the boundaries of a deletion or duplication mutation could suggest whether the mutation is expected to be beneficial or harmful the framework for reading If testing for deletion or duplication is required, In the case of a negative result, genetic sequencing should be used to screen for the mutations that are assigned to the other categories of mutations (about 25–30 percent) to DMD. Point mutations (nonsense or missense), minor deletions, and small duplications or insertions are among the mutations that can be detected with next-generation sequencing. Finally, if genetic testing does not support a clinical diagnosis of DMD, a muscle biopsy sample should be examined for dystrophin protein presence using immunohistochemistry of tissue cryosections or a western blot of a muscle protein extract.[52,53]

**Female carriers**

Genetic counselling for family members of people with DMD is recommended to determine who is at risk of becoming a carrier. Female relatives of a kid or man who has been genetically verified to have DMD should undergo carrier testing. If the relative is a child, the American Medical Association's recommendations for child genetic testing should be followed. Female carriers who have been found have a variety of reproductive options,

including preimplantation genetic diagnosis or prenatal genetic testing by chorionic villus or amniotic fluid sampling.[54]

**Newborn screening**

The feasibility of newborn screening for DMD was first demonstrated in the mid-1970s27 using dried blood spot measurements of creatine kinase concentrations.[55] A two-tier newborn screening diagnostic approach was recently reported, in which samples with elevated creatine kinase levels were then screened for dystrophin gene variants. DMD has been the subject of newborn screening trials in numerous countries, however the majority have been abandoned, and DMD is not now included on the Recommended Uniform Screening Panel, which is mostly limited to neonatal-onset illnesses for whom early treatment improves outcomes. However, increasing interest in newborn screening has grown as a result of stakeholder support and the fact that potential DMD medicines may be most effective if started before symptoms appear.[56]

**Neuromuscular management**

Following diagnosis, the neuromuscular specialist will act as the lead clinician, taking overall responsibility for the DMD patient's treatment and performing many duties and responsibilities throughout the patient's lifespan. The neuromuscular expert is especially suited to help patients and their families navigate the increasingly complicated and technology diagnostic and therapeutic environment of today's DMD treatment.[57, 58]

**Assessments**

The management of DMD is based on consistent and repeatable clinical assessments of neuromuscular function performed by skilled practitioners. Clinicians should employ a set of tests with which they are familiar and for which they understand the clinical correlations, as outlined in the 2010 care considerations. To maximize consistency and eliminate excessive test duplication, multidisciplinary team members must collaborate. The appendix contains suggested assessments, which are covered in the section on rehabilitation management. Newer research has demonstrated the usefulness of standardized functional evaluations across a patient's lifespan, as well as the predictive capacities of standardized functional assessments and ranges of optimum responsiveness. New evaluation technologies are also assisting in the care of elderly, non-ambulatory adults, demonstrating the value of clinical testing throughout one's life.[59,60,61]

**Interventions**

Physiotherapy and glucocorticoid medication, as outlined in the section on rehabilitation management, remain the pillars of DMD treatment and should be continued following loss of ambulation. Long-term glucocorticoid medications have been found to have a number of advantages, including delayed ambulation, retained upper limb and respiratory function, and the avoidance of scoliosis surgery. Recent studies have confirmed the benefits of commencing glucocorticoids in younger children before they show signs of physical degeneration; a current trial of weekend dose in boys younger than 30 months will shortly provide more information. Despite the fact that the benefits of glucocorticoid therapy are well known, there is still some debate over which glucocorticoids to use and at what doses. Recent studies have confirmed the benefits of commencing glucocorticoids in younger children before they show signs of physical degeneration; a current trial of weekend treatment in boys younger than 30 months will provide more information soon. Despite the fact that the benefits of glucocorticoid therapy are well known, there is still some debate over which glucocorticoids to use and at what dosages.[62] These uncertainties raise the danger of under- or over-treatment, which could skew the results of clinical studies of novel medicines. Large-scale natural history and cohort studies show that ambulation is extended from a mean of 103 years in those treated with corticosteroids for less than a year to a mean of 112 years in those treated with daily prednisone and 139 years in those treated with daily deflazacort. Prednisone medication over the weekends has been demonstrated to be equally effective as daily dosing in a few studies. Deflazacort 09 mg/kg per day and deflazacort 12 mg/kg per day were evaluated in a phase 3 double-blind RCT. In a few studies, weekend-only prednisone administration was found to be equally effective as daily dosing. A phase 3 double-blind RCT compared deflazacort 0·9 mg/kg per day, deflazacort 1·2 mg/kg per day, prednisone 0·75 mg/kg per day, and placebo. When compared to placebo, all therapy groups improved muscle strength, and deflazacort was linked with less weight gain than prednisone. In a current double-blind experiment, the benefit-to-risk ratio of deflazacort against prednisone is being investigated further.[63,64]

