Update in Duchenne and Becker muscular dystrophy

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\textbf{Purpose of review}
The purpose of this review is to highlight updates in the standard of care recommendations for DMD, and to describe approaches to and recent advances in genetic therapies for DMD.

\textbf{Recent findings}
Treatment of DMD patients with the corticosteroids prednisone or deflazacort remains the standard of care, and recent data shows that early treatment (as young as 5 months) with a weekend dosing regimen results in measurable improvement in motor outcomes. A mutation-specific therapy directed at restoring an open reading frame by skipping exon 51 is FDA-approved, and therapies directed at other exons are in trials. Gene replacement therapy shows significant promise in animal models, and trials are underway. Genome editing has received significant attention because of results in animal models, but challenges to implementation in humans remain.

\textbf{Summary}
The mainstay of treatment remains meeting well defined standards of care that have been shown to influence morbidity and mortality. These include use of systemic steroids, early nocturnal ventilatory support, appropriate cardiac care and prophylaxis, and wherever appropriate, scoliosis surgery. Early and accurate molecular diagnosis, along with appropriate and multidisciplinary care, provides the best opportunity for maximum benefit of both current standard and upcoming novel therapies for boys with DMD. Among the most promising of these is AAV-based gene replacement therapy, which is currently in clinical trials.

\textbf{Keywords}
corticosteroids, Duchenne muscular dystrophy, gene therapy, genetic therapies

\textbf{INTRODUCTION}
The dystrophinopathies represent a range of phenotypes caused by mutations in the \textit{DMD} gene. The most common and severe phenotype, Duchenne muscular dystrophy (DMD), has an incidence of \(<1:5000\) male live births and has onset in early childhood with slowly progressive skeletal and cardiac muscle weakness where boys are often wheelchair bound in the second decade and succumb to cardiorespiratory compromise in the late second to early third decade of life [1–4]. The milder Becker muscular dystrophy (BMD) has a much wider range of severity. Symptoms may range from limb-girdle weakness leading to loss of ambulation late in the second decade, to uncommon syndromes consisting of myalgias in youth and young adulthood but little effect on ambulation until late adulthood [5]. Both DMD and BMD are because of mutations in the X-linked \textit{DMD}, with clinical variability due to whether the mutation truncates the reading frame, resulting in the absence of the protein product dystrophin, or whether it maintains a reading frame that allows expression of a partially functional dystrophin. The diagnostic and therapeutic implications of this ‘reading frame rule’ are discussed in detail later in this article [6].

DMD was first described clinically in the 1860s, but the gene was not identified until 1986 and the protein product, dystrophin, the following year [7–9]. One key function of dystrophin is to maintain the integrity of the myofiber membrane during...
force generation, which it achieves by providing linkage between cytoskeletal actin (via binding of the dystrophin N-terminus) and the extracellular matrix (via binding of the dystrophin C-terminus to the transmembrane dystroglycan complex). In the absence of dystrophin, loss of membrane integrity leads to fiber degeneration, exhaustion of regenerative capacity, and the fibrosis and fatty replacement of muscle that leads to the clinical features.

The standard therapy for DMD is oral corticosteroids. Although patients were first treated with prednisone in 1974, it was not until shortly after discovery of the protein, that several studies were conducted evaluating daily prednisone for use in DMD and a benefit in terms of functional improvements were seen [10–12]. However, because of concerns about side effects, prednisone was not widely used and management of the disease varied widely nationally and internationally [13]. Over time, practice parameters were developed, but it was not until 2010 that the first international guidance for the care and management of DMD was published [4,13,14]. This consensus guidance has recently been updated, and the interested reader is referred to these detailed recommendations [15**–17**]. In this review, we will highlight some of these current standard of care recommendations for DMD, and promising upcoming therapies for the dystrophinopathies.

