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Pharmacological therapeutics targeting the secondary defects and downstream pathology of Duchenne muscular dystrophy

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Abstract

Introduction—Since the identification of the dystrophin gene in 1986, a cure for Duchenne muscular dystrophy (DMD) has yet to be discovered. Presently, there are a number of genetic-based therapies in development aimed at restoration and/or repair of the primary defect. However, growing understanding of the pathophysiological consequences of dystrophin absence has revealed several promising downstream targets for the development of therapeutics.

Areas covered—In this review, we discuss various strategies for DMD therapy targeting downstream consequences of dystrophin absence including loss of muscle mass, inflammation, fibrosis, calcium overload, oxidative stress, and ischemia. The rationale of each approach and the efficacy of drugs in preclinical and clinical studies are discussed.

Expert opinion—For the last 30 years, effective DMD drug therapy has been limited to corticosteroids, which are associated with a number of negative side effects. Our knowledge of the consequences of dystrophin absence that contribute to DMD pathology has revealed several potential therapeutic targets. Some of these approaches may have potential to improve or slow disease progression independently or in combination with genetic-based approaches. The applicability of these pharmacological therapies to DMD patients irrespective of their genetic mutation, as well as the potential benefits even for advanced stage patients warrants their continued investigation.

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Keywords

Duchenne muscular dystrophy; corticosteroids; myostatin; NF-κB; TGF-β; fibrosis; phosphodiesterase inhibitors; therapeutics; oxidative stress; calcium

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked inherited progressive muscle-wasting disease caused by nonsense or frame shift loss of function mutations in the dystrophin gene. DMD affects approximately 1 in 3,500 male births, and is one of the most common and severe forms of muscular dystrophy. Diagnosis generally occurs between 2–4 years of age when children begin to demonstrate early hallmarks of the disease including delayed motor milestones and enlarged calf muscles. Independent ambulation is lost between 10–12 years of age, and premature death occurs in the late twenties to early thirties due to respiratory and/or cardiac failure[1]. Mutations that permit production of a partially functional dystrophin with reduced expression lead to the milder allelic variant, Becker muscular dystrophy (BMD)[2].

Dystrophin is a critical component of the dystrophin-associated protein complex (DAPC), a multimeric assembly of proteins localized at the sarcolemma of skeletal, cardiac, and smooth muscle cells[3]. The DAPC is thought to primarily serve a mechanical role, functioning as a physical link between the internal cytoskeleton and extracellular matrix (ECM)[4, 5], though roles in mediating signaling are emerging[6, 7]. In DMD, loss of functional dystrophin and the consequential loss of the DAPC enhances sarcolemma susceptibility to contraction-induced damage resulting in repetitive cycles of muscle degeneration and regeneration[8]. Continuous immune cell infiltration and necrosis ultimately results in replacement of muscle with adipose and fibrotic connective tissue, such that the muscle no longer possesses force-generating capacity and experiences functional ischemia[9, 10].

Although advances in respiratory and cardiac supportive care in the last decades have improved quality of life and extended life expectancy considerably, further advances must come from therapeutic approaches to ameliorate the underlying pathophysiology of DMD. There are several genetic, cell-based, and pharmacological DMD treatment approaches under investigation aimed at replacement or repair of the primary *DMD* gene defect and restoration of the DAPC including viral delivery of mini-dystrophin, read-through of translation stop codons, and exon skipping to restore the reading frame (reviewed by Fairclough[11] and Guiraud[12]). However, their widespread delivery at sufficiently high concentrations to elicit a meaningful effect without causing a systemic immune response has proved challenging. In addition, only a specific subset of patients can currently benefit from certain strategies based on their genetic mutation, and individualized medicine will be required to take full advantage of these potential therapies[13, 14]. Therefore, investigation of pharmacological therapies to improve pathology independently and/or to synergize with genetic-based therapies in a combinatorial approach is a worthwhile area of research.

