Expert Opinion on Orphan Drugs

ISSN: (Print) 2167-8707 (Online) Journal homepage: www.informahealthcare.com/journals/ieod20

Review of Phase II and Phase III clinical trials for Duchenne muscular dystrophy

Michele A Scully, Shree Pandya & Richard T Moxley

To cite this article: Michele A Scully, Shree Pandya & Richard T Moxley (2013) Review of Phase II and Phase III clinical trials for Duchenne muscular dystrophy, Expert Opinion on Orphan Drugs, 1:1, 33-46, DOI: 10.1517/21678707.2013.746939

To link to this article: https://doi.org/10.1517/21678707.2013.746939

Published online: 17 Dec 2012.

EXPERT OPINION

Review of Phase II and Phase III clinical trials for Duchenne muscular dystrophy

Michele A Scully, Shree Pandya & Richard T Moxley†

University of Rochester Medical Center, Rochester, NY, USA

Introduction: Evidence-based therapeutics in Duchenne muscular dystrophy (DMD) has been limited to corticosteroids for the past 30 years. There have been a host of other therapeutic interventions studied in mice, canines and more recently humans, but they are yet to show effectiveness in clinical trials. Newer genetic approaches are in early stages of clinical trials.

Areas covered: In this paper, the authors review evidence-based studies for corticosteroids as well as other Phase II and Phase III clinical trials involving potential pharmacologic treatments: myostatin and phosphodiesterase inhibitors, insulin-like growth factor 1 and replenishment of nutritional deficiencies. Finally, the authors briefly review the current status of treatments specific for genetic mutations and gene therapy.

Expert opinion: Since the identification of corticosteroids as an effective treatment for DMD, there has not yet been another pharmacologic intervention that has shown as much benefit, although further investigation is needed for some of the mentioned therapeutics. New therapies will need to show a significantly greater sustained benefit for our DMD patients with cost effectiveness, in order for them to supplant or reduce the use of long-term corticosteroid treatment. While studying new therapeutics, further study and trials in corticosteroids should not be lost.

Keywords: clinical trials, corticosteroids, Duchenne muscular dystrophy, therapeutics

Expert Opinion on Orphan Drugs (2013) 1(1):33-46

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked disorder characterized by progressive skeletal muscle weakness, accompanied by cardiac and smooth muscle dysfunction, due to an abnormality or deficiency of the protein dystrophin [1]. DMD patients are typically diagnosed between ages 4 and 5 years but may exhibit symptoms years earlier [2]. As the disease progresses, patients become wheelchair-bound by their teenage years and death ensues by early adulthood, although some patients can live into their thirties with ventilatory support [3]. Current evidence-based therapeutic interventions are limited to corticosteroids. Randomized controlled trials with prednisone or deflazacort, 6 – 24 months in duration, have improved strength and function in boys with DMD [4-12]. Non-randomized follow-up studies of patients receiving these two treatments have shown sustained benefits from 5 to 15 years [13-17]. In combination with supportive care based on census guidelines, corticosteroid therapy in DMD prolongs survival, changes the natural history of the disease, extends functional abilities and improves quality of life for patients [18-21]. Corticosteroids are an important component of standard care for DMD patients but more study is needed to improve their use and more research is necessary to develop curative treatments.

While optimal dosing, age of initiation and long-term side effects of corticosteroids need continued study, opportunities to target correction of the underlying genetic

informa healthcare

10.1517/21678707.2013.746939 © 2013 Informa UK, Ltd. e-ISSN 2167-8707

Article highlights.

- Evidence-based treatment for DMD is currently limited to corticosteroids; however, alternative therapies that show promise, specifically those focusing on genetic mutations, are in early clinical trials.
- Genetic interventions, including exon skipping and gene therapy to alter dystrophin expression, are currently focusing on dystrophin expression in skeletal muscle. Systemic administration, into respiratory, cardiac and brain tissues will be a challenge with high costs.
- Corticosteroid therapy is proven to slow progression of DMD; however, there needs to be a better understanding of optimal age of initiation, dose and regiment. We also need to continue investigation of the mechanism responsible for the beneficial effects of corticosteroids.
- A focus on outcome measures remains important in the treatment of DMD patients, as is the focus on the optimal methods for treating and measuring side effects in our older DMD population who are already on corticosteroids. There is a huge opportunity to improve the therapy of these nonambulatory patients, including heart, lung, joint and GI tract interventions.

This box summarizes key points contained in the article.

mutations need continued exploration. The DMD gene is the largest known human gene, 2.3Mb, and consists of a coding sequence of 79 exons, which accounts for 0.6% of the gene [22]. Due to the large size of the DMD gene, there are a variety of mutations, 4,700 [23]. A total of 90% of patients with a mutation that disrupts the open reading frame of the DMD gene will have the DMD phenotype [24,25]. This is in contrast to patients with the less severe phenotype, Becker muscular dystrophy (BMD), who have in-frame mutations resulting in a truncated but functional protein [24-28]. Given variability in mutations, genetic therapeutic approaches with the exception of gene therapy, must target specific mutations. The majority of mutations can be divided into three categories: deletion of one or more exons, duplication of one or more exons or point mutations. In this review we briefly discuss genetic therapeutic approaches, but our primary focus is on Phase II and Phase III studies involving corticosteroids and other pharmaceutical treatments. These other pharmaceutical approaches include: antibodies to myostatin, insulin-like growth factor 1 (IGF-1), phosphodiesterase inhibitors and nutritional supplements. Our review of genetic therapy based upon DMD gene mutations includes stop codon read-through, exon skipping and gene transfer therapy. At the close of our review we provide an opinion on the future of DMD treatments and clinical trials.

2. Corticosteroids

Corticosteroids, prednisone and deflazacort, are the only evidence-based effective treatment for DMD [4,5]. The specific cellular events responsible for the beneficial effect of corticosteroids remain unknown. An immunosuppressive mechanism seems unlikely since trials with azathioprine and cyclosporin A did not produce the beneficial effects observed with corticosteroids [6,29]. Despite our limited understanding of the underlying mechanism(s), corticosteroids do slow the rate of disease progression and extend functional abilities for 2 years or longer [4,5,21]. Outcomes documenting this beneficial action include improved pulmonary function, time to rise from supine to standing time to walk 9 m and time to climb four stairs [4,5,21]. The first clinical trial in DMD demonstrating efficacy of prednisone occurred in 1974 [30]. Numerous trials that followed have looked for optimal age of initiation, dose and frequency [6-12,31]. Conclusions, to date, are that prednisone 0.75 mg/kg/day and deflazacort 0.9 mg/kg are equally effective in improving muscle strength and function in the short term (6 months to 2 years), and that prednisone 0.75 mg/kg/day is optimal compared to other class I dosing studies (1.5, 0.3 mg/kg/day and 0.75 mg/kg/q. o.d. and 10 mg/kg/week) [6-12,31]. In these short-term treatment trials, adverse effects (weight gain, cushingoid appearance) were similar between studies. While these side effects were more common in the corticosteroid-treated patients compared to the placebo group, they did not prevent continuation of corticosteroid treatment and were not considered clinically severe. An analysis of long-term side effects occurring with treatment > 2 – 3 years duration was not possible because of the trial design [6-12,31]. However, in nonrandomized trials, boys treated longer than 2 years with prednisone or deflazacort showed significant benefits in ambulation, delayed onset of scoliosis and pulmonary dysfunction, preserved cardiac function, survival and quality of life compared to untreated counterparts [13-17,20,21]. Aside from side effects noted in short-term studies, boys treated with corticosteroids for a mean of 5.5 years were significantly shorter and had delayed puberty. Although patients have stated that their major concern with early corticosteroid treatment is the effect on growth rate, they feel that corticosteroid benefits outweigh this risk [17,20]. Side effects involving increased frequency of long bone fractures and vertebral fractures are important to consider, but there is a lack of consensus on how frequent and severe these side effects are and what interventions are optimal to prevent or manage them [15-17].