CORTICOSTEROIDS

Prednisone use has been the mainstay of treatment for DMD since the 1980s and more recently deflazacort, an oxazoline derivative of prednisolone, became an alternative option that is at least equally efficacious with a slightly different but similar side effect profile [10,12,18]. Corticosteroids are known to increase muscle strength and delay loss of ambulation, although use was largely variable until the first international guidance on care for patients with DMD was published [4,13,14,19]. Nevertheless, the optimal dosing regimen remains unclear and a clinical trial evaluating daily prednisone, daily deflazacort and the 10 days on, 10 days off regimen is underway (FOR-DMD, NCT01603407). Although not included in the FOR-DMD clinical trial, weekend dosing remains an attractive alternative as it is equally as effective as daily prednisone and may have fewer side effects [20]. Weekend dosing was also recently studied in infants showing that initiation of weekend dosing at ages as young as 5 months was well tolerated and resulted in improved motor outcomes [21]. Current recommendations include initiation before substantial physical decline, typically by the age of 5, using daily dosing of either prednisone 0.75 mg/kg/day or deflazacort 0.9 mg/kg/day [22]. As earlier treatment may result in larger benefit in regards to loss of ambulation, many clinicians elect to initiate weekend dosing around age 3 years, using 5 or 10 mg/kg of prednisone per week dividing into two doses delivered on each Saturday and Sunday.

CARDIAC CARE

Cardiomyopathy is a major cause of morbidity and mortality in DMD. The absence of dystrophin leads to progressive fibrosis, initially manifesting as a subclinical cardiomyopathy that progresses to a dilated cardiomyopathy and heart failure. Early cardiac screening with an electrocardiogram and an echocardiogram or cardiac MRI is recommended yearly starting at diagnosis [23]. Early initiation of an Angiotensin converting enzyme inhibitor (ACEi), prior to developmental of abnormal cardiac function, has been shown to have a protective effect and the current recommendation is to start an ACEi around age 10 [23]. A recent randomized double-blind placebo-controlled trial of enalapril and metoprolol in 38 DMD boys aged 10–14 years suggested a delay in progression of cardiac disease, but did not reach statistical significance [24]. As optimal timing of prophylactic medication is unknown, a randomized placebo-controlled trial comparing an ACE inhibitor versus a beta-blocker is underway in boys with DMD ages 5–13 years [25].

Outstanding questions remain for optimal cardiac care in DMD [26,27]. These include determining the optimum combinatorial regimens in DMD, and to determine the optimum patient population that may benefit from supportive or destination therapies with interventions, such as the left ventricular assist device [28,29]. A recent article of interest addressed therapy with allogenic cardiosphere-derived cells (CDCs), to which have been attributed antifibrotic, anti-inflammatory, and regenerative properties via...
secretion of growth factors and mRNAs [30]. A potential therapeutic benefit was suggested by a small (n = 25), open-label randomized trial of direct infusion of 75 million cells into the coronary arteries of patients, in which cardiac MRI suggested diminished fibrotic burden and improved regional myocardial function at 6 and 12 months; a subsequent randomized blinded trial is underway [31].

RESPIRATORY CARE
The progressive loss of skeletal muscle strength in respiratory and bulbar muscles leads to respiratory compromise and an increased risk for significant morbidity and mortality. Regular monitoring of pulmonary function via pulmonary function tests and monitoring of scoliosis are important screening measures [32]. Implementation of several preventive measures and interventions guided by the above assessments including nocturnal noninvasive ventilation, cough assist, and vest therapies, in addition to scoliosis surgery and management of dysphagia have greatly reduced complications and improved quality of life [14]. Corticosteroids, in addition to the interventions mentioned above, have greatly improved respiratory function via increased skeletal muscle strength and subsequent less severe scoliosis [33–35], which can directly impact ventilatory function. Although nocturnal ventilator support has long been known to have an impact on morbidity and mortality, the coincident correction of spinal scoliosis in appropriate patients can increase survival by nearly a decade [36,37].

GASTROINTESTINAL CARE
Dystrophin is also expressed in smooth muscle, and absence of dystrophin in the gastrointestinal tract can lead to several complications. Constipation, reflux and delayed gastric emptying are complications that can have a significant impact on quality of life. Gastrointestinal symptoms should be specifically assessed at each follow-up visit and, if unavailable in the multidisciplinary clinic, consultation with a gastroenterologist may be indicated [38]. Boys with DMD are at risk for both obesity (particularly earlier in the disease course, and while undergoing steroid treatment) and malnutrition (later in the disease); a registered dietician is thus an essential component of any multidisciplinary clinic [38]. However, nutritional management remains difficult in this population because of a lack of disease-specific growth charts and difficulty in assessing height once the patients are nonambulant which make it difficult to accurately determine BMI.