Muscle fiber degeneration and regeneration, fibrosis, inflammation, Ca^{2+} dysregulation, oxidative stress, and ischemia are associated with the absence of dystrophin absence and

contribute to DMD disease progression (Figure 1). Although correction of the primary defect and restoration of the sarcolemmal DAPC may ameliorate muscle pathology, it is likely that maximal benefit will be achieved by a multifaceted approach that addresses multiple aspects of the disease. In combination with supportive care, pharmacological interventions that counter the underlying pathological mechanisms and stimulate muscle growth, promote of blood flow, and reduce fibrosis and inflammation may be viable options for patients even at advanced stages of disease. This review will focus on pharmacological approaches that manipulate downstream targets that have emerged from our growing understanding of the pathological consequences of dystrophin absence in muscle.

2. Corticosteroids

Currently, there is no cure for DMD, and the only standing treatment is long-term corticosteroid dosing with the anti-inflammatory glucocorticoids prednisone, prednisolone, and deflazacort. These drugs are thought to function primarily by inhibiting the NF-κB pathway[14, 15], though there is also evidence that they activate the calcineurin/NF-AT pathway[16]. Corticosteroids have been shown to prolong independent ambulation, improve pulmonary function, and delay the onset of cardiomyopathy[17], but they also have significant side effects including weight gain, hypertension, and vertebral compression fractures[18]. Although they target the same pathway, the incidence and severity of side effects differs among them. A direct comparison of deflazacort and prednisone in DMD in a multicenter, double-blind, randomized trial of 18 patients over one year of treatment showed that while there was no significant difference in motor outcomes, there was less weight gain in the group treated with deflazacort compared to prednisone (2.17 kg vs. 5.08 kg)[19]. Deflazacort has also been reported to decrease incidence of gastrointestinal pain[19] and to prolong ambulation[20] compared to prednisone, but it is not currently FDA approved and cannot be purchased in the U.S. making its acquisition difficult and costly for many families. However, Marathon Pharmaceuticals has recently submitted a deflazacort new drug application to the FDA with orphan drug and rare pediatric disease designation, and it has been granted fast track status[21]. Interestingly, it has also been shown that the addition of prednisolone treatment to *mdx* mice actually has a detrimental impact on positive cardiac outcomes from treatment with other drugs[22]. This finding emphasizes that care must be taken when introducing new therapeutics in patients already on a corticosteroid regimen to achieve a synergistic positive effect.

Efforts to develop a glucocorticoid alternative with fewer side effects are being investigated. Recently, ReveraGen has developed Vamorolone (previously known as VBP15), a novel glucocorticoid analogue that retains the anti-inflammatory characteristics of traditional DMD corticosteroid treatments, but without significant side effects. *Mdx* mice treated with this drug are reported to have less inflammation and increased grip strength, and did not cause stunted growth or immunotoxicity associated with prednisolone[23]. Vamorolone underwent successful phase 1 safety and tolerability clinical trials in healthy adult, and preparation for a phase 2a multiple ascending dose study in DMD boys ages 4–7 is currently underway, presenting a promising new glucocorticoid-based treatment option[24].

3. Other approaches targeting NF-κB

NF-κB is a central transcription factor responsible for modulating immune and inflammatory responses. The NF-κB pathway is stimulated by pro-inflammatory cytokines TNF-α and interleukin 6 (IL-6), which permit and assembly of the I-κB kinase (IKK) catalytic complex subunits IKKα, IKKβ, and NEMO. Assembly of this complex permits NF-κB entry into the cell and subsequent transcription of genes encoding cytokines and other immune response factors[25]. Absence of dystrophin leads to increased sarcolemmal permeability and generation of reactive oxygen species, associated with chronic activation of NF-κB in DMD[26, 27] and in *mdx*[28] muscle fibers and macrophages, which is thought to be a significant contributor to muscle fiber necrosis and dystrophic pathology[29, 30]. Inhibition of NF-κB activation has been found to improve skeletal and cardiac dystrophic pathology in *mdx* mice[28]. Therefore, pharmacological approaches to block this pathway, including by preventing assembly of the catalytic complex or by inhibiting the stimulatory cytokines, have been investigated.