Despite the above progress, future randomized trials are needed to determine the optimal dose of steroids, age to initiate and dosing schedule to improve function with minimal side effects. Additionally, long-term controlled studies are needed to determine long-term effects on ambulation, bone, respiratory and cardiac functions, behavioral problems and quality of life. Uncontrolled studies of cohorts of DMD patients receiving prednisone or deflazacort for 5 years and longer offer encouraging results. A large multicenter clinical trial to address the question of the optimal corticosteroid regimen for DMD is underway and is recruiting patients this year [32].

A new area of corticosteroid investigation includes the finding that a subset of DMD patients appear to have

decreased or absent responsiveness to corticosteroid treatment, while others seem to have a therapeutic response [33,34]. Since the exact mechanism of action responsible for the beneficial effect of corticosteroids in DMD remains to be established, it is difficult to evaluate these varying responses to corticosteroid therapy in these subsets of patients. One interesting hypothesis under consideration involves the role of the intracellular glucocorticoid receptor (GRL) – an essential step in our current model of the glucocorticoid signaling cascade [33]. It is possible that polymorphisms, specifically studied at the amino acid position 363 (N363), may contribute to altered corticosteroid sensitivity in certain DMD patients [35,36]. Future investigation into these polymorphisms may identify glucocorticoid analogs that have less troublesome (or no) side effects, while at the same time may have equal or improved therapeutic efficacy. Of course, it is assumed that beneficial effects of corticosteroid therapy in DMD are mediated through the glucocorticoid receptor-controlled pathway. Another single nucleotide polymorphism under investigation that may predispose to disease modification and thus treatment response is a polymorphism is the osteopontin gene (SPP1). Researchers suggest that SPP1 genotyping should be considered for stratifying DMD patients in clinical trials since there may be a disease modifier effect by the mutation [37].

3. Myostatin inhibitors

Myostatin is a negative regulator of skeletal muscle growth and myostatin antibodies may offer a possible treatment for DMD [38,39]. MYO-29, the first antibody to myostatin evaluated in human clinical trials, has been trialed in muscular dystrophy patients (facioscapulohumeral muscular dystrophy; BMD; forms of limb girdle muscular dystrophy). Despite positive results in canines (increased muscle mass, decreased serum CK and decreased muscle fibrosis), human results showed no improvement in any endpoints of muscle strength or function [40,41]. Alternative agents under investigation to target myostatin include follistatin and ACE-031. Follistatin is part of the inhibin-activin-follistatin axis and a cellular participant in the pathway of myostatin inhibition [42]. In mice, an increase in follistatin has led to a marked increase in muscle mass [43,44]. To examine its potential therapeutic use in patients, clinical trials are currently in progress, injecting follistatin by vector transfer into the quadriceps femoris muscles of patients with BMD and sporadic inclusion body myositis [45]. ACE-031 is a recombinant fusion protein that creates a decoy version of the myostatin receptor, activin type IIB, which is linked to a human antibody that interferes with myostatin. Animal models showed increased skeletal muscle mass and strength in mdx mice [46]. Phase I studies in healthy postmenopausal women were well tolerated and there was an increase in lean body mass [47]. However, a Phase II clinical trial was recently terminated based on preliminary safety data [48].

4. Phosphodiesterase inhibitors

Based upon animal studies, nitric oxide (NO) plays a role in the regulation of skeletal muscle excitation–contraction coupling, myogenesis and muscle repair through a nitric oxide-cyclic guanosine monophosphate (NO-cGMP) signaling pathway [49]. In skeletal muscle NO is synthesized by neuronal NO synthase (nNOS), which is part of the dystrophin complex and in the absence of dystrophin, the concentration of nNOS decreases [50]. NO is deficient in mdx mice [51], and may also be deficient in DMD patients related to a loss of dystrophin which reduces nNOS expression [50,52]. Alteration in nNOS expression may in turn prevent its normal localization, inhibit its vasoregulatory role, cause sympathetic vasoconstriction during exercise, impair muscle perfusion and lead to damage following mild exercise [53]. This hypothesis for muscle damage as an alternative or additional hypothesis to the mechanical membrane stress/damage hypothesis may lead to new approaches for treatment in DMD. One approach related to this previous preclinical research might involve therapy to minimize a potential alteration in nNOS function.

Phosphodiesterase inhibitors, sildenafil and tadalafil, which prevent the inactivation of cGMP, are a potential therapeutic intervention to bypass the loss of nNOS in dystrophic muscle. In mdx mice, sildenafil has reversed left ventricle and diaphragm dysfunction and tadalafil has been shown to decrease contraction-induced ischemia in muscle [54]. Clinical trials are recruiting to test short-term treatment (2 weeks) of sildenafil with escalating doses. Primary outcomes will be measured by the decrease in muscle tissue oxygenation (near infrared spectroscopy) and blood flow (Doppler ultrasound) evoked by reflex sympathetic activation in exercising forearm muscle [55].

Pentoxifylline (PTX) is a phosphodiesterase inhibitor that has recently undergone Phase II clinical trial assessment [56]. In preclinical studies, PTX treatment slowed muscle strength deterioration by 51% in exercised mdx mice [57]. However, a recent multicenter, randomized, double-blinded, controlled trial comparing 12 months of daily treatment with PTX to placebo in corticosteroid-treated boys with moderate to late ambulatory stage DMD (age 7 and older) failed to show improvement or slow the decline in overall muscle strength and function [56]. Despite the results, the investigators point out that the PTX treatment group showed a trend toward slower rate of decline and raise the possibility that a longer study may identify a beneficial, synergistic role for PTX. They also emphasize that future studies need to evaluate higher doses of PTX since doses of 120 mg/kg were necessary to attenuate the upregulation of TGF beta pathways in non-human primates and the negative results in the current study evaluated a dose of 20 mg/kg [56].

5. Insulin-like growth factor 1

In DMD, the absence of dystrophin is associated with the progressive loss of muscle fibers in specific muscle groups

accompanied by fibrotic tissue replacement. IGF-1 plays a role in muscle contraction, in addition to its role in stimulating muscle anabolism and enhancing insulin action in skeletal muscle [58]. The dihydropyridine receptor (DHPR), which is reduced in dystrophic muscle, is a primary link between dystrophin and cytoskeletal proteins to excitation–contraction (E–C) coupling [59,60]. IGF-1 is an exogenous hormone produced by numerous tissues including skeletal muscle. It has been well studied in mdx mice and has been shown to mediate changes in E–C coupling associated with increased levels of the DHPR isoform [59]. It also stimulates the proliferation and differentiation of skeletal muscle cells, allowing them to fuse to existing muscle fibers and enhance muscle regeneration, promote hypertrophy and increase the force of muscle contraction [61-64]. IGF-1 treatment in the mdx mice led to a 40% increase in muscle mass and to similar percentage increase in the force generation of the extensor digitorum longus muscle [65]. There was also hypertrophy and decreased fibrosis of the mdx diaphragm [65,66]. In a dual-gene combination strategy, viral vectors of mini-dystrophin and IGF-1 injected into mdx mice resulted in increased muscle mass and strength, reduced myofiber action and increased protection against contraction-induced injury – results not seen with individual viral vectors [67].

