ABSTRACT: Duchenne muscular dystrophy (DMD) is one of the most commonly recognized dystrophinopathies. There are no approved therapeutic options available for this disease but recent discoveries have led to hope that effective therapies might be forthcoming. With funding from patient advocacy groups, private investors, and governmental bodies such as the Food and Drug Administration Office of Orphan Product Development (FDA/OOPD), gene modification and other molecular therapies are being actively investigated. However, since DMD patients are few in number and disease manifestations vary considerably in early and late stages of disease, obtaining the data needed for full evaluation of putative therapies may prove challenging. Should ambulation remain the focus of Phase 2/3 studies or should consideration be given to the primary causes of late-stage morbidity and mortality, e.g., cardiac and respiratory dysfunction related to reduced or absent dystrophin production? It seems reasonable to argue that clinical trials planned for DMD should consider the entire population.

Duchenne muscular dystrophy: drug development and regulatory considerations

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Therapies designed to cure or reduce the morbidities associated with Duchenne muscular dystrophy (DMD) dangle before us like Tantalus’ grapes. In recent years, molecular genetics has opened new avenues of discovery as well as potential therapeutic options. With the advances in genetic analysis and the promise implicit in our enhanced ability to identify pathology, we may anticipate new therapies for rare diseases. While we have no new approved therapeutic options to date, there are products in preclinical testing as well as in Phase 1–3 clinical trials. While this is gratifying, it causes us to face new challenges. Neurological diseases that used to lead to death in early adolescence no longer uniformly carry that dismal prognosis. With better supportive care the demographics of the patient population have changed; this change leads to new questions regarding the development of safe and effective new therapies.

DMD was first described by Duchenne, a French neurologist, in 1868. DMD is the result of an almost complete absence of dystrophin, a 427 kDa protein normally found in skeletal, cardiac and smooth muscle, brain, and retinal tissues. Becker muscular dystrophy (BMD), a closely related condition with a slower rate of disease progression, is the result of a reduction in dystrophin. The lack of adequate dystrophin in skeletal muscle results in an inexorable progression of muscle weakness throughout the body and leads to loss of independent ambulation by adolescence in DMD or early adulthood in BMD. While both conditions are predominantly inherited as X-linked recessive disease with identified abnormalities in...
the dystrophin gene (Xp21), there is known to be a high sporadic mutation rate.\(^1\) The muscle cell death is due to a repeated combination of insults that ultimately outpace any muscle regenerative activity. These insults, which include membrane instability, oxidative damage, excess proteolytic activity, and impaired mitochondrial activity, exhaust the regenerative capacity of the muscle tissue.\(^2,^3\) In addition to the overt symptoms caused by skeletal muscle insufficiencies of the limbs, clinicians see morbidity secondary to both scoliosis and cardiac muscle involvement resulting in worsening of restrictive lung disease and cardiomyopathy. Respiratory and/or cardiac failure is the usual cause of death.

With an incidence of 1/3,500 male births and an estimated prevalence of 17,951 cases in the United States,\(^1\) DMD is rare enough to qualify as an orphan disease under the 1983 Orphan Drug Act. Additionally, it is considered a “life-threatening and severely debilitating illness,” and as such it is eligible for special regulatory provisions under the Federal Regulations that govern the Food and Drug Administration (FDA; 21 CFR 312.80-88-Subpart E). These latter provisions reflect the recognition that patients with life-threatening illnesses and their physicians may accept greater risks or adverse effect rates from products intended to treat such illnesses. This recognition may affect the amount of safety data needed at the time of drug approval.

Considerations for proprietary information constrain the authors from discussions of investigational new drug applications (INDs) and New Drug Applications (NDAs) for DMD and BMD, but we will summarize publicly available Orphan Drug Designations/Grant information as well as some information on potential therapies that have been reported in the medical literature.