NEMO binding domain (NBD), an NF-κB inhibitory peptide, decreases activation of NF-κB by blocking phosphorylation of the IKKα and IKKβ subunits and subsequent binding to NEMO, preventing assembly of the IKK catalytic complex. Systemic administration of NBD in *mdx* mice has been shown to decrease necrosis and increase regeneration in hind limb and diaphragm muscles, and improve generation of specific force[31]. NBD also improved cardiac contractile function in the more severely affected *mdx;utr*$^{-/-}$ mice, although histopathology including collagen content and inflammatory markers remained unchanged compared to vehicle-treated mice[32]. NBD does not completely inhibit NF-κB activity, such that theoretically, other essential cellular processes are not disrupted, which may be important given the central role of this pathway in many tissues. Intravenous delivery of NBD to the Golden Retriever Muscular Dystrophy (GRMD) model over 4 months was found to improve pelvic limb muscle force and improve histopathologic indices for inflammation and necrosis. However, administration over time led to infusion reactions and an immune response[33]. Given the preclinical success of NBD in the *mdx* mouse model, NBD was poised to enter human trials, but safety concerns identified in the GRMD dog model warrant further assessment of doses and formulation of NBD for human application. Long-term preclinical toxicology studies in non-human primates are being pursued.

Catabasis has developed a NF-κB inhibitor, CAT-1004, that reduced muscle degeneration and improved muscle regeneration and function in skeletal, diaphragm, and cardiac muscle in *mdx* mice. In comparison with prednisolone, CAT-1004 yielded greater number of regenerating fibers and reduced muscle inflammation without reducing muscle weight. In GRMD dogs, a single dose of CAT-1004 reduced NF-κB activity and production of plasma TNF-α[34]. Phase 1 trials in healthy adults reported safety, tolerability, and significant reduction in NF-κB activity[35]. Recruitment for a CAT-1004 phase 1/2 clinical trial in DMD boys ages 4–7 with 3 different doses is ongoing[36].

A repurposed drug under early investigation for DMD is infliximab (Remicade®), an antibody to human TNF-α that is commonly used for treatment of rheumatoid arthritis and Crohn's disease. Long-term administration of infliximab in *mdx* mice was found to reduce

skeletal and cardiac muscle fibrosis, but negatively impacted Akt activation and cardiac function. Interestingly, infliximab treatment also correlated with reduced cardiac myostatin mRNA levels[37] (further expanded on in section 4).

Inhibition of NF-κB signaling has demonstrated positive effects in ameliorating dystrophic pathology. However, therapies delivered systemically could potentially lead to negative outcomes due the central role of NF-κB signaling in many other tissues. In addition, although it is generally observed that heightened NF-κB activity negatively impacts myogenic potential, there is also some evidence that NF-κB signaling is important for expression of antioxidant enzymes in response to oxidative stress[38] (see section 7). Taking these considerations into account, targeted NF-κB inhibition in skeletal and cardiac muscle may be a desirable approach for DMD treatment given its consistent positive outcomes.

4. Promoting muscle mass

4.1 Myostatin inhibition

Myostatin is a member of the transforming growth factor-beta (TGF-β) superfamily of secreted growth and differentiation factors, and is a potent negative regulator of skeletal muscle growth. Its function is evident in animals with genetic mutations in the *Mstn* gene, including Belgian Blue cattle[39], the Bully whippet dog[40], and *Mstn* knockout mouse[41], all of which exhibit dramatic muscle hypertrophy. In DMD, muscle mass is lost due to severe muscle fiber degeneration and eventual exhaustion of regenerative capacity. Thus, myostatin was implicated as an attractive target for DMD therapy, leading to research efforts directed at stimulating muscle growth via its inhibition.

Myostatin binds activin type II receptors (ActRII) which recruit, phosphorylate and activate activin type I receptor propagating signals via the receptor-associated proteins Smad 2 and Smad 3[42, 43]. Smad 2/3 translocate into the nucleus, and activate the transcription of target genes through interaction with DNA and other nuclear factors muscle cell proliferation and differentiation. Several methods, including genetic knockdown[44], blocking antibodies[45, 46], and propeptide-mediated inhibition[47], have been explored to inhibit its activity. Crossing myostatin null mice with *mdx* mice showed double knockout $mstn^{-/-}/mdx$ mice had reduced diaphragm fibrosis and improved grip strength compared to *mdx* mice. However, cycles of muscle degeneration and regeneration as well as fibers with centrally located nuclei and elevated serum creatine kinase were still evident[48]. Inhibition of myostatin by injection of anti-myostatin monoclonal antibodies into *mdx* mice muscles yielded more promising results. Three months of weekly injections increased muscle mass up to 35% due to fiber size enlargement, and strikingly, serum creatine kinases levels decreased to near normal levels[45]. Propeptide-mediated myostatin inhibition also yielded functional benefits including increased tetanic force production[47]. Interestingly, reduced myostatin in dystrophin-deficient $Mstn^{+/-}$ dogs did not yield beneficial results, instead causing unequal muscle growth and greater joint contractures[49].