IGF-1 is available as recombinant human IGF-1 in a free form and as IGF-1 complexed with IGF-1 binding protein-3. There have been several treatment trials using free IGF-1 therapy in amyotrophic lateral sclerosis (ALS) [68-70] and one trial of free IGF-1 complexed with IGF-1 binding protein-3 in myotonic dystrophy [71]. ALS trials failed to show a clear beneficial result but demonstrated safety of IGF-1 in these patients. The trial in myotonic dystrophy showed encouraging results in seven of the nine patients treated. Two patients discontinued treatment because of cardiovascular complications [68-72]. More recently, treatment trials in children with primary growth hormone deficiency, elderly women following hip replacement surgery and patients with complications of HIV infection, have trialed IGF-1 complexed with IGF-1 binding protein-3, and each group of patients have tolerated this preparation without significant side effects [74-76]. An open trial of subcutaneous injections of IGF-1 complexed with IGF-1 binding protein in 15 moderately affected ambulatory patients with myotonic dystrophy type 1 using an escalating dosing treatment schedule for 24 weeks (0.5 mg/kg/day for 8 weeks, 1.0 mg/kg/day for 8 weeks and 2.0 mg/kg/day for 8 weeks) was well tolerated and showed an increase in lean body mass and improvements in metabolism. However there was no increase in muscle strength or function [71]. These clinical trials in adult neuromuscular disease and in children with primary growth hormone deficiency provide encouragement that boys with DMD will tolerate IGF-1 treatment without major side effects. The first DMD clinical trial is currently underway injecting SQ IGF-1 for 6 months in ambulatory DMD boys, 5 years and older, simultaneously receiving daily glucocorticoids for more than 12 months. The primary outcome is the 6-min walk test [77]. It should be noted that IGF-1 enhances insulin action and its use in patients with DMD receiving corticosteroid therapy is especially appropriate since corticosteroids decrease insulin action.

6. Nutritional

In certain protein-wasting conditions, acute postsurgical patients having major surgical procedures, and in critical care patients, glutamine, a nonessential amino acid, delivered intravenously has had beneficial effects in lessening acute infection and in slowing catabolism [78]. There have also been small-scale, short-term studies in chronic muscle wasting disorders, including DMD. In 1998 six DMD patients (ages 8 – 13 years) had improvement in protein anabolism after acute ingestion of glutamine [79]. In a similar study the following year, investigators found improved anabolism after 10 days of ingestion [80]. These results encouraged further evaluation into glutamine as a potential treatment and supplement for DMD patients. Initial investigation found a decrease in intramuscular concentration and *de novo* synthesis of glutamine in DMD patients, suggesting that glutamine may be acting as a conditional essential amino acid [80]. However, in a subsequent double-blind, randomized crossover trial of glutamine in 30 boys with DMD, there was no improvement at 4 months in the primary outcome, walking speed and no improvement in muscle mass or reduction in the rate of muscle breakdown. Thus, the short-term benefits of glutamine ingestion were not sustained with 4 months of treatment and it was proposed that glutamine does not serve as a conditional essential amino acid in DMD [81]. A factor that could have lessened any potential therapeutic benefit of glutamine is the possibility that increased glutamine intake led to increased removal and breakdown. An example to support this interpretation arises from a previous trial in boys with DMD of the essential branch chain amino acids, leucine, isoleucine and valine that failed to show any benefit. Blood level analysis of the branch chain amino acids revealed that after 2 weeks the patients had virtually converted all the ingested branched chain amino acid therapy into their oxidized forms which were in turn used for short chain fatty acid and energy production [82].

Other supplements (creatine monohydrate, conjugated linoleic acid, alpha-lipoic acid, and beta-hydroxy-beta-methylbutyrate) when given in combinations, were found to improve strength in the mdx mouse, with greatest efficacy in combination with prednisone [83]. Oral creatine supplementation alone showed efficacy in trials in mdx mice but demonstrated no beneficial effects on strength and function in patients with DMD [84-86]. However, despite a lack of functional improvement, a trial in 2010 with steroid-naïve patients given oral creatine versus controls, reported that 50% of parents having a son receiving creatine described subjective improvement in strength, while 57% of parents

having sons receiving placebo reported worsening of muscle strength [87]. There is no clear evidence of a benefit on strength and function with creatine supplementation and there are no carefully designed studies to determine if creatine has a long-term benefit. This question may go without an answer since there are other treatments having a higher priority for evaluation.

7. Treatments specific to genetic mutations

7.1 Stop codon read-through: gentamicin and ataluren

Up to 20% of patients with DMD have a premature stop codon, resulting in premature cessation of the mRNA translation and protein truncation [22]. Aminoglycosides, in prokaryotes, interfere with stop codons by introducing a nucleotide sequence at the aminoacyl transfer RNA acceptor site, allowing the translational machinery to continue the mRNA translation into a full length protein [88,89]. This approach to treatment has offered promise in a few genetic disorders, including cystic fibrosis and DMD. *In vitro* exposure of mdx mouse muscle cells to gentamicin showed stimulation of increased dystrophin and led to selection of a dosing schedule for the first 14 days *in vivo* trial. This trial demonstrated that daily subcutaneous gentamicin injection resulted in the production of a full-length dystrophin protein (10 – 20% of normal in all striated muscles examined) and a reduction in the serum creatine kinase levels [90]. However, subsequent clinical trials in DMD patients in 2001 (four patients received daily intravenous gentamicin 7.5 mg/kg for 14 days) and in 2003 (four patients received two cycles of 6 daily infusions separated by 7 weeks) showed less encouraging, varying results with no increase in dystrophin in the first trial and an increase in staining of three out of four biopsies following the latter treatment trial [91,92]. A more recent trial set out to address questions surrounding gentamicin therapy in DMD, including its biopotency, variability in dosing and long-term dosing [93]. Two cohorts (one with patients having stop codon mutations, the second, a control cohort having frameshift mutations), received intravenous infusion of gentamicin 7.5 mg/kg/day for 14 days. Two additional cohorts of patients having stop codon mutations underwent 6 months of intravenous treatment, with one cohort receiving gentamicin 7.5 mg/kg/day once a week and the other cohort receiving 7.5 mg/kg/day twice weekly [93]. The dosing was based on the known prolonged half life of dystrophin, 6 – 8 weeks [94]. The results revealed that the initial 14-day protocol in the stop codon cohort showed reduction in serum creatine kinase levels by 50%. After 6 months of 7.5 mg/kg/day dosing either once or twice weekly, muscle biopsy specimens showed an increased dystrophin up to 13 – 15% of normal ($p = 0.027$). No significant improvement in muscle strength or function was seen with weekly or twice weekly infusions. There was no significant renal, otological or vestibular toxicity after either of the 6-month treatment regimens [94]. Nevertheless, due to its known systemic side effects there are concerns and clinical limitations in considering the future use of gentamicin therapy. Alternative agents, which also work on nonsense mutation suppression, are being investigated, including new aminoglycosides and nonaminoglycosides [95,96].

Ataluren, formally known as PTC-124, is an oral nonaminoglycoside nonsense mutation suppressor, chemically different from aminoglycosides with no antibiotic properties. It is being used in clinical trials for both DMD and cystic fibrosis [96]. *In vitro*, Ataluren is thought to be superior to gentamicin in terms of bioavailability [97]. Phase I clinical trials have been completed in healthy individuals, and preliminary data from Phase II trials in DMD patients revealed an encouraging safety profile. However, the primary endpoint, 6-min walk testing, while trending toward improvement, failed to reach statistical significance and the study was completed early [98]. Refinements in study design have occurred and current studies are recruiting 110 patients for a 9-month trial (three times daily doses of 10, 10, 20 mg/kg up to 36 weeks) to establish Ataluren's long-term safety profile. Safety and efficacy measures will be made throughout the trial and 6 weeks following the final dose of Ataluren [99].

