Approaching a New Age in Duchenne Muscular Dystrophy Treatment

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Summary: Duchenne muscular dystrophy is the most common and severe form of muscular dystrophy. The cornerstones of current treatment include corticosteroids for skeletal muscle weakness, afterload reduction for cardiomyopathy, and noninvasive ventilation for respiratory failure. With these interventions, patients are walking and living longer. However, the current status is still far from adequate. Increased private and federal funding of studies in Duchenne muscular dystrophy has led to a large number of novel agents with propitious therapeutic potential. These include agents that modify dystrophin ex-

pression, increase muscle growth and regeneration, and modulate inflammatory responses. Many of these agents are already in clinical trials. Challenges to the development of additional novel therapeutics exist, including lack of validated animal models and lack of adequate biomarkers as surrogate endpoints. However, these challenges are not insurmountable and the next decade will likely see meaningful, new treatment options introduced into the clinical care of patients with Duchenne muscular dystrophy. **Key Words:** Duchenne, muscular dystrophy, clinical trials, therapeutics, myostatin, gene therapy.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is one of the most common and devastating disorders of humankind. One out of approximately 3500 boys worldwide is born with a mutation in the gene for dystrophin leading to loss of dystrophin protein from the muscle membrane. This loss of dystrophin results in muscle membrane fragility with repeated rounds of muscle fiber necrosis and regeneration and progressive replacement of muscle by fibrosis and fat. Skeletal and cardiac muscle lose strength and elasticity, which functionally translates into quadriplegia with joint contractures, respiratory insufficiency, and cardiomyopathy.

Young men with DMD are living significantly longer than in previous decades due to a combination of factors that include more aggressive respiratory and cardiac care and potentially long-term corticosteroid use. Still, the average age in adult DMD clinics is only in the mid-20s, and life beyond the 30s has not been reported. Therapeutic interventions clearly prolong strength beyond the

untreated natural history, but few would disagree that with most children losing ambulation by age 13, these current therapies are still woefully inadequate.

Despite the current lack of adequate therapies, there is good reason to be optimistic that novel therapeutic agents will positively impact this disease in the near future. This article reviews the current pharmaceutical treatment options, novel therapies in preclinical and clinical trials, and challenges to therapeutic development in DMD. Due to space limitations, this review does not include nonpharmacological interventions that are also critical to the successful orthopedic, respiratory, and psychosocial management of DMD.

CURRENT TREATMENT

Treatment of both pediatric and adult DMD varies widely among countries and even between clinics in the United States. Practice measurements regarding corticosteroid use have been developed by the Quality Standards Subcommittee of the American Academy of Neurology.¹ Furthermore, the Centers for Disease Control and Prevention (CDC) is facilitating the development of care considerations for individuals who have DMD using the RAND/UCLA Appropriateness Method, which combines peer-reviewed literature with the collective judg-

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ment of experts to yield a statement regarding the appropriateness and necessity of clinical interventions.² Results of eight expert panels addressing clinical issues of diagnostic, neuromuscular, cardiovascular, respiratory, gastrointestinal/nutritional, orthopedic/surgical, psychosocial, and rehabilitative management are expected to be released in early 2009. The following discussion highlights a subset of important issues in current management.

Neuromuscular concerns

The earliest signs and symptoms of DMD are progressive decline in limb muscle function. This is due primarily to skeletal muscle weakness and secondarily to joint contractures. Interventions can be made that have a modest effect on both of these processes.

The only class of drug that has been found to have a consistent positive effect on muscle strength in DMD is glucocorticosteroids. Randomized controlled clinical trials of daily oral prednisone demonstrated an improvement in muscle strength and function in children with DMD compared to baseline and to controls.^{3,4} From these trials, a dose of 0.75 mg/kg/day of prednisone is considered an appropriate starting dose.^{1,5} Long-term neuromuscular benefits to corticosteroid use include prolonged independent ambulation and a lower prevalence of scoliosis.⁶⁻⁸ Adverse effects of prednisone, in particular to behavior, weight, and bone density often necessitate reduction of dose over time. For example, the American Academy of Neurology practice measurements recommend decreasing prednisone to 0.5 mg/kg/day if weight gain >20% over estimated normal weight for height occurs over a 12-month period with further decrease to 0.3 mg/kg/day if excessive weight gain continues.¹ Many adolescent and adults with DMD either discontinue prednisone or decrease to a low dose due to cumulative side effects and the perception of little benefit after loss of ambulation.^{9,10}