**Emerging treatments**

Since the publication of the 2010 care considerations, the drug development pipeline for DMD has changed dramatically, and the full list of DMD treatment trials changes on a regular basis; updated information is available at Clinical Trials. gov and the WHO International Clinical Trials Registry Platform. DMD is a rare condition, and the growing

number of DMD studies is putting a strain on clinical trial capacity due to the small number of patients that qualify. Patient registries, the development of clinically meaningful outcome indicators, and natural history studies are all predicted to be boosted as a result of the need to improve patient recruitment.[65] The researchers were granted conditional marketing authorization by the European Commission for use in the European Union, targeting the approximately 11% of boys with DMD caused by a stop codon in the dystrophin gene.[66] The US Food and Drug Administration (FDA) approved Eteplirsen via an accelerated approval pathway in September 2016, targeting the approximately 13% of boys with a mutation in the dystrophin gene that is responsive to exon 51 skipping45. The first of a series of mutation-specific treatments, ataluren and Eteplirsen, has been approved by the FDA. Other dystrophin restoration therapies are in the works, with some nearing or undergoing regulatory approval. The FDA has also given deflazacort full approval, making it the first glucocorticoid with a stated indication for DMD. Medications targeting myostatin, anti-inflammatory and antioxidant molecules, and compounds to reduce fibrosis, pharmaceuticals to promote vasodilation, drugs to improve mitochondrial function, and drugs to regulate utrophin are among the other therapeutic types in studies for DMD. 46 None of these medications can be prescribed for people with DMD until clinical trials are finished and regulatory permission is obtained.[67, 68]

**Rehabilitation management**

Dystrophin deficiency causes well-known patterns of gradual muscle degeneration and weakening, postural compensations, the risk of progressive contracture and deformity, and functional losses. Improvements in DMD care have resulted in increased ambulation, 47 a lower prevalence of severe contracture and deformity, including scoliosis, 37 and increased function and engagement in all aspects of life. Physicians, physical therapists, occupational therapists, speech-language pathologists, orthotists, and durable medical equipment providers are among the rehabilitation personnel. An overview of potential assessments and interventions is included in Panel 2 and the appendix. Understanding DMD pathology, pathokinesiology, natural history, and disease progression is required for rehabilitation therapy; practitioners should consider each individual's goals and lifestyle to optimize quality of life across the lifespan. To minimize contractures, deformity, loss of function, compromised skin integrity, pain, and compromised cardiorespiratory status, assessment and anticipatory management must be provided across all domains of the International Classification of Functioning, Disability, and Health (ICF) from diagnosis onwards.[69]

**Endocrine management**

Impaired growth, delayed puberty, and adrenal insufficiency are endocrine consequences of DMD and its therapy. Monitoring growth and development, identifying and diagnosing hormone shortages, providing endocrine hormone replacement treatment when necessary, and preventing a life-threatening adrenal crisis are all goals of endocrine care.[70] There have been a few relevant expert opinion pieces and reviews published, 94–96 but data on the safety and efficacy of growth hormone and testosterone therapy in people with DMD is limited. The following recommendations are based on evidence and experience gained from the use of various medicines in other diseases, with adjustments for DMD.

**Growth**

Individuals with DMD often have impaired linear development, which is aggravated by glucocorticoid therapy. Linear growth should be measured every 6 months until puberty is complete and the final height is reached. In ambulatory people, standing height is the most acceptable measurement. On a standardized growth curve, height should be plotted and followed. Additionally, regular growth evaluation using a non-standing height measure should begin during the ambulatory period to allow for more reliable measurement once people lose their ability to walk. Arm spread, ulnar length, tibia length, knee height, and segmentally measured recumbent length have all been used to quantify growth in non-ambulatory children; however, none of these measurements have been validated in the DMD population, and they all require specialized training or equipment. We recommend that each institution choose and implement the metric that performs best in its own clinical setting.[71, 72]

**Puberty**

Delayed puberty due to hypogonadism is a potential complication of glucocorticoid therapy and can be psychologically distressing, impairing quality of life. If pubertal development has not occurred by the age of 14, an endocrinologist should be consulted right once. Individuals with evidence of delayed puberty should undergo biochemical testing using appropriate paediatric or ultrasensitive assays to confirm the diagnosis of hypogonadism. A radiograph of the left hand should also be investigated to determine bone age. Testosterone replacement treatment is advised for patients over the age of 14 who have verified hypogonadism, and it can also be explored for boys over the age of 12 who are on glucocorticoids and have not reached pubertal development. Despite the fact that no clinical trials have specifically

evaluated the use of testosterone in boys with DMD, it is regarded the gold standard for treating pathological pubertal delay in children and is indicated for the treatment of glucocorticoid-induced hypogonadism in adult men.[73] Behavioral changes, acne, body odour, quick growth spurt, and epiphyseal closure are usually outweighed by the potential benefits of testosterone on emotional and physical health. Individuals with DMD and their families found testosterone to be typically well tolerated and useful, according to a recent retrospective review. Testosterone replacement should be started at a modest dose and gradually escalated to adult replacement doses over several years to imitate normal pubertal development. It is possible to employ intramuscular or topical medicines. Testosterone levels in all individuals should be constantly checked. Lipids, haemoglobin, haematocrit, and blood glucose should all be measured in people who have been treated. If a person's functional status or heart function deteriorates, the clinician should consider stopping testosterone medication or reducing the dose.[74,75]