7.2 Exon skipping

A second genetic treatment strategy, exon skipping, involves excluding specific exons from the dystrophin mRNA transcript during the pre-mRNA processing to bypass mutations and restore the reading frame, resulting in a less severe phenotype, similar to BMD. This approach to treatment applies to about 72% of patients with DMD [100]. Oligonucleotides against splicing enhancer sequences (antisense oligonucleotides or AONs) have been developed to skip exons and restore the open reading frame as described. These AONs are 20 – 30 nucleotides in length and complimentary in sequence to regions of the pre-mRNA transcript. They bind to specific intronic or exonic sites of pre-mRNA, and promote specific exon exclusion from the mature mRNA. They can be given systemically or directly injected into muscle. Two examples of AONs are: 2′O-methyl-ribo-oligonucleotide-phosphorothioate (2′OMe) and phosphorodiamidate morpholino oligomers (PMOs). In preclinical studies, there were positive results (production of dystrophin staining muscle fibers) seen following AON treatment of cultured muscle cells from DMD patients with varying mutations [100]. Currently, two AONs, Drisapersen and Eteplirsen, show promise for targeting exon 51. Additionally, multiple intravenous AON trials in preclinical studies (canines) resulted in functional improvement and provided the groundwork for studies using a multiexon skipping approach [101].

In 2007, four patients received an intramuscular dose of the AON PRO051, a 2′OMePS by Prosensa (now entitled Drisapersen), into their tibialis anterior muscle, targeted at exon 51 [102]. Muscle biopsies revealed increased sarcolemma dystrophin expression. Functional improvement was not

observed following this isolated injection. One severely affected patient had a poorer result with less dystrophin, and this was thought to be related to the patient's advanced disease, suggesting the importance of performing clinical trials earlier in disease progression before muscle develops marked tissue replacement by fibrotic and adipose tissue [102]. Overall this investigation provided encouraging results and further studies including a Phase I or Phase IIa study in which 12 patients received 5 weeks of subcutaneously injections. These injections were well tolerated and there was dystrophin expression present in each patient as well as functional improvement in the 6-min walk in 10 of 12 patients. Although there were no serious adverse events, all treated patients developed proteinuria [103]. A Phase III randomized, double blind, placebo-controlled clinical study is currently ongoing, to assess the efficacy and safety of Drisapersen in DMD patients [104].

Eteplirsen, the second PMO targeted at exon 51 exclusion, is also showing promise as an exon skipping agent. In a study of seven DMD patients receiving subcutaneous injections of Eteplirsen into extensor digitorum brevis muscle (two received a dose of 0.09 mg and five received 0.9 mg), there was a 44 – 79% increase in dystrophin positive fibers on biopsy in the five patients receiving the high dose injections [105]. A Phase II, open label, dose escalation study with systemic Eteplirsen showed that 7 of 19 patients had modest improvement with positive staining of sarcolemmal dystrophin, ranging from 8.9 to 16.4%. There was a dose–response effect and no drug-related side effects [106]. October 2012, the manufacturer of Eteplirsen, Sarepta Therapeutics, announced that its Phase IIb randomized, double-blind, placebo-controlled multiple dose efficacy trial had met its primary efficacy endpoint. The preliminary unpublished data includes an average of 47% increase in dystrophin reverent fibers in biopsies of biceps in all Eteplirsen-treated patients (n = 8) compared to placebo/delayed treatment (n = 4), over 48 weeks. There was also significant clinical benefit on their primary clinical outcome measurement, the 6-min walk test in the 50 mg/kg treated group (n = 4) compared to the 30 mg/kg group (n = 4) and placebo (n = 4). All patients treated with Eteplirsen noted vomiting and balance disorder. Although this study has too low a number of subjects for sufficient power to determine efficacy, Eteplirsen has the potential to alter disease course [107].

Treatment approaches using direct muscle injection provide an important therapeutic groundwork; however, going forward, they are likely to be bypassed or relatively low in priority compared to systemic therapies. If direct injection therapy becomes more appropriate in future care of patients, the number of doses and dosing schedules for the different AONs will need further investigation. Another important part of groundwork for exon skipping therapy involves evaluation of patients with BMD to determine the exact relationship between the specific mutation in a given BMD patient, the structure and function of the altered dystrophin in that patient, and the phenotypic features of these BMD patients compared to patients with DMD. One recent study by Anthony *et al.* characterized 17 BMD patients including 4 who were asymptomatic, 12 mildly affected, and 1 severely affected patient with in-frame deletions and all having dystrophin levels > 40% of control [108]. Interestingly, the investigators point out that the group of BMD patients with deletions with an endpoint of exon 51 (the skipping of which could rescue the largest group of DMD deletions) showed significantly higher dystrophin levels than those with deletions ending with exon 53. However, taking together all of their results, Anthony *et al.* indicate that, 'all varieties of internally deleted in-frame dystrophin assessed in these 17 BMD patients have the functional capability to provide a substantial clinical benefit to patients with DMD' [108].

8. Gene therapy

The dystrophin gene was identified in 1987, and is the largest human gene, > 2.4M base pairs [109]. Due to its size, it is not possible to fit the entire gene into an adeno-associated virus, which is the best vehicle for gene transfer because of its persistence in healthy muscles without pathogenicity [110]. The packing capacity of the adeno-associated virus is ~ 4.7 kb [110]. Researchers have developed different mini-dystrophin genes [111-113], and studies have demonstrated that following their transfer there is protection of the plasma membrane of myofibers in adult mdx muscle [111].

Mendell *et al.*, in 2010, reported the first clinical gene therapy trial in a study of six boys with frame-shift mutations [114]. They delivered a mini-dystrophin gene in an adeno-associated virus into one biceps muscle and the opposite biceps received saline. Muscle biopsy specimens were assessed on day 42 (four patients) and day 90 (two patients). In all patients, vector DNA was detected, although functional protein was not visualized [114]. Future studies are in progress including plans for systemic as well as regional intraarterial delivery of vector containing mini-dystrophin gene. However, immune reaction to viral vector transfer remains an important concern that researchers need to address.

9. Conclusion

Table 1 summarizes current therapeutic interventions for DMD. Evidence-based treatments for DMD are limited to corticosteroids. They provide the only effective treatment at slowing the progression of the disease. However, important unanswered questions remain. How does corticosteroid therapy produce its beneficial effect in patients with Duchenne dystrophy, and do these beneficial effects depend, to a significant degree, on the age of the patient and severity of the disease when treatment is undertaken? Are there coexisting genetic factors that influence corticosteroid responsiveness and can they assist in defining the beneficial action of corticosteroids, as well as help in developing more optimal

Table 1. Summary of current therapeutic interventions evaluated for DMD.

