Neurology Care, Diagnostics, and Emerging Therapies of the Patient With Duchenne Muscular Dystrophy

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abstract

Duchenne muscular dystrophy is the most common form of childhood muscular dystrophy. A mutation in the *DMD* gene disrupts dystrophin (protein) production, causing damage to muscle integrity, weakness, loss of ambulation, and cardiopulmonary compromise by the second decade of life. Life expectancy has improved from mid-teenage years to mid-20s with the use of glucocorticoids and beyond the third decade with ventilator support and multidisciplinary care. However, Duchenne muscular dystrophy is associated with comorbidities and is a fatal disease. Glucocorticoids prolong ambulation, but their side effects are significant. Emerging investigational therapies have surfaced over the past decade and have rapidly been tested in clinical trials. Gene-specific strategies include nonsense readthrough, exon skipping, gene editing, utrophin modulation, and gene replacement. Other mechanisms include muscle regeneration, antioxidants, and antifibrosis and anti-inflammatory pathways. With potential therapies emerging, early diagnosis is needed to initiate treatment early enough to minimize morbidity and mortality. Newborn screening can be used to significantly improve early diagnosis, especially for gene-specific therapeutics.

Duchenne muscular dystrophy (DMD), although rare, is the most common muscular dystrophy, with a prevalence of 1 in 5000.[1–3] It is an X-linked disorder caused by a mutation in the *DMD* gene located on Xp21. The *DMD* gene is large (79 exons; 427 Kilodalton) and encodes a 427-Kilodalton muscle isoform protein dystrophin. The gene is located in the dystrophin-associated glycoprotein complex at the sarcolemma and in the cytoskeleton. The protein is located primarily in skeletal muscle to stabilize the plasma membrane and maintain the strength of muscle fibers.[4,5] The absence of dystrophin causes muscle membrane damage, elevated serum creatinine kinase (CK),

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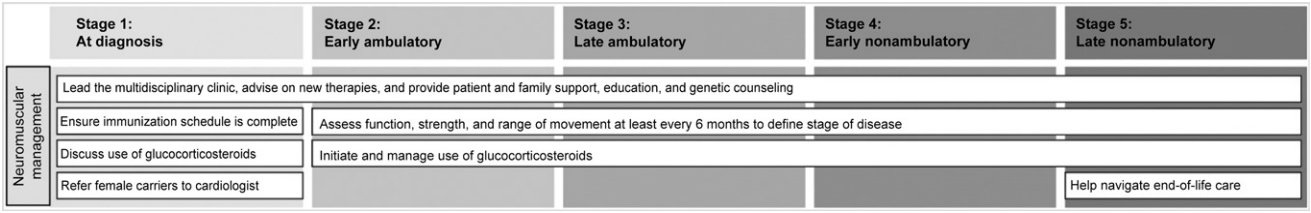

FIGURE 1
Summary of neuromuscular management depending on the clinical stage of patients with DMD. (Adapted with permission from Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol.* 2018;17(3):252.)

fiber necrosis, muscle degeneration, and regeneration.[6] Constant muscle breakdown and necrosis is followed by inflammatory processes that lead to the replacement of muscle with fibrotic tissue and fat accumulation.[7–9]

Common presenting symptoms include delayed motor milestones, inability to run or hop, toe walking, difficulty climbing stairs, and hypertrophy of calf muscles. Nonmotor presentations include failure to thrive, speech delay, fatigue, abnormal transaminases, myoglobinuria, and complications because of anesthesia (ie, malignant hyperthermia or rhabdomyolysis). Global developmental delay is a common presentation. Early signs and symptoms present at ages 2 to 3 years, whereas the average age of diagnosis is 3 to 5 years. It has been reported that it takes >1 year to confirm DMD from the time of presentation,[10] which is far too long in a disease for which time means loss of muscle function and the familial risk of recurrence is up to 50% in male siblings.