BONE HEALTH
Decreased bone mineral density is a known feature of DMD, which is worsened by corticosteroid use placing these patients at a higher risk of vertebral compression fractures and long bone fractures. A daily multivitamin is recommended in patients with poor variety in their diet and assessment of vitamin D status yearly to ensure the level is maintained above 30 ng/ml [38,39]. Monitoring of bone health should begin at diagnosis or onset of corticosteroids with lateral spine films at least every 2 years and a dual-energy X-ray absorptiometry yearly in addition to a thorough history at every follow-up visit. In patients who have had fractures, consultation with an endocrinologist or complex care pediatrician for consideration of a bisphosphonate is recommended [39].

MOLECULAR DIAGNOSIS
The diagnosis of DMD requires an appropriate clinical suspicion based on clinical features, which typically include parental recognition of weakness or gait abnormalities in the years 2–5. However, motor function is clearly affected earlier, and can lead to a delayed onset of ambulation, and there is growing recognition that cognitive features may be an early presenting syndrome. The presence of either, but particularly of motor delay, should lead to testing for an elevated serum creatine kinase.

The presence of an elevated creatine kinase typically leads to mutational testing from lymphocyte-derived DNA by one of a variety of methods. The key principle for an accurate genotyping of the specific DMD mutation is that in the presence of a deletion of one or more exons in the gene, all 79 exons should be assessed for copy number in order to establish the extent of the deletion. Such deletions account for around 65% of all dystrophinopathy mutations, and exon duplications account for another 6–11%. Accurate exon copy number assessment can be obtained via several different approaches, including multiplex ligation-dependent probe amplification (MLPA), complementary genomic hybridization (CGH) array analysis, PCR-based assays or determination of comparative read depths on next generation exon sequencing assays. If no exon deletion or duplications are found, sequencing of the entire coding region is required to establish the presence or absence of point mutations, such as nonsense mutations, or small insertions or deletions.

Muscle biopsy still has a place in the diagnosis of dystrophinopathies. Importantly, up to 7% of dystrophinopathy patients may have mutations that are undetectable by genomic DNA analysis, and
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many have deep intronic mutations that result in the inclusion of intronic sequence as pseudoexons that require analysis of muscle-derived mRNA to identify. Muscle biopsy also remains useful in assessing dystrophin protein expression in patients where the observed phenotype does not correlate with the phenotype predicted by the application of the ‘reading-frame rule.’ The most common category of these mutations consists of predicted nonsense, out-of-frame mutations (as identified from blood) that actually affect mRNA splicing and resulting in significant amounts of in-frame transcript and sufficient protein expression to alter the clinical course.

GENETIC THERAPIES

A major rationale for accurate genetic diagnosis is to make use of a growing number of mutation-specific therapies. In general, these are directed toward restoring an open-reading frame. The first of these is the antisense phosphorodiamidate morpholino oligomer (PMO) eteplirsen, directed toward altering splicing of the dystrophin mRNA to exclude exon 51. This therapy is applicable to multiple mutations; as examples, deletions of exons 45–50, 48–50, 50, or 52 (among others) are all predicted to have reading frames restored by exon 51 skipping, and are thus, amenable to eteplirsen treatment. Although treatment restores a relatively small amount of dystrophin, the antisense PMO showed improved ambulation and respiratory effects [40,41], and more favorable results than a trial of a competing 2’O-Me phosphorothioate antisense oligonucleotide [42]. Following its Food and Drug Administration (FDA) approval, eteplirsen is in widespread clinical use; further treatment trials with morpholinos directed to other exons (casimersen for exon 45, and goldeisen for exon 53) are underway. Cocktails of PMOs may ultimately be combined to generate a therapy that addresses skipping larger regions, such as exons 45–55, which would be therapeutic for up to 63% of patients [43]. Another mutation-specific therapeutic approach is nonsense suppression, such as with the drug ataluren, although that drug failed to show a convincing benefit in a randomized placebo controlled trial and is not marketed in the United States [44].