Intervention	Name	Known role	Presumed role in DMD	Effectiveness	Clinical side effects
Corticosteroid	Prednisone Deflazacort	Suppression of inflammation	Unknown, but unlikely suppression of inflammation	Slows progression of muscle weakness	Long-term studies show short stature, weight gain, vertebral fractures; no difference in long bone fractures.
Myostatin inhibition	MYO-29 Follistatin ACE-031	Myostatin is a growth differentiation factor that is a member of the TGF beta protein family and inhibits muscle differentiation and growth	Inhibition of myostatin	MYO-29 in canines showed increased muscle mass, decreased CK and decreased muscle fibrosis. MYO-29 was ineffective in humans. Follistatin and ACE-031 are currently in clinical trials.	Follistatin first clinical trial in BMD is enrolling ACE-031 is in Phase II clinical trials. It was tolerated in Phase I healthy controls.
Phosophodiesterase Inhibitors	Sildenafil Tadalafil PTX	Inhibit inactivation of cGMP	Prevents inactivation of nitric oxide-stimulated cGMP pathway, preventing contraction coupling damage	Sildenafil reversed left ventricle and diaphragm muscle dysfunction in mdx mice. Tadalafil decreased contraction-induced ischemia in mdx mice. PTX showed no clinical efficacy in a randomized clinical trial in combination with corticosteroids in DMD boys.	Sildenafil clinical trial currently recruiting; PTX significant adverse effects include gastrointestinal symptoms and reported hemorrhage/bleeding.
IGF-1	GF-1	Proliferation and differentiation of muscle precursor cells to induce hypertrophy of muscle fibers and enhance muscle regeneration	Increase DHPR, a primary link between dystrophin and cytoskeletal proteins to E–C coupling	In myotonic dystrophy patients, an increase in lean body mass and improvements in metabolism were seen, but no increased muscle strength or function	Clinical trial currently running for DMD patients
Nutritional	Glutamine Creatine monohydrate	Protein Synthesis Increases ATP	Replete decreased muscle protein synthesis	Neither showed effective in class 1 clinical trials. Subjective improvement in functional strength per parents in oral creatine patients	No significant side effects

Table 1. Summary of current therapeutic interventions evaluated for DMD (continued).

Intervention	Name	Known role	Presumed role in DMD	Effectiveness	Clinical side effects
Stop codon read-through	Gentamicin Ataluren	Introduce a nucleotide sequence at the aminoacyl transfer RNA acceptor site, allowing the translational machinery to continue	Translation of mRNA into a full length protein	After 6 months of treatment with gentamicin patients showed increased levels of dystrophin. Average muscle scores were stable compared to control group, whose scores dropped Ataluren did not show significance in the primary outcome in the treated group	Concerns of oto- and renal toxicity in gentamicin treated patients, but these have not been demonstrated at the doses tested
Exon skipping	Drisapersen Eteplirsen	Removes specific exons from the dystrophin mRNA transcript during the pre-mRNA processing	Restore reading frame for a less severe phenotype	Drisapersen and Eteplirsen showed a dose–response effect with improvement in sarcolemmal dystrophin and functional improvement in the 6-min walk test	Proteinuria seen in all 12 patients treated with Drisapersen. Eteplirsen: Reported side effects in treatment group were vomiting and balance disorder
Gene therapy	Mini-dystrophin gene	Healthy gene transplantation by adeno-associated vector	Replace depleted dystrophin	Successful delivery of vector DNA in six DMD boys with frameshift mutations, but no functional protein was visualized	None, so far, in clinical trials

corticosteroid treatment? Alternative pharmacological agents have shown promise in the mdx mouse and canine models, but are yet to show effectiveness in clinical trials and long-term side effects are not known. Targeting genetic mutations is a promising therapeutic approach that has the potential to benefit more then 75% of patients, but it is in its early stages. How quickly such genetic treatments can be developed and how cost effective they will be remains to be determined. Regardless of new genetic therapies, classical pharmacological approaches, corticosteroids, will still be important as a dual therapy, especially since current genetic therapies target only a subset of patients.

10. Expert opinion

It is encouraging to see the spectrum of new therapeutic approaches that have developed in DMD over the past 10 years. The advent of genetic treatments and gene therapy offers promise for new, more effective treatments. However, as we look forward to the future, questions can be posed as to how to refine and improve the design of new Phase III clinical trials and bring them to the market. As noted, recent publications have raised the possibility that genetic factors may influence responsiveness to corticosteroids and may also become important in determining the efficacy of genetic treatments. In improving and enhancing the design of future treatment trials, are there specific endpoints and measures that we need to evaluate for certain stages of DMD? Have we fully explored and optimized treatments that already show efficacy?

It is clear that corticosteroids are an effective treatment for slowing the progression of DMD. The first prednisone trial was over 30 years ago and it took three randomized clinical trials and a number of additional trials since these initial studies to deem corticosteroids as effective evidence-based therapy for patients with DMD. Despite continued research and clinical trials, we still lack a clear understanding of how corticosteroids increase muscle mass and strength in the early stages of DMD, and how they prolong ambulation, pulmonary and cardiac function throughout the course of this disease. We do not know why some patients do not respond to them, the age of initiation that is most effective, and the long-term side effects of various doses. With all of these questions, in combination with the lack of Phase III evidence for many of the other therapeutic interventions, researchers and clinicians could consider prioritizing a focus on maximizing our understanding of the mechanism of corticosteroids and optimizing their use. With corticosteroids as the standard of care, it is going to be difficult to design randomized trials of steroid-naïve patients. Future treatment trials need to be designed with corticosteroids as a part of ongoing therapy and continued effort devoted to defining the 'natural history', or more appropriately, the rate of disease progression in boys receiving long-term corticosteroids. As a part of this ongoing, 'natural history' assessment, DMD patients in the middle and later stages of the disease need renewed evaluation of the optimal endpoints to measure the rate of progression of different disease manifestations, including more sensitive and reliable methods to evaluate upper extremity function, pulmonary function (including effectiveness of cough), functional capabilities while using assistive devices, gastrointestinal function and overall quality of life [115]. We also need to extend and increase the sensitivity of current treatments and therapeutics, including measurement of the different long-term effects of corticosteroid therapy, noninvasive ventilation, cough assist devices, cardiac and bowel management regimens. At the other end of the age spectrum in boys with DMD, we need to develop more reliable and feasible measures to assess response to treatment in the very young, perhaps from infancy to 4 years of age. We also need to adapt other outcome measures to be able to evaluate our young population where it is sometimes difficult to depend on their cooperation in motor and cognitive tasks [116]. An abundance of opportunities to increase and refine our endpoint measures and improve immediate care for patients are available to pursue. A recent example is the idea that a shorter distance walk and velocity correlate is as accurate in functional outcome measurements as the 6-min walk and allows for more patients to participate in trials in less time [117].

An opportunity to pursue evaluation of different long-term treatment regimens with corticosteroids relates to intermittent versus daily corticosteroid therapy. For the most recent trial, Escolar *et al.*, 12 months of high dose (5 mg/kg on Saturday and Sunday) weekend corticosteroid therapy were thought to be as effective as standard daily treatment (0.75 mg/kg/day). These studies need long-term follow up, including evaluation of treatment failure rate, assessment of those patients requiring a switch to daily treatment and measurement of long-term benefits to quality of life, as well as assessment of the side effects.

Of the therapeutic interventions studied in mouse, canine and early human trials, IGF-1 appears to have potential as a possible synergistic dual therapy with corticosteroids. IGF-1 counters insulin resistance, such as the decrease in insulin action often associated with long-term corticosteroid therapy. IGF-1 has important anabolic effects and can synergize with the net anabolic action that corticosteroids have in DMD, contrary to expectations. The time seems right for a trial to examine the potential combined benefit of IGF-1 and corticosteroid therapy in DMD patients.

Long-term studies will be needed for the new genetic therapies and for all of the other therapeutic approaches we have mentioned to compare side effects to functional improvement, including measurement of quality of life. To achieve this goal is a major challenge, especially with the limited funding that is available. Going forward, there is likely to be an increased emphasis on cost–benefit analysis of the different treatments we develop. New therapies will need to show a significantly greater sustained benefit for our DMD patients in order for them to supplant or reduce the use of long-term corticosteroid treatment.