**Adrenal insufficiency**

Adrenal insufficiency caused by suppression of the hypothalamic-pituitary-adrenal (HPA) axis is an uncommon but life-threatening syndrome that can occur when glucocorticoids are abruptly withdrawn owing to illness or therapy withdrawal. All people taking glucocorticoids should be informed about the signs, symptoms, and treatment options for adrenal crisis, as well as be given prescriptions for injectable hydrocortisone (50 mg for children under 2 years, 100 mg for children or adults under 2 years).[76,77,78] In the case of severe sickness, substantial trauma, or surgery, stress dosing with hydrocortisone at 50–100 mg/m2 per day may be required in persons receiving more than 12 mg/m2 per day of prednisone or deflazacort. To allow the HPA axis to heal, glucocorticoid therapy should be tapered over weeks to months rather than abruptly stopped. The PJ Nicholoff Steroid Protocol is a good way to taper glucocorticoids.[79]

**Gastrointestinal and nutritional management**

Weight gain or loss, dietary or nutrient imbalance, fluid imbalance, low bone density, swallowing difficulties, and mandibular contracture are common gastrointestinal or nutritional issues in people with DMD. Immobility, glucocorticoid therapy, and decreased energy expenditure are all contributing factors. The respiratory, skeletal muscular, and heart systems can all be harmed by dietary abnormalities.[80,81] Nutritional care tries to avoid overweight or obesity, as well as undernutrition or malnutrition, by assessing growth and

weight on a regular basis; it also aims to promote a healthy, balanced diet with adequate calories, protein, hydration, and micronutrients, particularly calcium and vitamin D. There is a scarcity of solid evidence-based nutrition studies on DMD. As a result, nutrition guidelines for people with DMD are altered from those for the general population.[82] A registered dietitian nutritionist with adequate experience should be part of the care team and should see a person with DMD at every visit, beginning with the diagnosis. During periods when weight gain or loss is expected, the dietitian nutritionist will need to monitor you more frequently. For people who are at risk of becoming overweight, a physical therapist should be contacted to create and implement safe exercise programs. Individuals with suspected dysphagia should be evaluated by a speech-language pathologist. Constipation, gastroesophageal reflux, and gastrointestinal motility issues should be addressed by a gastroenterologist, as should the installation of a gastrostomy tube.[83]

## CONCLUSION

Improved approaches to respiratory, cardiac, bone health and osteoporosis, and orthopedic and surgical management can now be offered to children and adults with DMD. However, despite advances in our knowledge and understanding of best approaches to management, progress is needed across these subspecialties to meet the needs of patients.

For respiratory management, diagnostic tools and measures that might have clinical relevance but need further study include assisted cough peak flow, maximum insufflation capacity, the difference between maximum insufflation capacity and FVC, supine FVC, highest flow generated during an inspiratory FVC maneuver, the rapid shallow breathing index, and sniff nasal inspiratory pressure. Therapies for which research is needed to establish efficacy and optimum use include high frequency chest oscillation, intrapulmonary percussive ventilation, and negative-pressure ventilation.

Improved understanding of pulmonary phenotypic variability and of the effect of cardiac function and nutritional status on the respiratory system is needed to optimize care and to develop pulmonary outcome measures to assess the efficacy of current and emerging DMD therapies. Prospective studies are needed to assess the criteria recommended in this document for initiation of cough assistance and non-invasive ventilation, with use of clinically relevant outcome measures to develop evidence-based guidelines. Cardiac outcomes should be included in clinical trials because survival will not improve if emerging therapies do not effectively treat DMD cardiomyopathy.

New dystrophin-restoring medications are becoming accessible, with more on the way, and more data on the optimal glucocorticoid regimens for DMD patients is becoming available. The importance of the novel compounds in the overall management of DMD will need to be addressed in future care decisions, especially in light of the known benefit of long-term glucocorticoid therapy. When some of these novel medicines have been shown safe and effective, treatment for DMD can be tailored, with selection of the optimal combination of medications for each individual's unique mutation. RCTs are needed in the field 'of endocrine management to better understand the risks and benefits of growth hormone and testosterone therapy, as well as to determine the most appropriate indications, timing, and dose re Improvements in clinical and functional assessments for rehabilitation treatment are still being developed, with a focus on the lifetime. With technological advancements, new medicines will almost certainly be evaluated more frequently through activity monitoring combined with measures of novel, clinically significant biomarkers. Robotics and other rapid technological breakthroughs will increase people's independence, participation, and overall quality of life. Dystrophin-restorative therapy, for example, may improve exercise or activity capacity and safety. Physical therapists, occupational therapists, speech-language pathologists, and orthotists will work together to improve musculoskeletal management and function, using innovative technologies.

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