VIRAL GENE THERAPY

Among the most promising experimental therapies is gene replacement using adenoassociated viral (AAV) vectors. AAV is not associated with human disease, and AAV genomes are essentially nonintegrating into chromosomes, factors that provide a margin of safety for considering therapeutic development. Different AAV serotypes have differential tropism to human tissues, and utilization of muscle tropic AAVs in combination with appropriate promoters has allowed the development of vectors designed for treatment of muscle diseases. The power of AAV gene replacement has recently been demonstrated by the remarkable results achieved in patients with spinal muscular atrophy type 1 (SMA1) treated with an AAV9 vector carrying the full-length SMN cDNA. The extraordinary survival benefit resulting from this treatment led to FDA approval of this viral therapy under the trade name Zolgensma in May 2019.

A challenge to viral gene replacement for DMD is that unlike the case with the SMN cDNA, the full-length DMD cDNA is too large, at approximately 11.5 kilobases (kb), to fit into an AAV genome, which has a maximum packaging capacity of around 5 kb. As a result, several groups have designed microdystrophin genes, essentially based upon the reading-frame rule; these encode miniaturized DMD constructs that encode protein products in which critical functional domains are included – generally, critical N-terminal and C-terminal-binding domains, with differing inclusion of other regions. A full discussion of the differences among these is beyond the scope of this article, but the interested reader is referred to a recent detailed review [45**] Three such competing microdystrophin trials are underway at present (sponsored by Pfizer, Sarepta Therapeutics, and Solid Therapeutics), with unpublished (to date) reports of early promising results. The ultimate durability of such therapy remains to be seen, as does the ultimate beneficial effect of the engineered microdystrophins, as no natural mutations as seen in BMD exist to inform expectations. Nevertheless, preclinical data for each construct is promising, and the results of each trial are eagerly awaited.

An alternate approach to viral gene therapy is the delivery of surrogate genes that can substitute for dystrophin function. One such example is the GALGT2 gene, which encodes an O-mannosyltransferase responsible for the terminal glycosylation of dystroglycan at the neuromuscular and myotendinous junctions, where utrophin replaces dystrophin in the dystroglycan-associated protein complex. Expression of exogenous GALGT2 via a viral vector results in expression of the glycosylated dystroglycan epitope and localization of utrophin across the entire myofiber, resulting in improvement in mouse models [46,47], and a pilot trial of gene delivery in humans is underway. Another promising approach delivers noncoding small nuclear RNAs (UT7snRNA) with antisense sequences directed toward exon definition elements; these RNAs can induce highly...
efficient exon skipping, with promising results in animal models and planning for near-term trials underway [48,49].

**GENOME EDITING**

Recent advances in genome editing at the DMD locus in model systems have raised interest in the prospect of somatic editing as a therapeutic approach. Most such approaches have utilized CRISPR/Cas9 systems, with guide RNAs (gRNAs) directing endonuclease activity to induce excision of one or more exons in order to restore an open reading frame [50]. CRISPR/Cas9 editing has shown promising results in both mouse and canine models [51,52*]. Such an approach can theoretically extend to excision of the entire region of exon 45 to exon 55, with the previously stated goal of treating the largest number of DMD patients [53]. However promising, multiple challenges remain prior to clinical trials. There are concerns regarding germline alteration, off-target effects, and immune responses to the bacterial Cas9 proteins used in many strategies. Depending upon the Cas9 used, packaging of the Cas9 gene along with the necessary gRNAs into a single AAV vector may not be possible, suggesting a need for dual vectors, and overall efficiency of editing remains a challenge. Despite enthusiasm of the patient community, genome editing therapy will likely require significant further preclinical studies.

**CONCLUSION**

There is great excitement around the possibility that in the near future there will be novel therapies that may significantly alter the course of DMD, and the impact of these therapies will be maximized by the development of newborn screening strategies that result in presymptomatic diagnosis [54]. Nevertheless, the mainstay of treatment remains meeting well defined standards of care that have been shown to influence morbidity and mortality. These include use of systemic steroids, early nocturnal ventilatory support, appropriate cardiac care and prophylaxis, and wherever appropriate, scoliosis surgery. Nevertheless, the promise of novel therapies is significant. Importantly, the prospect of gene-directed therapies, including viral gene replacement, supports the need for early diagnosis and treatment, the rationale for which is increasingly well documented. Early and accurate molecular diagnosis, along with appropriate and multidisciplinary care, will provide the best opportunity for maximum benefit of novel therapies in each boy with DMD.

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**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- **of special interest**
- **of outstanding interest**

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