The advancements in exon skipping are very exciting and give new life to potential treatment options for muscular dystrophies. However, many questions surround their use. It is unclear if the best preclinical models have been identified and whether those models allow a valid (human translatable) assessment of an optimal delivery system, as well as appropriate therapeutic targets. This uncertainty about our current use of animal or cell models as a reliable guide for therapeutic targets, along with the challenge of developing affordable therapeutic strategies, will require a thoughtful, thorough and extended study to identify optimal AON therapeutics, while providing the most patient friendly and cost-effective treatment regimens. During this time, corticosteroid research should not be lost.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Hoffman EP. Dystrophinopathies. In: Karpati G, Hilton-Jones D, Griggs R, editors. Disorders of voluntary muscle. Cambridge University Press; Cambridge: 2001. p. 385-432
2. Ciafaloni E, Fox DJ, Pandya S, et al. Delayed diagnosis in duchenne muscular dystrophy: data from the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet). J Pediatr 2009;155(3):380-5
3. Eagle M, Baudouin SV, Chandler C, et al. Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. Neuromuscular Disord 2002;12(10):926-9
4. Moxley RT III, Ashwal S, Pandya S, et al. Practice parameter: corticosteroid treatment of Duchenne dystrophy: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2005;64(1):13-20

• **Evidence-based guidelines for DMD.**

5. Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev 2008(1):CD003725

• **Evidence-based guidelines for DMD.**

6. Griggs RC, Moxley RT III, Mendell JR, et al. Duchenne dystrophy: randomized, controlled trial of prednisone (18 months) and azathioprine (12 months). Neurology 1993;43(3 Pt 1):520-7
7. Mendell JR, Moxley RT, Griggs RC, et al. Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. N Engl J Med 1989;320(24):1592-7
8. Fenichel GM, Florence JM, Pestronk A, et al. Long-term benefit from prednisone therapy in Duchenne muscular dystrophy. Neurology 1991;41(12):1874-47
9. Griggs RC, Moxley RT, Mendell JR, et al. Prednisone in duchenne dystrophy. A randomized, controlled trial defining the time course and dose response. Clinical Investigation of Duchenne Dystrophy Group. Arch Neurol 1991;48(4):383-8
10. Bonifati DM, Ruzza G, Bonometto P, et al. A multicenter double-blind randomized trial of deflazacort versus prednisone in Duchenne muscular dystrophy. Muscle Nerve 2000;23(9):1344-7
11. Mesa LE, Dubrosvky AL, Corderi J, et al. Steroids in Duchenne muscular dystrophy-deflazacort trial. Neuromuscular Disord 1991;1(4):261-6
12. Angelini C, Pegoraro E, Turella E, et al. Deflazacort in Duchenne dystrophy: a study of long-term effect. Muscle Nerve 1994;17(4):386-91
13. Balaban B, Matthews DJ, Clayton GH, Carry T. Corticosteroid treatment and functional improvement in Duchenne muscular dystrophy: long-term effect. Am J Phys Med Rehabil 2005;84(11):843-50
14. Biggar WD, Harris VA, Eliasoph L, Alman B. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. Neuromuscul Disord 2006;16(4):249-55
15. King WM, Ruttencutter R, Nagaraja HN, et al. Orthopedic outcomes of long-term daily corticosteroid treatment in Duchenne muscular dystrophy. Neurology 2007;68(19):1607-13
16. Houde S, Filiatrault M, Fournier A, et al. Deflazacort use in Duchenne muscular dystrophy: an 8-year follow up. Pediatr Neurol 2008;38(3):200-6
17. Merlini L, Gennari M, Malaspina E, et al. Early corticosteroid treatment in 4 Duchenne muscular dystrophy patients: 14 year follow-up. Muscle Nerve 2012;45(6):796-802
18. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial mangement. Lancet Neurol 2010;9(1):77-93

•• **Consesus-based guidelines for DMD.**

19. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lance Neurol 2010;9(2):177-89

•• **Consesus-based guidelines for DMD.**

20. Moxley RT III, Pandya S, Ciafaloni E, et al. Change in natural history of Duchenne muscular dystrophy with long-term corticosteroid treatment: implications for management. J Child Neurol 2010;25(9):1116-29

•• **This review is about long-term corticosteroid trials and highlights their important.**

21. McAdam LC, Mayo AL, Alman BA, Biggar WD. The Canadian experience with long-term deflazacort treatment in Duchenne muscular dystrophy. Acta Myologica 2012;31(1):16-20
22. Ahn AH, Kunkel LM. The structural and functional diversity of dystrophin. Nat Genet 1993;3(4):283-91
23. Aartsma-rus A, Van Deutekom JC, Fokkema IF, et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle Nerve 2006;34(2):135-44

24. Monaco AP, Bertelson CJ, Liechti-Gallati S, et al. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics 1988;2(1):90-5

25. Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet 1989;45(4):498-506

26. England SB, Nicholson LV, Johnson MA, et al. Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. Nature 1990;343(6254):180-2

27. Phelps SF, Hauser MA, Cole NM, et al. Expression of full-length and truncated dystrophin mini-genes in transgenic mdx mice. Hum Mol Genet 1995;4(8):1251-8

28. Deburgrave N, Daoud F, Liense S, et al. Protein- and mRNA-based phenotype-genotype correlations in DMD/BMD with point mutations and molecular basis for BMD with nonsense and frameshift mutations in the DMD gene. Hum Mutat 2007;28(2):183-95

29. Kirschner J, Schessl J, Schara U, et al. Treatment of Duchenne muscular dystrophy with ciclosporin A: a randomised, double-blind, placebo-controlled multicentre trial. Lancet Neurol 2010;9(11):1053-9

30. Drachman DB, Tokya KV, Myer E. Prednisone in Duchenne muscular dystrophy. Lancet 1974;2(7894):1409-12

31. Escolar DM, Hache LP, Clemens PR, et al. Randomized, blinded trial of weekend vs daily prednisone in Duchenne muscular dystrophy. Neurology 2011;77(5):444-52

32. Robert G. Finding the Optimum Regimen for Duchenne Muscular Dystrophy (FOR-DMD). In: clinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2012. Available from: http://clinicaltrials.gov/ct2/show/NCT01603407?term=NCT01603407&rank=1: NCT01603407

33. Huizenga NA, Koper JW, De Lange P, et al. A polymorphism in the glucocorticoid receptor gene may be associated with and increase sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab 1998;83(1):144-51

34. Reeves EK, Rayavarapu S, Damsker JM, Nagaraju K. Glucocorticoid anlogues: potential therapeutic alternatives for treating inflammatory muscle diseases. Endocr Metab Immune Disord Drug Targets 2012;12(1):95-103

35. Bonifati DM, Witchel SF, Ermani M, et al. The glucocorticoid receptor N363S polymorphism and steroid response in Duchenne dystrophy. J Neurol Neurosurg Psychiatry 2006;77(10):1177-9

36. De Bosscher K, Beck IM, Haegeman G. Classic glucocorticoids versus non-steroidal glucocorticoid receptor modulators: survival of the fittest regulator of the immune system? Bran Behav Immun 2010;7):1035-42

37. Bello L, Piva L, Barp A, et al. Importance of SPP1 genotype as a covariate in clinical trials in Duchenne muscular dystrophy. Neurology 2012;79(2):159-62

38. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 1997;387(6628):83-90

39. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci USA 1997;94(23):12457-61

40. Wagner KR, Fleckenstein JL, Amato AA, et al. A phase I/IItrial of MYO-029 in adult subjects with muscular dystrophy. Ann Neurol 2008;63(5):561-71

41. Bish LT, Sleeper MM, Forbes SC, et al. Long-term systemic myostatin inhibition via liver-targeted gene transfer in golden retriever muscular dystrophy. Hum Gene Ther 2011;22(12):1499-509

42. Schneyer AL, Sidis Y, Gulati A, et al. Differential antagonism of activin, myostatin and growth and differentiation factor 11 by wild-type and mutant follistatin. Endocrinology 2008;149(9):4589-95