With recent advancements in clinical trials, there is an unmet need to diagnose affected infants and children early. Mendell et al[1] presented data on newborn screening in Ohio initiated with a CK level from dried blood spot followed by genetic confirmation of those with elevated CK. Eagle et al[11] reported that life expectancy in the 1960s was 14 years; in the 1990s, life expectancy was 25 years for those receiving ventilatory support. The mean age at which patients lose ambulation is ~9 to 10 years.[11] Glucocorticoids are used to improve muscle strength and function in patients with DMD[12] and prolong ambulation[13] by an additional 2 to 3 years[14]; patients with DMD who are treated with glucocorticoids become wheelchair dependent by 12 to 13 years of age. Loss of ambulation is associated with greater morbidity and mortality as the disease progresses; not surprisingly, the primary objective of researchers in most clinical trials is prolonging ambulation. Cardiomyopathy and respiratory insufficiency can shorten the life span, but with ventilator support, survival in DMD has been extended to >30 years, and cardiac function is the remaining determinant of survival.[15] Investigational drugs that are used to improve cardiopulmonary function also have emerged in clinical trials (for additional details, see the specialty article on cardiac management that is part of this supplement[16]). Our purpose in this article is to guide clinicians in the diagnosis and treatment of DMD itself, expanding on principles outlined in the 2018 Duchenne Muscular Dystrophy Care Considerations sponsored by the Centers for Disease Control and Prevention.[17] In Fig 1, we provide an overall guide for the neuromuscular management of patients with DMD according to each patient's clinical stage.

NEUROLOGY

DMD is a proximal myopathy affecting muscles, such as the quadriceps and gluteals. Fast-twitch (type IIb) myofibers are preferentially affected, leaving the slow-twitch (type I) type to predominate.[18,19] The disease is characterized by muscle wasting, weakness, myofiber size variation with muscle necrosis, fat accumulation, connective tissue replacement, and paradoxical hypertrophy of calf muscles. Gait abnormality and difficulty getting up from a chair or from a supine position are common clinical features. However, the onset of symptoms can vary in age, severity, and presentation. Individuals affected by DMD typically present with delayed motor development or a gait abnormality, especially toe walking or delayed age of ambulation, and are often referred to physical therapy. Although the average age of presentation ranges from 2 to 5 years, the recognition of loss of milestone and the use of Bayley Scales of Infant Development, Third Edition can improve the identification of affected infants before motor impairment occurs.[20–22]

GLUCOCORTICOIDS

Prednisone prolongs ambulation and has been shown to have long-term benefits for respiratory and cardiac function in nonambulatory patients.[12,13,23–25] Some researchers suggest there may be fewer side

effects with deflazacort than prednisone.[26,27] However, the relative efficacy and side effects of different glucocorticoid regimens remains untested in well-designed, head-to-head trials. The standard dose for prednisone is 0.75 mg/kg per day and for deflazacort is 0.9 mg/kg per day (Fig 2). For a more detailed discussion on glucocorticoid management, see the Supplemental Information.

DIAGNOSIS

Clinical variability, genetic heterogeneity, and the large size of the *DMD* gene contribute to the complexity of diagnosis (Fig 3). Mutational analysis has made the confirmation of a molecular diagnosis possible in the majority of cases. However, diagnosis and prognosis are also dependent on clinical presentation and genetic profile. Genotype-phenotype correlation can help prognosticate, but age of presentation and family history are also important. For example, most exon deletions are correlated with disease severity on the basis of the "reading-frame rule" and apply to ~92% of cases.[28] The vast majority of whole-exon deletions or duplications are correlated with a pathogenic mutation. The reading-frame stop codon caused by an out-of-frame mutation disrupts the open reading frame and ablates the translation of dystrophin, causing a more severe *DMD* phenotype. An in-frame mutation can still translate the open reading frame to a partial-length dystrophin, resulting in Becker muscular dystrophy and a milder phenotype.[29] In some cases, in-frame mutations with worsening myopathy are more clinically consistent with DMD.

The methods used to diagnose DMD include clinical history, physical examination, serum CK, liver enzymes, genetic testing, and perhaps muscle biopsy. In the past, muscle biopsies were routinely performed to diagnose DMD. With advancements in genetic testing strategies, including multiplex ligation–dependent probe amplification, comparative genomic hybridization, Sanger sequencing, and next-generation sequencing, a molecular diagnosis can be obtained from genomic DNA so that muscle biopsies are rarely needed to diagnose DMD. If a *DMD* gene mutation cannot be identified by using genetic testing, clinicians may choose to perform a muscle biopsy to determine the percentage of dystrophin by using histopathological staining and immunoblot analysis. If dystrophin staining is equivocal, the muscle can be sent for RNA transcription testing to identify intronic changes.