Due to the expected and nontrivial side effects of long-term daily prednisone use, several other glucocorticoid regimens have been examined. These have included 10 days on followed by 10 days off of 0.75 mg/kg/day of prednisone, 10 mg/kg prednisone divided over 2 weekend days, and alternate-day prednisone. Clinical studies of these regimens suggest that side effects may be reduced, but efficacy compared with daily prednisone is unclear at best.¹¹⁻¹³ Deflazacort is a related corticosteroid not approved in the United States but available in Canada and many European countries. Deflazacort has been shown to prolong functional abilities with fewer side effects than prednisone.^{14,15} A double-blinded, randomized trial comparing daily doses of 0.9 mg/kg of deflazacort to 0.75 mg/kg of prednisone or placebo showed no statistically significant differences in the two treatment groups in strength or function.¹⁶

There is currently insufficient data on the long-term strength benefits and relative side effects of these various glucocorticosteroid regimens. To address this issue, an international group of investigators plan to conduct a randomized, controlled, double-blind trial over 3 years comparing three corticosteroid regimens: 1) daily prednisone, 2) daily deflazacort, or 3) intermittent prednisone with 10 days on and 10 days off. Results of this trial to find the optimal steroid regimens in DMD will be extremely valuable in creating an international consensus on care and management of DMD.

Respiratory and cardiac care

There is mounting evidence that glucocorticosteroids likely preserve cardiac and respiratory function. The majority of individuals with DMD eventually die from complications of respiratory muscle weakness. This includes weakness of inspiratory muscles resulting in hypoventilation and expiratory muscle weakness resulting in weak cough and inability to effectively clear secretions. The greatest contribution to improvement in respiratory care, and thus likely the greatest contribution to extending longevity in DMD, has been noninvasive ventilation.¹⁷⁻¹⁹ Pharmacologically, the near lifelong treatment of DMD with corticosteroids appears to have produced improvements in pulmonary function.^{15,20} A case-controlled study that compared DMD patients treated with steroids for a mean duration of 8.2 years to untreated patients found that peak cough flow ($p = 0.047$) and maximum expiratory pressure ($p = 0.021$) were significantly higher in the steroid-treated patients.²¹

Dilated cardiomyopathy begins early in DMD with an estimated 25% of 6-year-olds and 59% of 10-year-olds affected.²² By adulthood, nearly all DMD patients have significant cardiac involvement.^{10,22} Recently, a historical cohort study of DMD cases undergoing serial cardiac evaluations over 3 years showed that corticosteroid treatment begun prior to ventricular dysfunction was protective against development of ventricular dysfunction, defined as a shortening fraction less than 28%.²³ The probability of freedom from ventricular dysfunction by Kaplan-Meier estimates was a striking 93% for the steroid-treated cohort *versus* 53% for the untreated cohort at 1500 days.²³

Early angiotensin-converting enzyme inhibitor (ACEi) treatment may also be indicated in DMD as suggested by a trial of perindopril by Duboc and colleagues.²⁴ In this double-blind study, children from 9.5 to 13 years of age with DMD and normal cardiac function were treated with perindopril or placebo for 3 years, and then all participants received open-label perindopril for an additional 2 years. Although there was no difference between groups after 3 years, at 5 years there was a significant reduction in left ventricular dysfunction in the ACEi-treated group ($p = 0.02$).²⁴ There are several, large,

prospective trials of ACEi and angiotensin II–type 1 receptor blockers in patients with dilated cardiomyopathy from a variety of etiologies, indicating improved survival and decreased symptoms.^{25–29} Such studies argue for early and continued use of corticosteroids and ACEi/angiotensin II–type 1 receptor blockers in patients with DMD.