43. Nakatani M, Tekehara Y, Sugino H, et al. Transgenic expression of a myostatin inhibitor derived from follistatin increases skeletal muscle mass and ameliorates dystrophic pathology in mdx mice. FASEB J 2008;22(2):477-87

44. Benabdallah BF, Bouchentouf M, Rousseau J, et al. Inhibiting myostatin with follistatin improves the success of myoblast transplantation in dystrophic mice. Cell Transplant 2008;17(3):337-50

45. Jerry RM. Follistatin gene transfer to patients with becker muscular dystrophy and sporadic inclusion body myositis. In: clinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2012. Available from: http://clinicaltrials.gov/ct2/show/NCT01519349?term=NCT01519349&rank=1: NCT01519349

46. Cadena SM, Tomkinson KN, Monell TE, et al. Administration of soluble activin type IIB receptor promotes skeletal muscle growth independent of fiber type. J Appl Physiol 2010;109(3):635-42

47. Acceleron Pharma, Inc. A safety, tolerability, pharmacokinetic and pharmacodynamic study of ACE-031 in healthy postmenopausal women. In: ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); Available from: http://clinicaltrials.gov/ct2/show/NCT00952887?term=ace-031&rank=1: NCT00952887

48. Acceleron Pharma, Inc. Study of ACE-031 in subjects with Duchenne muscular dystrophy. In: ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2012.Available from: http://clinicaltrials.gov/ct2/show/NCT01099761?term=ace-031&rank=3 NCT01099761

49. Lee KH, Baek MY, Moon KY, et al. Nitric oxide as a messenger molecule for myoblast fusion. J Biol Chem 1994;269(20):14371-4

50. Brenman JE, Chao DS, Xia H, et al. Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. Cell 1995;82(5):743-52

51. Wehling M, Spencer MJ, Tidball JG. A nitric oxide synthase transgene ameliorates muscular dystrophy in mdx mice. J. Cell Biol 2001;155(1):123-31

52. Chang WJ, Iannaccone ST, Lau KS, et al. Neuronal nitric oxide synthase and dystrophin-deficient muscular dystrophy. Proc Natl Acad Sci USA 1996;93(17):9142-7

53. Sander MB, Chavoshan B, Harris SA, et al. Functional muscle ischemia in neuronal nitric oxide synthase deficient skeletal muscle of children with Duchenne muscular dystrophy. Proc Natl Acad Sci USA 2000;97(25):13818-23

54. Percival JM, Whitehead NP, Adams ME, et al. Sildenafil reduces respiratory muscle weakness and fibrosis in the mdx mouse model of Duchenne muscular dystrophy. J Pathol 2012;228(1):77-87

55. Ron V. Tadalafil and sildenafil for duchenne muscular dystrophy. In: clinicaltrials.gov [Internet] National Library of Medicine (US), Bethesda (MD): 2012.Available from: http://clinicaltrials.gov/ct2/show/NCT01359670?term=sildenafil+duchenne&rank=2: NCT0135970

56. Escolar DM, Zimmerman A, Bertorini T, et al. Pentoxifylline as a rescue treatment for DMD. A randomized double-blind clinical trial. Neurology 2012;78(12):904-13

57. Granchelli JA, Pollina C, Hudecki MS. Pre-clinical screening of drugs using the mdx mouse. Neuromuscul Disord 2000;10(4-5):235-9

58. Jorgensen JO, Moller L, Krag M, et al. Effects of growth hormone on glucose and fat metabolism in human subjects. Endocrinol Metab Clin North Am 2007;36(1):75-87

59. Schertzer JD, van der Poel C, Shavlakadze T, et al. Muscle-specific over expression of IGF-1 improves E-C coupling in skeletal muscle fibers from dystrophic mdx mice. Am J Physiol Cell Physiol 2008;294(1):C161-8

60. Ampong BN, Imamura M, Matsumiya T, et al. Intracellular localization of dysferlin and its association with the dihydropyridine receptor. Acta Myol 2005;24(2):134-44

61. Rosenthal SM, Cheng ZQ. Opposing early and late effects of insulin-like growth factor I on differentiation and the cell cycle regulatory retinoblastoma protein in skeletal muscle. Proc Natl Acad Sci USA 1995;92(22):10307-10311

62. Engert JC, Berglund EB, Rosenthal N. Proliferation precedes differentiation in IGF-1-stimulated myogenesis. J Cell Biol 1996;135(2):431-40

63. Musaro A, McCullagh K, Paul A, et al. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet 2001;27(2):195-200

64. Barton-Davis ER, Shorturma DI, Musaro A, et al. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. Proc Natl Acad Sci USA 1998;95(26):15603-7

65. Barton ER, Morris L, Musaro A, et al. Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. J Cell Biol 2002;157(1):137-48

66. Gregorevic P, Plant DR, Leeding KS, et al. Improved contractile function of the mdx dystrophic mouse diaphragm muscle after insulin-like growth factor-I administration. Am J Pathol 2002;1(6):2263-72

67. Abmayr S, Gregorevic P, Allen JM, Chamberlain JS. Phenotypic improvement of dystrophic muscles by rAAV/microdystrophin vectors is augmented by Igf1 codelivery. Mol Ther 2005;12(3):441-50

68. Sacca F, Quarantelli M, Rinaldi C, et al. A randomized controlled clinical trial of growth hormone in amyotrophic lateral sclerosis: clinical, neuroimaging, and hormonal results. J Neurol 2012;259(1):132-8

69. Sorenson EJ, Windbank AJ, Mandrekar JN, et al. Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. Neurology 2008;71(22):1770-5

70. Borasio GD, Robberecht W, Leigh PN, et al. A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-1 Study Group. Neurology 1998;51(2):583-6

71. Heatwole CR, Eichinger KJ, Friedman DI, et al. Open-label trial of recombinant human insulin-like growth factor 1/recombinant human insulin-like growth factor binding protein 3 in myotonic dystrophy type 1. Arch Neurol 2011;68(1):37-44

72. Vlachopapadopoulou E, Zachwieja JJ, Gertner JM, et al. Metabolic and clinical response to recombinant human insulin-like growth factor 1 in myotonic dystrophy. J Clin Endocrinol Metab 1995;80(12):3715-23

73. Lai EC, Felice KJ, Festoff BW, et al. Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo controlled study. The North America ALS/IGF-1 Study Group. Neurology 1997;49(6):1621-30

74. Cohen P, Rogol AD, Howard CP, et al. Insulin growth factor-based dosing of growth hormone therapy in children: a randomized, controlled study. J Clin Endocrinol Metab 2007;92(7):2480-6

75. Weissberger AJ, Anastasiadis AD, Strugess I, et al. Recombinant human growth hormone treatment in elderly patients undergoing elective total hip replacement. Clin Endocrinol 2003;58(1):99-107

76. Rao MN, Mulligan K, Tai V, et al. Effects of insulin-like growth factor (IGF)-1/IGF-binding protein-3 treatment on glucose metabolism and fat distribution in human immunodeficiency virus-infected patients with abdominal obesity and insulin resistance. J Clin Endocrinol Metab 2010;95(9):4361-6

77. Children's Hospital Medical Center, Cincinnati. Safety and efficacy study of IGF-1 in duchenne muscular dystrophy. In: ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2012.Available from: http://clinicaltrials.gov/ct2/show/NCT01207908?term=IGF-1+duchenne&rank=1: NCT01207908

78. Novak F, Heyland DK, Avenell A, et al. Glutamine supplementation in serious illness: a systemic review of the evidence. Crit Care Med 2002;30(9):2022-9