While people with DMD have been treated off-label with agents such as steroids and gentamicin, there are now multiple investigational agents entering preclinical and clinical trials. Newer genetic methods hold promise for finding the disease-causing mutation(s) within the dystrophin gene in almost all cases. Genetic diagnostic techniques and potential treatments are now leading to cautious optimism that disease modifying therapies may be forthcoming. However, to date, no therapies have received FDA approval for the specific treatment of any of the morbidities associated with Duchenne/Becker muscular dystrophy. In an effort to advance the development of potential therapies for muscular dystrophy, the Office of Orphan Product Development (OOPD) has granted Orphan Drug Designation to the following products:

- 1997 Oxandrolone (Savient Pharmaceuticals)
- 2004 Recombinant human anti-growth and differentiation factor 8 (Wyeth)
- 2004 PTC124 (PTC)
- 2005 PRO046 (Proensa BV)
- 2005 L-aminoacarnityl-succinyl-leucyl-argininal-diethylacetal (Ceptor)
- 2006 Idebenone (Santhera)
- 2007 AVI-4658 (AVI BioPhama)

**THERAPIES**

**Current “Gold Standard” Therapy?** Off-label prescription of corticosteroids, specifically prednisone and deflazacort, has been utilized for decades to increase the size and/or number of muscle cells and potentially slow muscle breakdown.\(^4,^5\) Studies of these treatments have generally been historically controlled, which can cause problems if the drug effect is not large; however, there is evidence from randomized controlled trials that glucocorticoid therapy may be associated with retention of muscle strength and function for up to 2 years.\(^5\) Boys treated for 2 or more years with deflazacort are reported to have experienced a 3–5-year postponement in wheelchair use, decreased scoliosis, and a decreased need for nocturnal ventilator support; however, growth stunting and cataract formation were noted.\(^6\)

**Newer Therapies Being Explored.** The DMD phenotype may arise from deletions, duplications, or mutations of one or more exons within the large dystrophin gene. BMD patients are known to be less severely affected than patients with DMD. These observations have led to interest in exon-skipping therapies designed to create a BMD phenotype.\(^7\) In preliminary in vitro studies, investigators were able to produce dystrophin in 75% or more of the myotubes from two patients with DMD by inducing exon 45-skipping in less than 20% of mRNA.\(^8\) In 2007, this same group reported induction of dystrophin production with intramuscular injection of an antisense oligonucleotide in a group (\(n = 4\)) of patients with DMD.\(^9\) Other researchers have combined variants of antisense oligonucleotides with various cell-penetrating peptides to facilitate cell delivery. One such conjugate,
which skips the stop codon found in dystrophin exon 23, demonstrated that dystrophin production could be enhanced in cardiac (up to 7 weeks) and diaphragmatic/skeletal muscle (up to 17 weeks) when the conjugate was administered to mdx mice, a model with a nonsense mutation that leads to a DMD phenotype.10

Viral-mediated gene transfer therapy has been considered a possible treatment modality. Since the size of the dystrophin gene exceeds the 4.5-kb carrying capacity of recombinant adeno-associated viruses, “microdystrophins” of 4 kb or less have been developed.1,11 While microdystrophin transfer has had varying degrees of success in the mdx mouse, concern has been raised about effect duration. Immunosuppression, which has been considered an adjunctive therapy to gene therapy, has had demonstrated efficacy in animal models.12 Since many males with DMD/BMD are living into adulthood, the discovery that even 20-month-old (elderly) mice demonstrated enhanced dystrophin expression upon administration of gene vectors containing microdystrophin has raised the hope that patients with advanced disease may benefit.13 Townsend et al.11 were able to ameliorate aspects of diastolic dysfunction using recombinant adeno-associated virus based gene transfer of micro-dystrophin into an mdx mouse model.