Despite the positive clinical effects of corticosteroids to dystrophic skeletal and cardiac muscle, the physiological basis for this benefit is unknown. CD4+ and CD8+ lymphocytes are found in dystrophic muscle; however, it is unlikely that the effects of prednisone are due to immune modulation alone. A controlled clinical trial of azathioprine did not show beneficial effect, although it did decrease mononuclear cell populations in muscle to a similar degree as prednisone.^{30,31} Prednisone also has anabolic effects in dystrophic muscle and induces transcriptional changes of a large number of genes.³² It is possible that one or a combination of target genes induced by prednisone is primarily responsible for its effects. Identifying such factors may be a more direct approach to treatment of DMD without inducing the side effects of long-term glucocorticosteroid treatment.

NOVEL THERAPEUTIC APPROACHES

Due to increased advocacy from parent and patient groups, increased government and foundation funding, and growing interest from pharmaceutical companies in muscular dystrophy, there has been a recent surge in the development of promising, novel therapeutic agents. These agents are at various stages of development from preclinical studies in animal models to drug manufacturing and toxicity studies to early clinical trials in muscular dystrophy. A variety of approaches are being explored by the scientific community that will likely have benefits to patients, cumulatively providing both near treatments and future cures. In addition, such a broad approach will likely lead to a treatment involving more than one therapeutic agent, such as the “cocktail” of drugs used to treat other severe or fatal disorders, including malignancies.

Molecular modification

Since the cloning of dystrophin in 1987,³³ hopes have ridden on a cure related to restoring dystrophin to the plasma membrane. The task of delivering the dystrophin gene, one of the largest in the human genome, has proven monumental. Over the years, important advances have been made in identifying key regions of the gene in truncated dystrophin gene construction, optimal vectors for delivery of the gene and methods of delivery to restore dystrophin expression and reduce muscle necrosis. Excellent recent reviews of viral and nonviral gene therapy for DMD can be found in M.S. Swanson, “The

Muscular Dystrophies: Molecular Basis and Therapeutic Strategies.”^{34,35} A few recent and novel approaches to deliver genes, modify transcription of genes, or alter translation to protein are described here.

Adeno-associated virus (AAV) has emerged as the leading viral vector for human gene therapy. However AAV has a maximum packaging limit of 5 kb, and the dystrophin gene stripped of its noncoding regions is approximately 11.5 kb. From genotype/phenotype studies, followed by strategic deletion and animal trials of various noncritical domains, truncated dystrophin gene constructs have been produced to fit the AAV packaging limitations. Mini-dystrophins and micro-dystrophins have been shown to ameliorate the dystrophic pathology of the mdx mouse.^{36–40} Another novel strategy to overcome the capacity limitations of AAV is through the use of trans-splicing and overlapping vectors (reviewed in Duan et al.⁴¹). With co-infection, trans-splicing vectors undergo head-to-tail recombination through viral inverted terminal repeats, whereas overlapping vectors have common sequences that undergo homologous recombination to recover the full-length gene. The trans-splicing and overlapping vector features have recently been combined in a single hybrid vector system allowing expression of even larger mini-dystrophin genes.⁴²

A phase I trial of a micro-dystrophin under a cytomegalovirus promoter in a modified AAV is now in clinical trials in boys with DMD (NCT00428935, clinicaltrials.gov). This trial will provide important information regarding the safety of AAV in this patient population, as well as insight into immune responses to possible nonmuscle dystrophin expression. Efforts to target dystrophin expression selectively to skeletal and cardiac muscle and potentially reduce toxicity are also ongoing and include the generation of modified creatine kinase promoters.⁴³

While AAV gene therapy attempts to introduce an exogenous dystrophin gene, another molecular approach using antisense oligonucleotides (AON) attempts to modify the expression of DMD genes at the level of pre-mRNA. Approximately two-thirds of individuals with DMD have deletions or duplications that destroy the reading frame. Antisense oligonucleotides are constructed to target the exons flanking an out-of-frame mutation, inducing splicing of the pre-mRNA to “skip over” one or more exons and restore the reading frame, producing an internally truncated but functional dystrophin. Preclinical studies in the mdx mouse model, which harbors a nonsense mutation in exon 23 of the dystrophin gene, and in a transgenic hDMD mouse model with a full-length human DMD gene, have demonstrated that AONs can be constructed to target specific exons to allow correction of the reading frame and dystrophin expression at the sarcolemma.^{44–46} Recently, an open trial of a 2-O-methyl phosphorothioate antisense