The success of myostatin inhibition in the *mdx* mouse model has led to multiple clinical trials, however, drug development in this effort has been challenging. Initial therapeutic strategies aimed to systemically abrogate myostatin/ActRIIB signaling to ensure widespread

effect on musculature. However, there have been concerns regarding efficacy, interference with ActRIIB signaling in non-muscle tissues, and potential adverse side effects. MYO-029, a high-affinity myostatin binding antibody, was shown to increase muscle mass in mice by 30% over 3 months of treatment[50]. However, clinical studies of BMD, LGMD and FSHD patients treated with MYO-029 administered intravenously every 2 weeks for 6 months were ceased after they did not improve strength or function despite being well-tolerated[50]. Efforts to develop ACE-031, a recombinant pseudo ActIIB receptor that increased muscle mass and whole body pulling tension in *mdx* mice[51], were terminated due to incidents of dilated blood vessels, nosebleeds, gum bleeding in treated DMD boys[52]. Subcutaneous ACE-031 delivery every 2–4 weeks to ambulatory DMD boys in a recent ascending dose trial was not associated with serious or severe adverse events, and demonstrated trends for pharmacodynamic effects on lean body mass and body mass density. However this study was also ceased due to safety concerns of epistaxis and telangiectasias[53]. A large phase 2 clinical study of over 100 DMD boys with monthly intravenous infused doses of PF-06252616, an anti-myostatin monoclonal antibody from Pfizer, is currently ongoing in the U.S., Canada, Europe, and Japan[54]. Unlike ACE-031, PF-06252616 is highly specific to myostatin, which may confer fewer negative side effects.

Despite variable clinical outcomes to increase muscle size and strength through myostatin inhibition, there is still active research in this pursuit. Fortunately, more directed approaches are now being developed to be delivered directly via intramuscular injection which may minimize negative side effects. Acceleron has developed ACE-083, a locally acting myostatin trap, to be delivered by single muscle injection. Phase 1 trials of ACE-083 in post-menopausal women showed safety and a 7–15% increase in muscle mass[55]. BMS-986089, a human anti-myostatin antibody developed by Bristol-Myers Squibb, yielded a 4.5–5% increase in muscle mass in healthy subjects following thigh muscle injection[56]. Recruitment for a phase 2 trial of BMS-986089 in ambulatory DMD boys ages 5–10 delivered weekly by subcutaneous injection is ongoing[57]. Given these positive preliminary results with highly specific antibodies and/or local delivery approach, myostatin inhibition as a target for DMD therapy may still be a viable treatment option.

4.2 Insulin growth factor-1

Insulin Growth Factor-1 (IGF-1) is a key mediator of skeletal myoblast differentiation that is also under investigation as a DMD therapy to increase muscle mass and retain strength. IGF-1 promotes muscle hypertrophy by activation of the PI3K/Akt/mTOR pathway stimulating protein synthesis and inhibiting protein degradation[58]. IGF-1 is well-tolerated in clinical studies for treatment of osteoporosis[59] and Rett syndrome[60] making it an attractive potential therapy. In addition, insulin-like growth factor was among a group of compounds found to increase tetanic force generation in an *ex vivo* bioartificial muscle *mdx* model[61]. Preclinical studies in *mdx* mice demonstrated that IGF-1 overexpression increases EDL and diaphragm muscle mass, enhances force generation, and reduces necrosis and fibrosis[62]. These promising mouse studies led to a controlled phase 2 clinical trial of Increlex (an FDA-approved recombinant IGF-1 delivered by daily subcutaneous injection) conducted in glucocorticoid-treated DMD boys to examine its efficacy to maintain muscle function. Preliminary analysis suggested that although the treatment improved height growth and

measures of insulin resistance, there was no change observed in motor function following 6 months of treatment[63]. Although no follow-up studies have been reported, IGF-1 may be a valuable therapy in younger DMD patients to maintain muscle mass and strength.