In a child with a family history of DMD, a serum CK level is recommended. In a child without a family history, the presence of global development delay, psychomotor delay, and/or motor delay prompts a serum CK level (CK is elevated 50–100 times normal in DMD). The liver enzymes, aspartate aminotransferase, and alanine aminotransferase are also elevated but from the muscle instead of the liver. γ-glutamyl transferase is the preferred laboratory test to check for liver disease in DMD. If CK is elevated, then DNA should

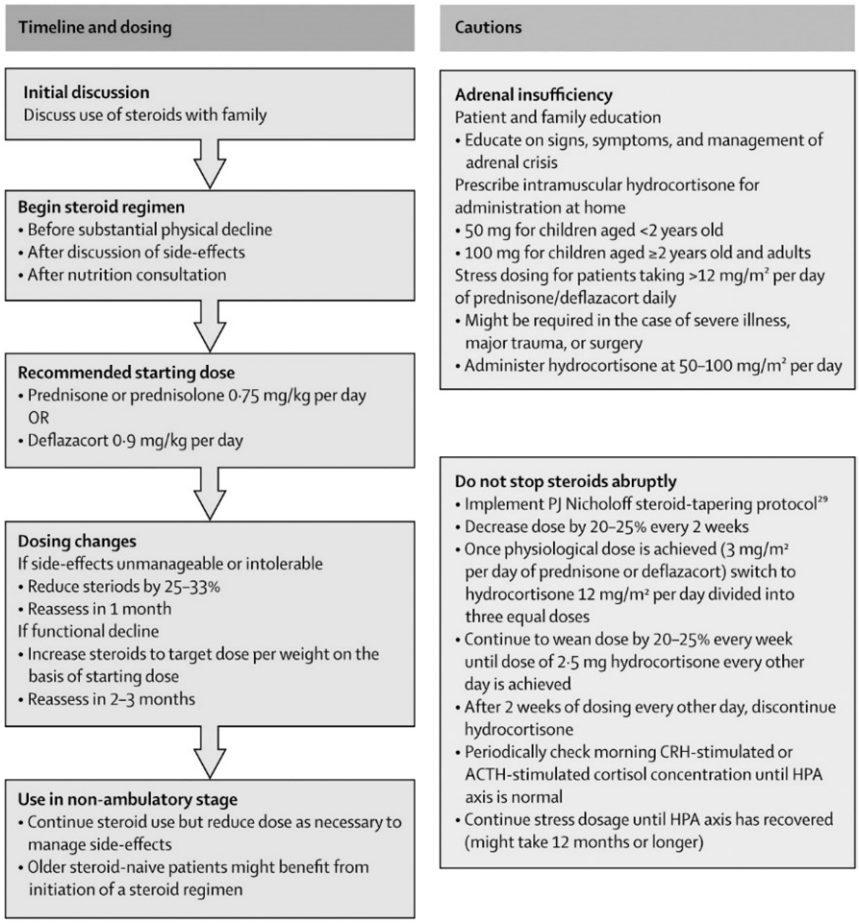

FIGURE 2

Care considerations for glucocorticoid (steroid) initiation and use for patients with DMD. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal. (Reproduced with permission from Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol*. 2018;17(3):255.)