oligonucleotide demonstrated skipping of exon 51 and sarcolemmal dystrophin expression 28 days after single injection into the tibialis anterior muscle of 4 patients with DMD.⁴⁷ The amount of dystrophin produced ranged from 3% to 12% that of control specimens by Western blot and 17% to 35% of control specimens by ratio of dystrophin to laminin $\alpha 2$ by immunohistochemistry.⁴⁷ To enhance expression, different modifications of the AON backbone are currently being developed with higher affinity for their nucleic acids and greater resistance to degradation. These modifications include phosphorodiamidate morpholino oligomers (or “morpholinos”) and a trial of a phosphorodiamidate morpholino oligomers also targeting exon 51 has begun in DMD (NCT00159250, clinicaltrials.gov).

Approximately 10 to 15% of DMD is due to a nonsense mutation that causes premature termination of the translation of the dystrophin gene, resulting in a truncated or absent protein. “Nonsense suppression” refers to the strategy attempting to stabilize the ribosome to “read-through” the mutation, inserting a random amino acid at the premature termination codon and producing a full-length protein with a single missense mutation. The antibiotic gentamicin was found in some studies,⁴⁸ but not others,⁴⁹ to induce efficient read-through and dystrophin expression in the mdx mouse model. In an open clinical trial of four Duchenne and Becker muscular dystrophy patients with nonsense mutations, 2 weeks of daily 7.5 mg/kg i.v. gentamicin failed to induce dystrophin expression.⁵⁰ A phase I trial of twice weekly i.v. gentamicin for 6 months in DMD is currently ongoing (NCT00451074, clinicaltrials.gov).

Gentamicin has significant drawbacks as a long-term treatment for nonsense suppression including the fact that it is an intravenous medication and can cause nephrotoxicity and ototoxicity, which are dose-limiting, and thus may be limiting in achieving efficacy. A small molecule, orally available agent, PTC124 was identified through a high throughput drug screen that specifically allows read-through of nonsense mutations, but not normal translational stop signals.⁵¹ PTC124 was reported to restore dystrophin expression in cultured DMD human and mdx myoblasts with nonsense mutations, as well as in mdx muscle *in vivo*.⁵¹ PTC124 was well-tolerated in phase 1 healthy volunteers.⁵² A phase 2A trial of PTC124 is currently evaluating muscle dystrophin expression and a randomized, controlled, 48-week treatment phase 2B trial in DMD and Becker muscular dystrophy with nonsense mutations has begun, which will use total distance during a 6-minute walk test as a primary outcome measure (NCT00592553, clinicaltrials.gov).

The genetic treatments previously described, AAV-mediated gene transfer, AON-mediated exon skipping, and nonsense suppression are examples of molecular modifications that have recently made the leap from lab-

oratory preclinical studies to clinical trials. Each experimental therapeutic has its own specific promises, limitations, and challenges. For example, nonsense suppression with PTC124 has the advantage of being an orally available agent with the potential to induce systemic expression of dystrophin, but with the obvious limitations of being applicable to only a small percentage of DMD patients. AONs would be applicable to the majority of DMD patients and are anticipated to show good safety profiles. However, intravenous or intramuscular treatments will need to be repeated at regular intervals due to the limited half-life of the AON, mRNA, and dystrophin protein, which over the course of a lifetime increase the risk of drug toxicity and procedural side effects. AAV-delivered gene therapy has a distinct advantage of promising long-term treatment effects. However, there remains many unanswered questions in AAV therapy that may prove limiting, including the potential immune response to the vector and transgene, as well as the ability to effectively deliver systemically.

Modulation of muscle growth and regeneration

Muscle has an inherent ability to regenerate via resident progenitor stem cells. However, regeneration in many muscle disorders, including DMD, is incomplete and results in remodeling of muscle with fibrosis and fatty infiltration. Stimulating growth and regeneration of dystrophic muscle does not address the underlying pathophysiology of DMD, lack of dystrophin, and subsequent muscle fiber necrosis, but may provide functional benefits by increasing size and strength of minimally affected muscle or by improving the quality of the composition of dystrophic muscle. Postnatal growth and regeneration are regulated by a variety of endogenous growth factors, most notably insulin-like growth factor-1 (IGF-1) and members of the transforming growth factor- β (TGF- β) superfamily.