In experimental models, aminoglycosides have been shown to allow read-through of premature stop codons as a result of nonsense mutations. Since some DMD patients are known to have a nonsense mutation within the dystrophin gene, gentamicin has been used in an attempt to allow appropriate gene transcription. In the mdx mouse model, subcutaneous injection of gentamicin led to full-length dystrophin production at low levels; a study in humans did not reproduce the results of the mouse study.14 Like aminoglycosides, PTC 124 allows ribosomes to read through nonsense mutations to the appropriate termination (stop) codons. Welch et al.15 reported that PTC124 allowed increased dystrophin production in both rodent and human models of muscular dystrophy due to nonsense mutations. This product is currently being evaluated as a treatment for nonsense mutation-associated DMD.

Therapies that target end-organ damage are also under active investigation. MYO-029 is designed to inhibit the negative regulation of muscle growth by myostatin. An early dose-ranging safety study in adult humans was not powered to demonstrate efficacy but did show a trend toward increased muscle bulk; it remains to be seen whether this therapy will result in clinically meaningful results for patients.16 Debiopharm-025 (D-MeAla³EVal⁴-cyclosporin), a nonimmunosuppressive inhibitor of cyclophilin, was able to partially reduce necrosis in both diaphragm and soleus muscles in a mouse model of muscular dystrophy.3 Idebenone, an analog of coenzyme Q10, has been considered a potential treatment due to its protective effect against lipoperoxidation, its stimulation of mitochondrial function, and its improvement of the myocardial energy production in cardiac hypertrophy.17 Although the published studies were done in Friedreich ataxia patients, there is thought to be a potential for benefit for DMD-associated cardiac and skeletal muscle morbidity.18,19

DISCUSSION

Challenges and Concerns. Our enthusiasm about the research avenues being investigated must be tempered by acknowledgement of the constraints and challenges of clinical trials in this population. Pediatric neuromuscular disease presents a unique challenge, because patients lose muscle function as they grow into adolescence. The therapies, if not definitively curative, must provide a risk/benefit ratio acceptable to patients as well as their caregivers; these two parties may not calculate risk/benefit in the same way. There are effectively two distinct patient populations in DMD: ambulatory persons and nonambulatory persons. Ambulatory boys and their parents/caregivers are likely to value preservation of independent ambulation as long as possible. In the nonambulatory population, the major concerns turn to maintaining upper body muscle function as well as potential reproductive function. Additionally, consideration must be given to the social implications of obesity and delayed secondary sex characteristics associated with the long-term use of steroids. To date, most DMD studies have focused on the ambulatory group, generally using a measure of ambulation as the primary endpoint. However, even if drugs fail to preserve independent ambulation but slow progression to wheelchair use, a significant benefit will have been achieved in that the disease will have stabilized.

It may be argued that the preservation of ambulation, an outcome that is undeniably important to the ambulatory preadolescent boy and his parents, is irrelevant to the older nonambulatory population. Since the cardiac and pulmonary complications usually do not manifest in prepubescent
boys, these aspects of the disease cannot be adequately monitored in studies of ambulatory boys.20,21,22,23 The standard use of corticosteroids in an attempt to preserve skeletal muscle strength and ambulation may lead to obesity and elevations in blood pressure with attendant cardiac effects; there have been no formal long-term studies to assess the potential cardiovascular effects of chronic steroid use in this population. In an mdx mouse model, genetic repair of skeletal muscle (as the sole target) increased motor activity, but that increased activity led to additional stress on the diseased heart muscle.24 Dilated cardiomyopathy and/or cardiac arrhythmias are the predominant cardiac abnormalities seen in these patients. Although heart failure may have an early and insidious onset, it is still detected late in the clinical course due to physical inactivity and the lack of classic signs and symptoms of heart failure.25 In addition, there are often changes in body habitus (e.g., scoliosis, contractures) that may limit standard echocardiogram evaluation.