79. Hankard RG, Hammond D, Haymond MW, Darmaun D. Oral glutamine slows down whole body protein breakdown in Duchenne muscular dystrophy. Pediatr Res 1998;43(2):222-6

80. Hankard R, Mauras N, Hammond D, et al. Is glutamine a 'conditionally essential' amino acid in Duchenne muscular dystrophy? Clin Nutr 1999;18(6):365-9

81. Mok E, Letellier G, Cuisset JM, et al. Lack of functional benefit with glutamine versus placebo in Duchenne muscular dystrophy: a randomized crossover trial. PLoS ONE 2009;4(5):e5448

82. Mendell JR, Griggs RC, Moxley RT III, et al. Clinical investigation in Duchenne muscular dystrophy: IV. Double-blind controlled trial of leucine. Muscle Nerve 1984;7(7):535-41

83. Payne ET, Yasuda N, Bourgeois JM, et al. Nutritional therapy improves function and complements corticosteroid intervention in mdx mice. Muscle Nerve 2006;33(1):66-77

84. Passaquin AC, Renard M, Kay L, et al. Creatine supplementation reduces skeletal muscle degeneration and enhances mitochondrial function in mdx mice. Neuromuscul Disord 2002;12(2):174-82

85. Pulido SM, Passaquin AC, Leijendekker WJ, et al. Creatine supplementation improves intracellular Ca2+ handling and survival in mdx skeletal muscle cells. FEBS Lett 1998;493(3):357-62

86. Tarnopolsky MA, Mahoney DJ, Vajsar J, et al. Creatine monohydrate enhances strength and body composition in Duchenne muscular dystrophy. Neurology 2004;62(10):1771-7

87. Banerjee B, Sharma U, Balasubramanian K, et al. Effect of creatine monohydrate in improving cellular energetics and muscle strength in ambulatory Duchenne muscular dystrophy patients: a randomized. placebo-controlled 31P MRS Study. Magn Reson Imaging 2010;28(5):698-707

88. Anderson WF, Gorini L, Breckenridge L. Role of ribosomes in streptomycin-activated suppression. Proc Natl Acad Sci USA 1965;54(4):1076-83

89. Davies J, Gilbert W, Gorini L. Streptomycin, suppression, and the code. Proc Natl Acad Sci USA 1964;51:883-90

90. Barton-Davis ER, Cordier L, Shotuma DI, et al. Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. J Clin Invest 1999;104(4):375-81

91. Wagner KR, Hamed S, Hadley DW, et al. Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. Ann Neurol 2001;49(6):706-11

92. Politano L, Nigro G, Nigro V, et al. Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results. Acta Myol 2003;22(1):15-21

93. Malik V, Rodino-Klapac LR, Viollet L, et al. Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. Ann Neurol 2010;67(6):771-80

94. Ghahramani Seno MM, Graham IR, Athanasopoulous T, et al. RNAi-mediated knockdown of dystrophin expression in adult mice does not lead to overt muscular dystrophy pathology. Hum Mol Genet 2008;17:2622-32

95. Nudelman I, Rebibo-Sabbah A, Cherniavsky M. Development of novel aminoglycoside (NB54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations. J Med Chem 2009;52(9):2836-45

96. Lee HL, Dougherty JP. Pharmaceutical therapies to recode nonsense mutations in inherited diseases. Pharmacol Ther 2012;136(2):227-66

97. Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447:87-91

98. . PTC Therapeutics. Phase 2b Study of PTC124 in Duchenne/Becker Muscular Dystorphy (DMD/BMD). In: ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2011. Available from: http://clinicaltrials.gov/ct2/show/NCT00592553?term=NCT00592553&rank=1: NCT00592553

99. PTC Therapeutics. Study of Ataluren for Previously Treated Patients With nmDBMD in the US. In: ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2011.Available from: http://clinicaltrials.gov/ct2/show/NCT01247207?term=NCT01247207&rank=1: NCT01247207

100. Aartsma-Rus A, Janson AA, Kaman WE, et al. Therapeutic antisense-induced exon skipping in cultured muscle cells from six different DMD patients. Hum Mol Genet 2003;12:907-14

101. Yokota T, Lu QL, Partridge T, et al. Efficacy of systemic morpholino exon-skipping in duchenne dystrophy dogs. Ann Neurol 2009;65:667-76

102. Van Deutekom JC, Janson AA, Ginjaar LB, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 2007;357(26):2677-86

103. Goemans NM, Tullinius M, van den Akker JT, et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. N Engl J Med 2011;364(16):1513-22

104. GlaxoSmithKline. A clinical study to assess the efficacy and safety of GSK2402968 in Subjects with Duchenne Muscular Dystrophy (DMD114044). In: ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD); 2010. Available from: http://clinicaltrials.gov/ct2/show/NCT01254019?term=NCT01254019&rank=1 NCT01254019

105. Cirak S, Feng L, Anthony K, et al. Restoration of the dystrophin-associated glycoprotein complex after exon skipping therapy in Duchenne muscular dystrophy. Mol Ther 2012;20(2):462-7

106. Cirak S, Arechavala-Gomeza V, Guglieri M, et al. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. Lancet 2011;378(9791):595-605

107. Sarepta Therapeutics. Full 48-week Phase IIb data from eteplirsen study in Duchenne presented at World Muscle Society. [October 16, 2012]. Retrieved on October 26 2012. Availble from http://issuu.com/parentprojectmd/docs/mendell_eteplirsen_48_week_data_wms2012_final

108. Anthony K, Cirak S, Torelli S, et al. Dystrophin quantification and clinical correlations in Becker muscular dystrophy: implications for clinical trials. Brain 2011;134(Pt 12):3547-59

109. Koenig M, Hoffman EP, Bertelson CJ, et al. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 1987;50(3):509-17

110. Scott JM, Li S, Harper SQ, et al. Viral vectors for gene transfer of micro-, mini-, or full-length dystrophin. Neuromuscul Disord 2002(Suppl 1):S23-9

111. Wang B, Li J, Xiao X. Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in the mdx mouse model. Proc Natl Acad Sci USA 2000;97(25):13614-719

112. Kornegay JN, Li J, Bogan JR, et al. Widespread muscle expression of an AAV9 human mini-dystrophin vector after intravenous injection in neonatal dystrophin-deficient dogs. Mol Ther 2010;18(8):1501-18

113. Zhang Y, Duan D. Novel mini-dystrophin gene dual adeno-associated virus vectors restore neuronal nitric oxide synthase expression at the sarcolemma. Hum Gene Ther 2012;23(1):98-103

114. Mendell JR, Campbell K, Rodino-Klapac L, et al. Dystrophin Immunity in Duchenne's Muscular Dystrophy. N Eng J Med 2010;363(15):1429-37

115. Florence J, Connolly A, Miller JP, et al. Outcomes measure reliabiliity in non ambulatory boys and men with Duchenne Muscular Dystrophy (DMD): Results from the Muscular Dystrophy Association DMD clinical research network. Neurology 2012;78(Meeting Abstracts 1):P04.085

116. Lowes L, Alfano L, Viollet L, et al. Moderate walking distances and velocity correlate with function comparable to 6 minute walk test. Neurology 2012;78(Meeting Abstracts 1):S49.005

117. Connolly A, Florence J, Cradock M, et al. Motor and cognitive assessment of infants and young boys with duchenne muscular dystrophy; results from the muscular dystrophy association dmd clinical research center network. Neurology 2012;78(Meeting Abstracts 1):P04.084

Affiliation

Michele A Scully MD, Shree Pandya MS PT & Richard T Moxley[†] MD
[†]Author for correspondence
University of Rochester Medical Center,
601 Elmwood Ave, Box 673,
Rochester, NY 14642, USA
E-mail: RichardT_Moxley@urmc.rochester.edu