IGF-1 promotes regeneration by stimulating proliferation and differentiation of muscle progenitor cells.^{53,54} IGF-1 also promotes protein synthesis and inhibits protein degradation by blocking transcription of the ubiquitin-ligases (reviewed in Glass⁵⁵). Mdx mice expressing an IGF-1 transgene (mdx/mIGF^{+/+}) have increased muscle mass, force, and reduced fibrosis compared with their mdx littermates.⁵⁶ Furthermore, mdx/mIGF^{+/+} animals appear to have less susceptibility to contractile damage and reduced myonecrosis than mdx littermates.^{56,57} Postnatal treatment of mdx mice with subcutaneous IGF-1 demonstrated increased specific force and resistance to fatigue of diaphragm muscles.⁵⁸ Clinical trials of IGF-1 isoforms have not yet been attempted in DMD. However, recombinant human IGF-1 plus IGF-1 binding protein 3 is currently being studied in a phase II trial of myotonic muscular dystrophy (NCT00577577; clinicaltrials.gov). Unfortunately, this trial will not assess muscle pathology

or muscle mass that might have provided indication of the direct biological effect on the muscle from the drug, and if positive, would have encouraged future clinical trials in DMD. Nevertheless, if an IGF-1 plus IGF-1 binding protein 3 effect can be demonstrated in muscle strength or function, there will be a high level of enthusiasm for subsequent clinical trials in DMD as well as other muscle degenerative and wasting disorders.

Myostatin is a member of the TGF- β superfamily and an endogenous negative regulator of muscle growth.⁵⁹ Loss of function mutations in myostatin result in massive muscle hypertrophy in a variety of species, including humans.^{60,61} Mdx mice lacking myostatin have increased muscle mass, grip strength, and improved muscle histological features with decreased fibrosis.⁶² In acute injury models, muscle regenerates more quickly and robustly in the absence of myostatin.^{63,64} De-repression of quiescent muscle progenitor cells is a possible mechanism for enhanced muscle regeneration in the absence of myostatin for which there is accumulating evidence.^{65–69} In addition, the direct modulation of myostatin fibroblast proliferation likely contributes to the observed effects on fibrosis.^{70,71}

Myostatin can be inhibited postnatally by a variety of mechanisms that similarly induce muscle growth.⁷² Most current myostatin inhibitors have been designed to bind to myostatin and inhibit interaction with its endogenous receptor, putatively the activin IIB receptor.⁷³ Inhibition of myostatin with a neutralizing antibody⁷⁴ and with a modified myostatin pro-peptide, delivered via AAV,⁷⁵ ameliorate the dystrophic phenotype of the mdx mouse model. The first clinical trial of a myostatin inhibitor in a disease population was recently completed.⁷⁶ This phase I/II trial demonstrated safety without target-related side effects of a neutralizing antibody to myostatin, MYO-029, in subjects with diverse adult muscular dystrophies, including Becker muscular dystrophy, which is a disorder allelic to DMD.⁷⁶ Improvement in muscle strength and function were not demonstrated in this trial but biological activity of MYO-029 was suggested by trends in increased muscle mass and fiber diameters.⁷⁶ DMD has a different pathophysiology and natural history than the diseases included in the MYO-029 trial and may respond to a myostatin inhibitor quite different than adult muscular dystrophy. In addition, the rapid progression and more uniform disease course of DMD compared with adult muscular dystrophy are adventitious features for clinical trial design, which argue for a separate clinical trial of a myostatin inhibitor in DMD.