Concerns Related to the Nonambulatory Subset of the Population. Even if a given product delays progression to wheelchair use, DMD patients will spend a significant part of their lives in a nonambulatory state. It seems clear that the consequences of DMD-associated weakness other than in the lower limbs should be assessed, such as the primary causes of late-stage morbidity and mortality in this population, e.g., cardiac and respiratory dysfunction related to reduced or absent dystrophin production. Drugs that might show primarily cardio/pulmonary preservation or improvement may be missed by focusing solely on ambulation; conversely, any potentially detrimental effects on cardiac/pulmonary function might not be appreciated. Without data on the cardiac and pulmonary effects of the drugs in the patients who experience them the most, the risk/benefit assessments in these older subjects will be limited.

During the July 2008 Parent Project Muscular Dystrophy (PPMD) meeting, one of the authors (D.E.M.) had occasion to have a discussion with a group of nonambulatory young men with DMD/BMD, a group that has an interest in advocating therapies. These young men were very interested in clinical trials that included assessments of drug effects on distal (for keyboarding and hand/finger grip) and proximal upper body function (for lifting things including telephones, eating and drinking, etc.). They also hoped that device manufac-

Clinical Trial Considerations. DMD is a serious and life-threatening orphan disease with no currently approved therapies. Potential DMD therapies are good candidates for 6-month priority NDA review. While usually two adequately controlled clinical trials with prospectively controlled, clinically meaningful endpoints are needed to support efficacy, there are specific situations in which a single trial with strong evidence of efficacy accompanied by confirmatory evidence can be accepted as substantial evidence of effectiveness.26 The submission of a single, potentially small trial adds pressure to make certain that regulatory bodies have the best possible information on potential risks incorporated into that proposed trial, since there is a chance that they will not have a second controlled study to evaluate before making a decision on marketing approval. At the time of marketing approval it would be optimal for the full range of the target population, i.e., ambulatory and nonambulatory patients, to have been evaluated (at more than one dose) and to have available nonclinical data on carcinogenicity and reproductive issues, especially in products that could reasonably be expected to extend life into early adulthood or beyond.

In selected cases, drug companies may be asked to do Phase 4 (postmarketing) studies in order to accrue additional information on long-term use or to evaluate use in earlier/later disease stages. However, that may mean drugs will come to market without information on safety and efficacy in a significant portion of the target population, a fact that spotlights the significant issue of rights to inclusion in investigatory trials. It may be argued that nonambulatory subjects can be evaluated by their inclusion in Phase 4 studies. However, postponing the inclusion of nonambulatory patients may mean that drugs may be marketed without adequate assessment of potential risks in older subjects who will almost certainly begin to use the drugs off-label. Additionally, postapproval, it may be difficult to recruit subjects, ambulatory or
nonambulatory, into the Phase 4 trials if these studies have a placebo arm. Many drugs under investigation for DMD have been given the funding and regulatory advantages that are conferred with orphan drug status; these products are eligible for special regulatory considerations such as 7 years of marketing exclusivity upon approval, exemption from review filing fees, and tax credits for clinical trial expenses. Unless these drugs are intended only for treatment of early stages of the disease, the trials should include all segments of the patient population that might require the drug at least to establish safety but also to assess effectiveness in later stages of the disease.

Remaining Questions to Consider. As we go forward in drug development and consideration of drugs for persons with DMD, the expectation that that they may live into early adulthood at least and face major morbidities that do not involve ambulation should lead to reevaluation of how drugs are assessed. Although preserving ambulatory ability is unequivocally an important goal, preservation of cardiac muscle function and/or respiratory muscle function are of importance to survival. It is possible that there will be treatments for muscular dystrophy that do not affect the traditional primary target-preservation of ambulation but do affect the primary causes of late-stage morbidity and mortality, e.g., cardiac and respiratory dysfunction related to reduced or absent dystrophin production. These treatments will not be discovered unless later-stage patients are included in clinical trials. Since it is possible that only one subset will have benefit from a given product, the only way to ensure that we have adequate data to inform drug approval for use in one or both subsets is to have studies performed in both the ambulatory and nonambulatory DMD/BMD populations.