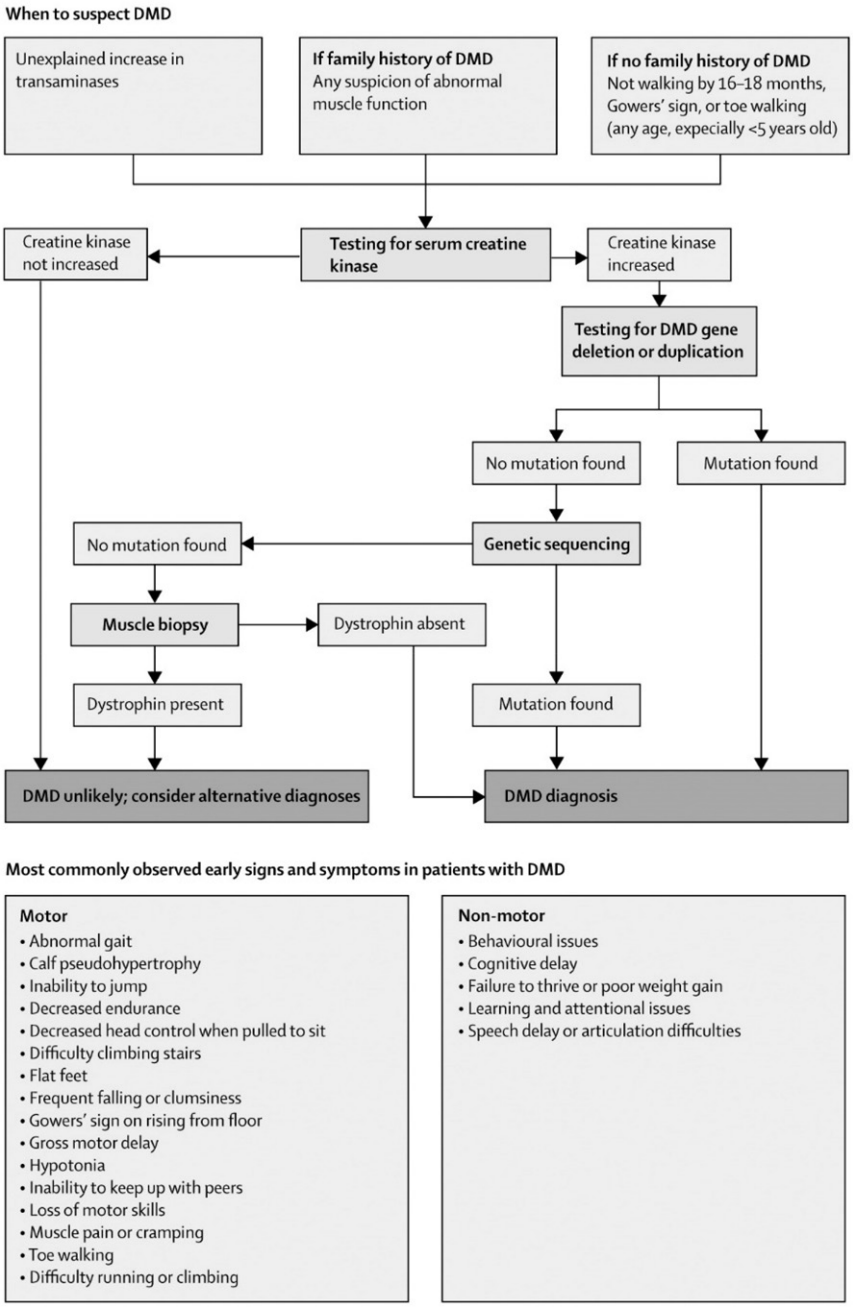

FIGURE 3
Diagnosis of Duchene muscular dystrophy. Described early signs and symptoms of DMD are based on Ciafaloni et al.[10] (Reproduced with permission from Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol.* 2018;17(3):253.)

be sent to test for exonic deletions and duplications, which are seen in ~65% of cases.[30,31] Table 1 includes the types of gene mutations in DMD. Multiplex ligation–dependent probe amplification is the most widely used technique and can be used to test all 79 exons. Comparative genomic hybridization is an oligoclonal-based method that can be used to detect complex rearrangements, intronic alteration, or mutation break points.[30] The evolution of next-generation sequencing has enabled the sequencing of millions of copies of DNA fragments simultaneously and has reduced time and cost.[32]

TABLE 1 Types of *DMD* Mutation

Mutation	%
Deletions	65
Duplications	6–10
Point mutations	25
Complex mutations	<2

If the deletion and/or duplication test result is negative, then sequencing is conducted to identify smaller mutations. Sequencing the entire coding region can reveal small mutations, splicing mutations, or single base changes. If no mutation is identified after sequencing, a muscle biopsy can be obtained to determine the presence or absence of dystrophin by using histologic staining. The absence of dystrophin confirms a diagnosis of DMD. Reduced dystrophin could be associated with Becker muscular dystrophy or DMD depending on the presence and severity of muscle weakness on examination. Dystrophin <3% is consistent with DMD, whereas ≥20% on muscle biopsy suggests that DMD is unlikely. Some variation in muscle biopsy results occurs depending on the age of the child and what part of the affected muscle was biopsied. Alternatively, tissue from the muscle can be tested through RNA transcription analysis to assess for intronic mutations that may have been identified in the other genetic strategies. Immunoblotting of the muscle biopsy also can be used. If no *DMD* mutation is identified, the clinician can consider an alternative diagnosis, such as limb-girdle or congenital muscular dystrophy. Imaging with quantitative magnetic resonance and magnetic resonance spectroscopy reveal an increase in fat fraction with disease progression.[33,34] Given the heterogeneity of affected skeletal muscle in DMD, an