4.3 Other drugs to promote muscle mass

Although traditionally used for treating asthma and COPD, β2-agonists have also been found to induce satellite cell proliferation and muscle protein production to increase lean body mass in models of muscle injury. In *mdx* mice, β2-agonists reduced muscle degeneration and increased muscle force[64–66]. The β2-agonist albuterol has been tested extensively in FSHD patients and showed only limited positive effects, but was well-tolerated[67, 68]. More recently, albuterol was shown to increase lean body mass in ambulatory DMD and BMD boys, but has not been pursued further. Urocortin, a corticoid releasing factor receptor agonist, functions similarly to β2-agonists by elevating cAMP to inhibit calapins. Urocortin 2 administered by 6 subcutaneous injections over 2 weeks rescued *mdx* diaphragm muscle force loss compared to saline treatment[69], and was also found to improve *mdx* smooth muscle GI motility[70]. Urocortins have also shown efficacy in treating heart failure[71], and thus may elicit both skeletal and cardiac muscle benefits in DMD.

DT-200 (formerly known as GLPG0492) is a non-steroidal selective androgen receptor modulator originally investigated to treat sarcopenia and cachexia[72, 73]. In *mdx* mice, DT-200 improved running performance and increased diaphragm force[74]. DT-200 had positive phase 1 clinical data in healthy subjects[75] and has broad potential for multiple muscle wasting diseases.

A consideration for clinical trials aimed at promoting muscle mass remains that increased muscle mass may not necessarily translate to improved muscle strength[41]. In addition, later stage DMD patients may not have sufficient muscle tissue remaining to elicit meaningful outcomes with some of these therapies. Despite variable efficacy in earlier attempts to inhibit myostatin, some of the more recent therapeutics aimed at increasing muscle mass show promise.

5. Drugs targeting fibrosis

Fibrosis is defined as the deposition of ECM components, and in healthy muscle, it is critical for providing a scaffold on which to build and structure new tissue during growth and repair. However, even minor insults to this process can lead to detrimental pathological effects. In DMD, loss of dystrophin leads of sarcolemmal fragility, muscle fiber degeneration, and uncontrolled activation of profibrotic signaling pathways. Muscle tissue is progressively replaced by excessive accumulation of fibrotic and adipose tissue, which not only reduces contractile function, but also decreases the available muscle tissue for therapeutic intervention[76]. Thus, significant DMD research has been directed at preventing the development of this excessive fibrotic tissue.

Transforming growth factor-beta (TFG-β) is a potent profibrogenic factor that is expressed in regenerating muscle after injury and in dystrophic muscle of *mdx* mice[77], GRMD dogs[78] and DMD patients[79, 80]. TFG-β is normally stored in the ECM as a latent precursor, and

upon activation by tissue damage or specific growth signals, binds to serine/threonine kinase receptors types I and II to initiate transcription of several profibrotic genes including collagen and fibronectin. TFG-β is also produced by infiltrating immune and inflammatory cells, and can decrease the production of enzymes that degrade the ECM[81]. Given that pathological fibrosis is a hallmark feature of dystrophic muscle and that TFG-β levels correlate with the extent of muscle fibrosis in DMD patients[82, 83], the TFG-β pathway has emerged as an important therapeutic target to increase muscle regeneration and reduce excessive fibrotic tissue deposition in DMD.

One of the earliest investigated TFG-β inhibitors was losartan, which was an attractive candidate because of its extensive use as a safe antihypertensive drug. Losartan is oxidized in the liver and its metabolite acts to block angiotensin II receptor activation, which is required to initiate TFG-β signaling. Initial studies in *mdx* mice showed treatment with losartan for 6–9 months reduced fibrosis in the diaphragm and gastrocnemius muscles, increased grip strength[84], decreased cardiac muscle fibrosis, and improved cardiac function[85]. However, subsequent studies showed minimal functional benefit[86]. Losartan entered a limited human clinical trial that showed one year of losartan treatment was safe and improved cardiac ejection fraction[87].