TGF- β 1, another member of the TGF- β superfamily, is homologous to myostatin and induces many similar effects on muscle, including inhibiting proliferation and differentiation of muscle precursor cells and stimulating fibrosis.^{77–79} In fact, TGF- β 1 is several-fold more potent than myostatin in its effects on cultured myoblasts and

fibroblasts *in vitro* (personal observations). Contrary to myostatin, TGF- β 1 is upregulated in early DMD.^{80–82} These features would suggest that TGF- β 1 would be an excellent therapeutic target. However, although myostatin expression is localized to skeletal muscle, TGF- β 1 is widely expressed and controls a range of processes in various tissues throughout the body, raising concerns of specificity and toxicity. The recent results of a well-tolerated, widely used, angiotensin II–type 1 receptor blocker, losartan, in mdx mice are therefore of particular interest.⁸³ Previous studies have suggested that losartan reduces TGF- β expression in heart and kidney.^{84,85} Mdx mice treated for 6 to 9 months with losartan demonstrated less fiber size heterogeneity, less fibrosis per area, and increased strength by grip strength and absolute force of explanted muscle.⁸³ Many older DMD patients are currently being treated with losartan for the presumptive cardiac benefits of angiotensin II–type 1 receptor blockers, as previously described. A clinical trial of losartan in children with DMD to evaluate its effects on skeletal muscle strength and function is anticipated to begin enrollment in 2009.

Drugs that stimulate muscle growth and regeneration have wide applicability to a number of primary and secondary disorders of muscle. For this reason, development of such drugs has become a focus of several pharmaceutical companies. It is anticipated that several different myostatin inhibitors, for example, will undergo phase I healthy volunteer testing. These drugs will not cure DMD, but it is hoped that they will provide functional benefits that alter the course of the disease. They may play an important role in adjuvant therapy to other “curative” treatments. For example, AON efficiency has been found to be higher in regenerating fibers,⁴⁴ which in the future might be stimulated by IGF-1, losartan, or myostatin inhibitors.

Emerging therapeutic targets

Dystrophic muscle is marked by chronic inflammation, which not only serves to reduce debris of necrotic muscle fibers, but has also been implicated as playing a direct role in pathogenesis. Nuclear factor-kappa B (NF- κ B) transcription factors have long been known to regulate immune and inflammatory responses.^{86,87} More recently, it has become clear that NF- κ B is involved in skeletal muscle development and regeneration.^{86,87} NF- κ B signaling is persistently elevated in immune cells, as well as regenerating muscle fibers in mdx muscle,⁸⁸ and evidence exists that this same pathway is also active in DMD patients.^{81,88,89} Pharmacological inhibition of I κ B kinase (IKK), a direct upstream positive regulator of NF- κ B, resulted in improved pathology and muscle function in mdx mice.⁸⁸ Activators of NF- κ B, such as TNF- α are upregulated in DMD muscle.⁹⁰ Such findings encouraged recent trials of pentoxifylline, a TNF- α inhibitor,

in DMD. Preliminary results of an open-label, pilot study of oral, immediate release pentoxifylline in steroid naïve DMD children (ages 4 to 9 years old) was poorly tolerated, but suggested a potential stabilization of strength in a small number of subjects who completed the protocol.⁹¹ A larger, randomized, controlled trial of pentoxifylline in boys older than 7 years of age who have DMD and were on prednisone, suggested no additional benefit over corticosteroids.⁹² Development of more selective NF- κ B modulators is clearly warranted.

Loss of the link between the extracellular matrix and the cytoskeleton is a common pathophysiologic process in a variety of muscular dystrophies including DMD. A variety of approaches, including overexpression of GalNAc transferase or alpha 7 beta 1 integrin have been used to attempt to stabilize this link in the absence of dystrophin and have demonstrated proof of concept by reducing dystrophic pathology in the dystrophic mouse models.^{93,94} Biglycan is a small, endogenous, extracellular proteoglycan, which may serve to enhance the stability of the extracellular matrix–cytoskeletal link in addition to other functions. Biglycan binds to components of the dystrophin associated glycoprotein complex, including α -dystroglycan, α and γ sarcoglycan, as well as collagens I and VI.^{95–97} It is highly expressed in developing and regenerating muscle.⁹⁸ Biglycan null mice exhibit a mild dystrophic phenotype and have reduction in the cytosolic dystrophin associated proteins dystrobrevin, syntrophin and neuronal nitric oxide synthase (nNOS).⁹⁹ Remarkably, recombinant biglycan can be injected into muscle, become stably associated with the sarcolemma and extracellular matrix, and restore expression of cytosolic dystrophin associated proteins to the plasma membrane in null mice.⁹⁹ Studies evaluating the effects of biglycan to ameliorate dystrophic features in the absence of dystrophin will be extremely interesting and potentially warrant pharmacologic development for clinical trials in DMD.