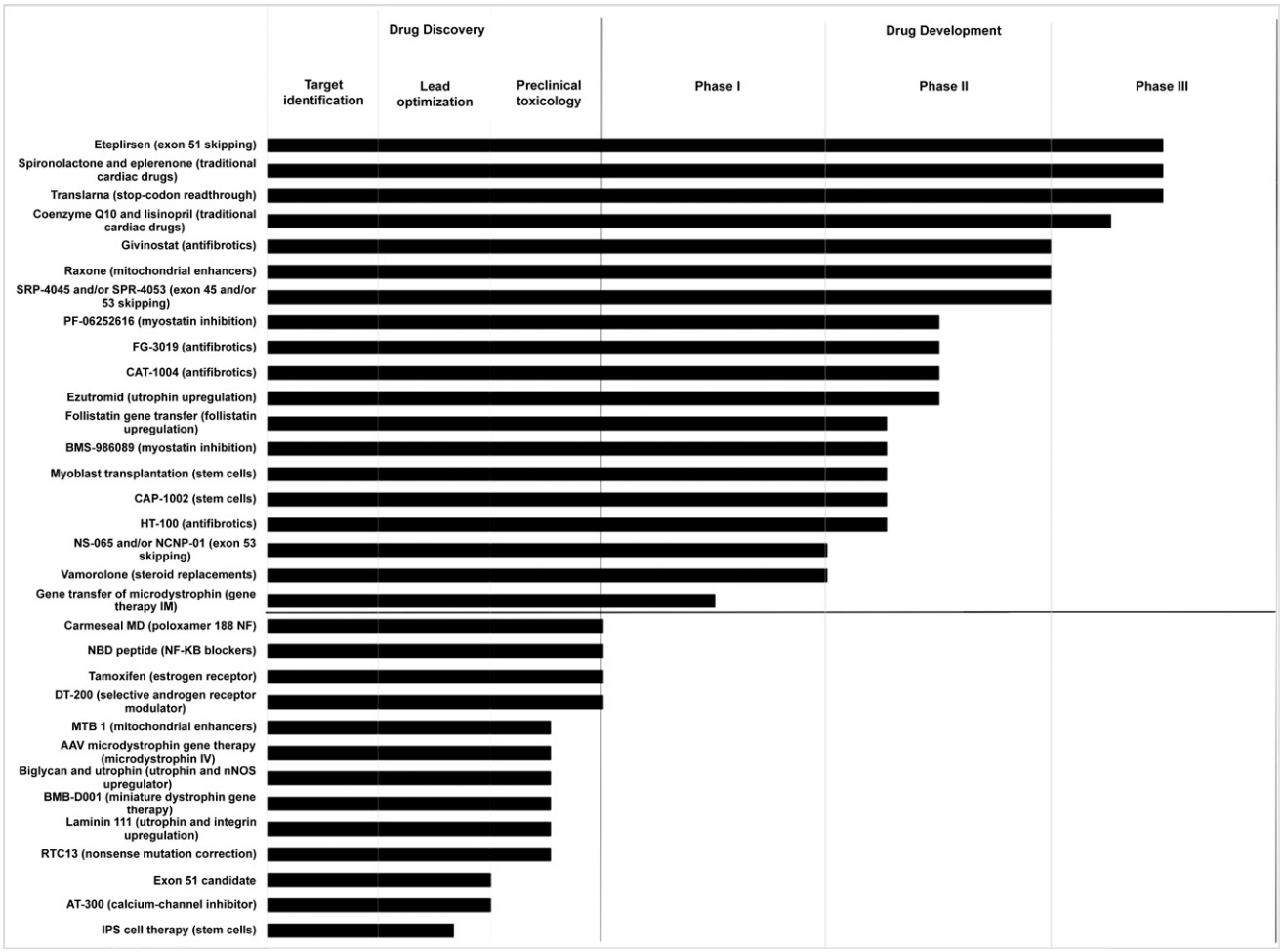

FIGURE 4
Drug discovery chart for DMD clinical trials. Illustrated is the active pace of drug discovery in DMD.

MRI-guided muscle biopsy can be applied in the diagnosis and/or confirmation of DMD when genetic testing is unrevealing.

EMERGING THERAPIES

Numerous treatment strategies have been investigated for DMD. After decades of searching for targets, identifying molecular pathways, and preclinical investigations in animal models, potential therapeutic targets have been translated to clinical trials in DMD. In this section, we review some of the main strategies and active clinical trials. Figure 4 is a chart of the potential drugs in the DMD drug discovery pipeline.