Each drug submitted for regulatory review is considered on an individual basis, and there is no standard formula that is used by a given review division to determine acceptability of trial designs. However, there are certain things that could be considered for inclusion in the development plan for DMD/BMD therapies:

- One of the key factors in the evaluation of an intervention in controlled clinical trials is the clinical meaningfulness of the suggested endpoints. Endpoints that assess functional benefit to the patient are preferred, since they provide practical meaning to patients and their healthcare providers. Subtle changes on neurologic tests are not sufficient even if a statistically significant effect is achieved, unless the patients feel those changes reflect a true functional benefit to their lives. Patients (and/or caregivers in certain cases) are the best persons to determine clinically meaningful change. Since they are the affected parties, their views must be taken into account.

- Trials in nonambulant males should assess effects on activities of daily living and other quality of life measures. While there are existing scales that may be suitable for use with or without modification, the FDA Study Endpoint and Labeling Development Team (SEALD) should be consulted early on to assist with the quality-of-life measures and other subjective endpoints within the clinical trial design.

- Cardiac monitoring should be flexible. Since some patients may not be suitable for echocardiograms, multigated acquisition (MUGA) scans or magnetic resonance imaging (MRI) could be used (where necessary) to provide baselines for comparison (using the same modality) at study completion. The American Academy of Pediatrics has a schedule of recommended cardiac evaluations for DMD/BMD patients, so historical data may be available to use as a baseline in many study participants. However, it must be noted that if this historical data is not standardized, there may be the potential for bias between treatment groups that may lead to difficulty in interpreting data.

- Since cardiac dysfunction is a known cause of morbidity in DMD/BMD patients, long-term controlled studies should consider evaluating for signs of clinical benefit such as a delay in progression to heart failure symptoms/hospitalizations. Additionally, the quality-of-life measurements that exist for heart failure patients might be of use in the older nonambulant population. Again, SEALD should be consulted early to evaluate use of these measures in the proposed trials.

- Since progressive pulmonary muscle weakness is known to occur in DMD/BMD and effects on forced vital capacity are relatively late findings, monitoring of maximal inspiratory and expiratory pressures as well as peak cough flow should be considered as possible indicators of potential drug effects. Quality of life measurements exist for pediatric asthma patients; those measures may be adaptable for the DMD/BMD population as well. The American Thoracic Society has recommended routine evaluations for DMD/BMD.
patients, so historical data may be available to use as a baseline in many study participants.\textsuperscript{21} The potential for bias from nonstandardized data must still be considered.

- Since mobility is an issue for some potential study participants, consideration could be given to having either designated investigators travel to the patients to perform appropriate testing or to utilize videoconferencing to perform examinations locally with secondary review at a central site.

- While some in vitro work is done on DMD fibroblasts, there may be value in considering evaluation of drug effect on DMD cardiac muscle, since it may be that a drug would benefit cardiac but not skeletal muscle. Knowing that there might be a tissue-specific effect could inform the selection of study endpoints.

\textbf{Where Do We Go from Here?} DMD and BMD remain incurable diseases, but the hope for effective drugs has never been better justified. Therapeutic strategies in active development employ small molecules and macromolecules; they target both the underlying genetic defect as well as the end-organs. The state of the art has transitioned from elucidation of the molecular lesions underpinning DMD and BMD to the generation of therapies designed to treat the consequences of those defects.

The good news is that DMD patients no longer experience mortality in prepubescence; the bad news is that we as a medical community have not fully revised our thinking to acknowledge the welcome reality of the prolongation in life span. Here we have considered the course of the illness as we currently understand it; we have briefly reviewed the products under investigation, and we have raised some of the questions that currently vex us. We must depend on the neuromuscular community, which has been so vigorous in its advocacy, to partner with us in charting the course forward by providing input as we try to find safe and effective treatments that will provide tangible benefits to DMD patients of all ages.

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\textbf{REFERENCES}