Pirfenidone is another small molecule designed as an antifibrotic agent and used clinically for treatment of idiopathic pulmonary fibrosis[88], and has received orphan drug status from the FDA tor treatment of scleroderma. Although the precise mechanism of action is unknown, it has been shown to suppress expression of TFG-β[89], though its efficacy in *mdx* mouse studies has been mixed. One study showed 4-weeks of pirfenidone treatment in *mdx* mice increased matrix metalloproteinases MMP-2 and MMP-9 with minimal changes to fibrosis, independent of TFG-β levels[90]. In another study, 7-months of high-dose pirfenidone administration to 8-month old mice improved cardiac contractility, but not fibrosis[91]. However, when targeting therapeutic modulation of the TFG-β pathway, or any signaling pathway in the treatment of DMD, one must consider that timing of treatment may be critical in determining its efficacy. For example, TFG-β is elevated at 6 and 9 weeks of age, but not at 12 weeks in the *mdx* diaphragm[77], suggesting that treatment with TFG-β inhibitors at this later stage may not be efficacious in this model. In DMD patient muscle derived fibroblasts, pirfenidone was shown to reduce synthesis and deposition of intracellular collagen[92].

Two antineoplastic drugs, suramin and FDA-approved imatinib mesylate (Gleevec®), have been shown to act as TFG-β antagonists in several chronic fibrotic diseases including kidney disease[93], myocarditis[94], and liver disease[95]. Intraperitoneal injection of suramin in 6-month old *mdx* mice has been shown to increase MMP-2 and MMP-9 activity[96], decrease creatine kinase, and to attenuate fibrosis in skeletal muscle, but not cardiac muscle[97]. In 8-month old *mdx* mice, however, suramin did improve cardiomyopathy and decrease myocardial fibrosis, inflammation, and myonecrosis[98]. Imatinib mesylate inhibited TFG-β pro-fibrogenic activity, decreased creatine kinase[99], reduced fibrosis, and improve hind limb grip strength[100] in *mdx* mice. Most recently, imatinib mesylate was found to ameliorate muscle pathology of the DBA/2-*mdx* mouse, a severe DMD mouse model that more closely recapitulates several human disease characteristics[101]. These results combined with the

observation that these drugs are well tolerated in non-DMD clinical trials make them attractive candidates for DMD therapy.

Among the anti-TFG-β drugs under investigation, halofuginone has demonstrated some of the most promising results. Halofuginone is a low molecular weight plant alkaloid that plays dual roles – it inhibits TFG-β-mediated collagen synthesis signaling by impeding Smad 3 binding to DNA[102], but also enhances Akt and MAPK/ERK signaling to promote myotube fusion in *mdx* and wild type primary myoblasts[103]. Ten weeks of halofuginone treatment in 4-week old *mdx* mice decreased diaphragm fibrosis, increased the number of revertant fibers[104], and promoted satellite cell activation and survival[105]. The pro-proliferative features of halofuginone, in addition to its anti-fibrotic action, could have positive implications for muscle regeneration in DMD patients. Clinical trials in DMD boys with HT-100, a oral delayed-release halofuginone, showed positive trends in serum biomarkers of collagen degradation, and a 11.7% mean increase in total muscle strength compared to baseline (study entry) over 18–22 months[106]. However, dosing and enrollment was recently suspended due to the death of a study subject[107].

A new approach under investigation is repurposing tamoxifen, a selective estrogen receptor modulator, to counter DMD-related fibrosis. Tamoxifen is most commonly known as an anticancer drug for treating and preventing breast cancer[108], but is also used clinically for treating of retroperitoneal fibrosis[109] and encapsulating peritoneal sclerosis[110]. In these diseases and others, tamoxifen demonstrates antifibrotic properties and is thought to work through inhibition of TGF-β[111, 112], and preclinical application in dystrophin-deficient mice has shown promising results. Oral tamoxifen treatment of 3-week old *mdx*^{5cv} mice for 15 months at a dose of 10mg/kg/day was found to diminish cardiac and diaphragm fibrosis, improved muscle structure and force, and conferred slower development of muscle phenotype[113]. In addition to its extensive use in adults, tamoxifen has also been tested for other conditions in adolescent boys ages 13–16 for up to 48 months and appears to be well tolerated and safe dosed up to 20mg twice daily[114, 115]. Of note, in these studies, tamoxifen did not cause pubertal delay, one of the side effects of long-term corticosteroid treatment in DMD boys[116]. The safety of tamoxifen in boys as young as 5 to 7 years old, the age at which early DMD treatment is often initiated, has not yet been established. However, 20mg/day of tamoxifen has been shown to be safe in young girls ages 3 to 10 over 12 months of treatment[114]. Given the safety of tamoxifen in children and its efficacy in decreasing fibrosis in other diseases, its repurposing in DMD boys is of considerable interest.