Restore Protein (Dystrophin)

Exon skipping and nonsense readthrough can restore the expression of the gene product dystrophin. These agents generate a functional or partially functional protein in DMD.

Exon Skipping

The concept behind exon skipping is targeted at the messenger RNA level and is based on the reading-frame rule. The intent is to skip (or delete) an out-of-frame mutation and restore the reading frame by making an in-frame mutation that encodes a more functional protein that is consistent with the milder, Becker muscular dystrophy–like phenotype. This technology uses antisense oligonucleotides. Two chemical constructs, 2'-O-methyl phosphorothioate and phosphorodiamidate morpholino oligomers, have been used in clinical studies to bind to exon-splice junctions and generate exon skipping.[35,36] The 2'-O-methyl phosphorothioate drug, drisapersen, for exon 51 skipping, which initially showed improvement on the 6-minute walk test in early clinical trials, progressed to a larger phase III study in which it did not reach its primary end point.[37,38] The phosphorodiamidate morpholino oligomers drug, eteplirsen, is also designed for exon 51 skipping. Researchers in an initial study enrolled 12 patients for >3 years, and these treated patients demonstrated

a slower rate of decline in ambulation compared with untreated historical controls.[39,40] Exon skipping–amenable mutations constitute ~80% of all DMD, of which ~13% of cases are amenable to exon 51 skipping[41] (Table 2). Drisapersen is administered subcutaneously, and eteplirsen is delivered by intravenous infusion. A drisapersen application to the US Food and Drug Administration (FDA) was not approved, but eteplirsen was granted accelerated approval by the FDA while an ongoing confirmatory study is underway.[42]

Nonsense Suppression

Nonsense mutations generate a stop codon, resulting in a truncated, nonfunctional protein. Nonsense (stop-codon) mutations comprise ~10% to 15% of DMD cases.[43,44] The readthrough strategy, which involves the suppression of the stop-codon mutation, encourages the ribosome to read through the stop codon, which promotes the production of dystrophin.[45] Proof of concept for nonsense suppression was initially established with an aminoglycoside: gentamicin.[46] Translarna (ataluren), an oral medication with a better safety profile compared with gentamicin (formerly referred to as PTC124), promotes the readthrough of premature translation termination codons. Safety and tolerability was established, and a phase IIa proof-of-concept trial revealed increased dystrophin posttreatment on muscle biopsy.[47] Due to the unmet need for treatments in DMD and promising early phase findings, this drug has been approved by the European Medicines Agency.[48] A phase III trial revealed enrichment of effect in a subgroup of patients with a baseline 6-minute walk test between 300 and 400 m at enrollment.[49] However, the FDA voted that the data used to support Translarna were inconclusive.

TABLE 2 Exon Skipping–Amenable Mutations

Exon Amenable to Skip	%
51	13.0
45	8.1
53	7.7
44	6.2
46	4.3
52	4.1
50	4.0
43	3.8
6 and 7	3.0
8	2.3
55	2.0

Replace Lost Dystrophin

Utrophin is an analog of dystrophin that shares ~80% sequence homology with dystrophin.[50–52] SMT C1100 is a molecule that works as a utrophin modulator and is an investigational drug with the potential to replace lost dystrophin with utrophin.[53] Safety and tolerability has been shown in a study of 12 patients with DMD.[54] A phase II study is ongoing to evaluate the activity and safety of this orally administered drug in a larger cohort of patients.

Target Signaling Pathways

Anti-inflammatory Approaches

Muscle fibers in DMD undergo inflammatory and fibrotic changes that have been responsive to glucocorticoids. As a result, anti-inflammatory approaches to treat DMD remain a focus in drug discovery. Myofiber necrosis is reported to result from chronic NF-κB activation and tumor necrosis factor α.[9,55,56] Prednisone and deflazacort, used to treat DMD, have anti-inflammatory effects and are presumed to inhibit the NF-κB pathways. However, these drugs are generally nonspecific anti-inflammatory agents with multisystem side effects. New drugs aimed at specifically targeting NF-κB or tumor necrosis factor α are being moved forward in drug discovery and are currently in the preclinical stages, poised to move to clinical trials. Vamorolone (also known as VBP15), an oral anti-inflammatory steroid with fewer side effects in preclinical and phase I trials compared with traditional glucocorticoids, has advanced to phase II in an ongoing, larger clinical trial to assess safety and efficacy.