CHALLENGES IN THERAPEUTIC DEVELOPMENT

As previously described, multiple novel therapeutic agents have recently entered clinical trials or are expected to in the near future. In addition, there are many promising lines of research, such as those involving cell-based therapies, which continue to be pursued in preclinical animal models. Despite this progress, several challenges to developing therapies in muscular dystrophy exist.

Lack of validated animal models

The mdx mouse is a genetic orthologue of DMD and, as such, is an excellent model system for molecular studies.^{33,100} However, as Victor Dubowitz, Emeritus

Professor of Pediatrics, University of London, Imperial College School of Medicine, frequently commented, the mdx mouse is a very poor model of the DMD phenotype (personal communications and Dubowitz¹⁰¹). It is well-recognized that the mdx mouse does not model the progressive and severe weakness, joint contractures, respiratory failure, and cardiomyopathy that are hallmarks of the human disease. Yet, essentially all novel therapeutics are screened by attempting to show some “amelioration” of this mild mouse disease. Significant emphasis is currently placed on whole animal and physiologic measurements of function in the mdx mice that may or may not correspond to increased strength, let alone, function in children with DMD. There is a current large disconnect in the field of DMD therapeutic development in which agents are proposed for the clinical trial based on non-validated rodent outcome measures, and then are asked to demonstrate not only improved muscle strength, but muscle function and quality of life. It is predictable that most agents will not be able to successfully jump this gap.

Alternative mouse models exist, such as the utrophin null/mdx mouse lacking both utrophin and dystrophin, which is not a pure genetic model of DMD, but more closely parallels the human disease in severity. In addition, with the recent establishment of the National Center for Canine Models of DMD, the capacity to test novel agents in large animal models has greatly increased. Dog models, such as the golden retriever muscular dystrophy dog with a splice site mutation in the dystrophin gene, are not without their own limitations, such as high cost, breeding limitations, and large phenotypic variability.¹⁰² However, the golden retriever muscular dystrophy has many advantages over the mdx mouse model, including severity of the disease resulting in early death, development of fibrosis and contractures, and homologous immune system.^{103,104} The predictive value of outcome measures in these various animal models for human disease is unknown, and essentially unknowable, until there have been enough clinical successes of agents for muscle disease to take from the bedside back to the laboratory.

Lack of biomarkers of muscle growth and regeneration

Biomarkers that can act as surrogate endpoints of disease accelerate drug development. Although drugs have certainly been developed without the aid of biomarkers, more drugs are developed for diseases where good biomarkers exist.¹⁰⁵ The classic example is glycosylated hemoglobin (hemoglobin_{A1C}) for diabetes, which is used to gauge average plasma glucose concentration over prolonged periods of time. Hemoglobin_{A1C} thus serves as a substitute for the clinical endpoint of good blood sugar control and is predictive of clinical benefit or harm, such as retinopathy, nephropathy, and neuropathy.

The clinical field of myopathy as a whole is in need of serum biomarkers for muscle health. Surrogate markers of muscle function currently exist, such as manual and quantitative muscle strength testing; however, these are time-consuming, require trained personnel, and raise concerns regarding reliability. Quantitative and qualitative analysis of muscle by various imaging modalities will be an important aspect of future clinical trials in muscle disease, but they are costly in time, money, and personnel. Creatine kinase is a muscle serum biomarker, but is dependent on activity and fluctuates dramatically in DMD patients. Most importantly, creatine kinase only provides an imperfect picture of muscle necrosis, but not at all of muscle atrophy or muscle regeneration. A blood test or panel that measures the balance of muscle necrosis and atrophy versus growth and regeneration, and is predictive of changes in muscle function, would be a huge advantage in the development of novel therapeutic agents for DMD.

CONCLUSION

Each decade in recent history has seen a small improvement in the lives of those with DMD. The coming decade holds even greater promise as best clinical practices in DMD are evaluated and disseminated, and several novel therapeutic agents enter clinical trials and use. Although it is unlikely that any of the current approaches will independently cure this devastating disease, the broad base of approaches tackling many different aspects of the disease will serve the community well, providing options and synergistic therapies.

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