6. Calcium regulation and mitochondria

One of the earliest and most well studied hypotheses on the mechanisms leading to DMD muscle degeneration and myofiber necrosis is elevated cytosolic Ca^{2+}. Stretch-induced membrane permeability due to dystrophin absence causes both passive and active Ca^{2+} entry through physical membrane tears and through stretch-activated channels, with subsequent mitochondrial dysfunction[117] and activation of Ca^{2+}-dependent degradative pathways[118]. Myofibers from dystrophic mice and DMD patients exhibit heightened resting and active Ca^{2+} levels[119, 120], and also show defects in SR-Ca^{2+} handling and reuptake during

relaxation[121, 122]. This excess cytosolic Ca^{2+} has been shown to cause pathology in several ways including by altering excitation contraction-coupling, causing mitochondrial rupture by overactivation of the mitochondrial permeability transition pore (MPTP), and by hyperactivation of calpains, which can exacerbate dystrophic pathology by cleaving critical intracellular proteins. Given that Ca^{2+} levels are influenced by several factors in dystrophic muscle, there have been pharmacological efforts to correct it on multiple levels including at the level of the plasma membrane, the SR, and the mitochondria.

6.1 Calcium channel blockers

Early investigation of the hypothesis of Ca^{2+}-induced pathology in DMD led to a series of clinical trials involving various Ca^{2+} channel blockers including flunarizine, nifedipine, and verapamil[123–125]. Despite being well tolerated in DMD patients, there were no clinically significant effects, but other Ca^{2+} channel blockers have since been investigated. Streptomycin is a broad range antibiotic that primarily functions to inhibit protein synthesis, but also acts as a non-specific blocker of Ca^{2+} channels. Streptomycin has shown variable efficacy in *mdx* mice - initially it was demonstrated to decrease muscle degeneration, improve force and significantly reduce the uptake of procion orange dye into fibers from *mdx* muscles after eccentric contractions[118, 126]. However, a later study showed streptomycin did not show positive effects in diaphragm or heart muscle and actually worsened heart pathology[127] and its suitability of streptomycin as a DMD therapeutic has been questioned due to the side effects associated with prolonged antibiotic use. An intriguing drug in this category recently gaining attention is AT-300 (previously known as GsMTx4), a peptide originally discovered in the venom of the Chilean Rose Tarantula, which was found to specifically block mechanosensitive Ca^{2+} channels[128]. AT-300 was first tested side-by-side with streptomycin in *mdx* mice and was shown to have similarly positive effects[118, 126]. Moreover, GsMTx4 was found to block transient receptor potential canonical 6 (TRPC6) channel activation *in vitro*[129], and hyperactivation of TRPC6 channels has been linked to the adverse mechanical stress response and intracellular Ca^{2+} in dystrophic heart[130]. The rights to AT-300 were recently acquired by Akashi Therapeutics, and they are investigating its therapeutic potential for DMD[131]. AT-300 has been granted orphan drug designation by the FDA, but will require thorough preclinical testing before clinical application.

6.2 Repairing the plasma membrane

The absence of dystrophin permits Ca^{2+} to enter dystrophic muscle fibers through physical membrane tears. Mitsugumin 53 is a TRIM family protein that is principally expressed in skeletal and cardiac muscle, and plays an essential role in cell membrane repair evidenced by mitsugumin 53 knockout mice exhibiting a progressive muscular dystrophy[132]. In striated muscle, mitsugumin 53 facilitates vesicle translocation to sites of membrane injury to patch the membrane in damaged muscle cells[133]. Preclinical evidence showed that injection of recombinant human mitsugumin 53 decreased muscle pathology in *mdx* mice[134].