Antioxidants

Oxidative stress can damage cellular function, specifically mitochondria. The overproduction and/or accumulation of reactive oxygen species can lead to mitochondrial dysfunction in neuromuscular disease. Studies have revealed that increased reactive oxygen species in DMD contribute to membrane permeability and protein degradation and activates inflammatory pathways, thereby exacerbating necrosis and fibrosis. Idebenone, an antioxidant that inhibits lipid peroxidation, has shown efficacy in a phase III trial, with improvement seen in respiratory function (ie, forced vital capacity).[57] Idebenone is orally administered and currently undergoing regulatory review.

Antifibrosis

In DMD, endomysial fibrosis is a hallmark clinical feature associated with muscle weakness and poor long-term outcome; transforming growth factor β (TGF-β) is a target in antifibrosis therapy. Therapeutic agents aimed at blocking cytokine signaling by inhibiting the TGF-β pathway in preclinical models have been shown to decrease fibrosis in some studies, although not in others.[58–61] Losartan, an antihypertensive drug with a known safety profile, was an attractive therapeutic target, but the preclinical studies that initially revealed reduced fibrosis later revealed minimal functional benefit.[62,63] Another agent, oral halofuginone hydrobromide (also known as HT-100), is currently in a phase II trial to assess safety, tolerability, and dose selection for future trials. Givinostat, an oral inhibitor of histone deacetylases

aimed at reducing fibrosis of muscle fibers and promoting muscle regeneration in DMD, is in a phase III global clinical trial.[64]

Phosphodiesterase 5A Inhibition

Diminished blood flow can cause muscle damage. Mendell et al[65,66] showed in the mouse model that functional ischemia produces similar changes seen in muscle fiber degeneration and regeneration in DMD. Researchers in more studies have since implicated the nitric oxide synthase pathway in ischemia and thus inhibition of phosphodiesterase (PDE) as a potential therapeutic target.[67] Sildenafil, an oral PDE 5 inhibitor, was studied in adult patients with DMD and cardiomyopathy. The trial was terminated early due to worsening left-ventricular end-systolic volume in subjects on sildenafil.[68] Tadalafil, another oral PDE inhibitor, was shown to restore blood supply to muscle in the mdx mouse. However, tadalafil had no effect on the primary outcome and did not lessen the decline in ambulatory ability in patients with DMD.[69]

Myostatin Inhibition

The inhibition of the myostatin pathway is a muscle-building therapeutic approach for muscular dystrophy. Myostatin regulates muscle growth by breaking down muscle protein, and blocking myostatin has been shown to increase muscle mass and reduce fibrosis.[70,71] Wagner et al[72] conducted an early phase clinical trial in adult patients with muscular dystrophy using a recombinant human antibody and showed safety, although not efficacy. New strategies and the formulation of an antimyostatin antibody, PF-06252616, have since occurred, and the agent is being used in an ongoing phase II clinical trial in patients with DMD. This agent is delivered intravenously. Similarly, an antimyostatin adnectin, BMS-0986089, is currently in phase I or II trials in ambulatory DMD patients. This agent is delivered subcutaneously.

Improve Cardiac Function

CAP-1002 is a novel therapeutic approach in which cell therapy is used to treat cardiomyopathy in DMD. This investigational treatment is already being investigated as a treatment of postmyocardial infarction. The agent is an allogenic cell therapy derived from human heart tissue that is administered directly to the heart through the coronary arteries by cardiac catheterization. This drug is currently in the phase I or II study stage to investigate safety and tolerability.

Eplerenone, a drug that is already used in heart failure and has a known safety profile, has been investigated in a randomized, placebo-controlled clinical trial in patients with DMD and early cardiomyopathy who are already taking an angiotensin-converting enzyme inhibitor. After 12 months, it was reported that a statistically significant reduction in left-ventricular strain occurred in the treatment arm compared with a placebo.[73] Although long-term benefits cannot be concluded from these findings, longitudinal data from this cohort will provide greater insight on the potential benefit of this cardiac intervention in DMD. Results after a 2-year study revealed that eplerenone can be cardioprotective in patients with DMD.[74]

Restore Gene Function

In a monogenetic disorder, such as DMD, the idea of replacing a defective gene with a "corrected" gene seems simple, but many challenges have been encountered to date. Strategies on how to target the desired muscle, circumvent the immune response, and achieve efficient systemic delivery are some of the major hurdles.[75] The discovery of an adeno-associated virus (AAV) to be a safe and effective therapeutic tool in gene transfer has addressed some of the technical barriers.[76,77] However, AAV has a limited capacity to package the entire *DMD* gene.

Gene Replacement

In DMD, the large size of the *DMD* gene poses the challenge of packaging into an AAV. Mini-dystrophins (ie, miniaturized versions of the *DMD* gene) have been developed.[78,79] Investigators also have packaged the gene into 2 vectors in a dual-AAV approach and showed that it restored sarcolemmal neuronal nitric oxide synthase expression in dystrophin-deficient mice.[80] Transgene delivery by an AAV through intramuscular injection in a phase I clinical trial in humans revealed an immune response to the transgene product, bringing attention to the role of T-cell immunity to self- and nonself-dystrophin in the study design of future gene therapy trials.[81] Limb vascular delivery is another method to regionally deliver the gene.[82] Systemic delivery can be achieved intravenously, but targeting the gene to muscle and limiting its off-target delivery are important aspects of the gene delivery. Although gene therapy is still in early stages, the knowledge gained from the pioneering work of many dedicated investigators over several decades has taken the field closer to becoming a potential therapy. Phase I and II studies to deliver microdystrophin C with AAV vectors are anticipated to begin this year.

Gene Editing

A novel genetic engineering technology, clustered regularly interspaced short palindromic repeats (CRISPR) coupled with an endonuclease (CRISPR-associated protein 9), has been applied to edit mutations in the *DMD* gene. Researchers in several laboratories have reported that gene editing can be used to improve muscle function in the mouse model and muscle stem cells. Investigators have shown

that the editing tool was used to resect the faulty exons and partially restore protein in mice.[83–87] The mice treated with CRISPR did better on functional tests compared with the untreated group but not as well as the normal mice. Although gene editing with CRISPR is closer to clinical translation now than ever before, it may be years before these technologies achieve efficiency toward clinical trials.

Target Genetic Modifiers

The vast clinical variability in DMD is well known, and environmental factors can contribute to the genetic modifier effect and heterogeneity of phenotypic features. Latent TGF-β binding protein 4 can be used to predict the age of loss of ambulation.[88,89] *SPP1*, a gene encoding osteopontin, acts as a pharmacodynamic biomarker of steroid response.[89] These 2 pathways converge in the regulation of TGF-β. *ANXA6* encodes annexin A6, a calcium-binding protein, and is correlated with sarcolemmal repair in the mouse model.[90,91] These modifier genes are correlated with phenotypic variation and serve as novel therapeutic targets for future drug discovery.

FUTURE DIRECTIONS

As disease-modifying treatments emerge, patients with DMD will live longer, and they will need to be prepared to live as independently as possible. Care centers will need to incorporate health service considerations into their care plans. The molecular diagnoses stored in "unified" databases, which include molecular profiles and well-characterized phenotypes, can be used to augment the identification and selection of more homogeneous candidates for specific therapies as gene-derived strategies are translated to the clinics. As genetic modifiers are validated, biomarkers and novel therapeutic targets can be developed. Putative disease-modifying strategies related to cardiac function and neuronal involvement in DMD warrant further exploration for novel targets.

CONCLUSIONS

The *DMD* gene was identified ∼3 decades ago, and the presteroid era changed after prednisone was shown to prolong ambulation.[12] Glucocorticoids remain the standard of care, but significant side effects limit their therapeutic window.

As survival has improved with the launch of specialized care centers and better respiratory support, cardiac disease has become a more important cause of death. The majority of the investigational drugs improve skeletal and pulmonary function, and cardiomyopathy remains a critical area in need of effective treatment. Furthermore, addressing neurodevelopmental needs and providing early intervention are necessary. As the translational community shepherds novel therapeutics into the clinic, other initiatives are equally important, including newborn screening, management strategies for infants and toddlers, neurobehavioral management for school-aged children, transition management, and adult considerations for affected men. Newborn screening and effective treatments may ultimately extend survival in DMD beyond the third decade.

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ABBREVIATIONS

AAV: adeno-associated virus
CK: creatinine kinase
CRISPR: clustered regularly interspaced short palindromic repeats
DMD: Duchenne muscular dystrophy
FDA: Food and Drug Administration
PDE: phosphodiesterase
TGF-β: transforming growth factor β

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