Poloxamer 188 is a membrane sealant polymer that localizes into lipid monolayers and areas of damaged membranes. In cardiomyocytes from *mdx* mice, poloxamer 188 prevented stretch-mediated Ca^{2+} overload into the muscle cell,[135] and chronic infusion in GRMD dogs

was found to reduce myocardial fibrosis[136]. Most recently, chronic administration of national formulary grade poloxamer 188 in *mdx* and *mdx/utr*$^{-/-}$ dko mice was shown to improve diaphragm muscle including decreasing the number of centralized nuclei and variation in fiber size, improving fiber density, and decreasing collagen deposition[137]. However, contradictory evidence in *mdx* skeletal muscle shows that poloxamer 188 does not prevent exercise-induced membrane breakdown in quadriceps muscle[138] and may actually increase susceptibility to contraction-induced injury in tibialis anterior muscle[139]. These studies present the possibility that poloxamer 188 may have differing effects in cardiac and diaphragm muscle versus limb muscles.

6.3 Clearing calcium overload

BGP-15 is a pharmacological inducer of Hsp72 that binds sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). SERCA normally pumps Ca^{2+} out of the cytosol and into SR stores, but this function is perturbed in dystrophic muscle[121]. Subsequent studies showed that increasing the level of SERCA protein in *mdx* skeletal muscle using transgenic or viral delivery significantly mitigated the dystrophic pathology[140], supporting SERCA as a DMD treatment target. Treatment of *mdx* mice with BGP-15 increased SERCA activity, improved muscle architecture, strength and contractile function in severely affected diaphragm muscles. In severely affected *mdx;utrn*$^{-/-}$ mice, BGP-15 decreased kyphosis, improved TA force to wild type levels, and extended lifespan[141]. In non-DMD mouse and rat models, BGP-15 was also shown to protect against heart failure and to alleviate ventilation-induced diaphragm dysfunction, respectively[142, 143]. It has yet to undergo clinical trials, but has strong preclinical evidence relevant to DMD.

In dystrophic muscle, ryanodine receptors (RyR1 in skeletal muscle and RyR2 in the heart) are overactive causing leaking of Ca^{2+} into the cytosol[144], which can be stabilized by small molecules termed rycals. Rycal S107 has been shown to improve muscle strength in models of bone metastases[145], lower SR Ca^{2+} leakage, and prevent cardiac arrhythmias *in vivo* in *mdx* mice[146]. ARM210/S48168, a proprietary rycal candidate from ARMGO Pharma, was found to improve exercise capacity, muscle specific force, grip strength and muscle histology in preclinical mouse models. It was recently advanced into a clinical stage program for DMD and received FDA orphan drug designation[147].

Enhanced Na^+/H^+ exchanger type-1 (NHE-1) activity resulting in intracellular Na^+ overload has been shown to contribute to dystrophic pathology[148] and may contribute to Ca^{2+} overload by reducing Ca^{2+} extrusion through the Na^+/Ca^{2+} exhanger[149]. NHE inhibitors cariporide and EIPA have been shown to elicit protective effects against muscle degeneration in dystrophic BIO14.6 hamsters and *mdx* mice that significantly reduce both elevated Na^+ and Ca^{2+} in dystrophic myotubes[148]. Rimeporide, a selective Na^+/H^+ exchanger type-1 (NHE-1) inhibitor, was found to reduce cardiac necrosis in cardiopmyopathic hamsters[150], and improve functional strength, and reduce diaphragm inflammation[151]. EspeRare Foundation is currently recruiting participants for a phase Ib clinical trial[152].

6.4 Mitochondria

Intracellular Ca^{2+} overload and oxidative stress cause permeabilization of mitochondria by formation of large pore complexes termed mitochondrial permeability transition pores (MPTP) on the membrane, which is mediated by cyclophilin D. Subsequent disruption of cellular ATP generation causes mitochondrial swelling, rupture, and ultimately cell death[153]. Cyclosporin A is a fungal cyclic peptide that has been shown to inhibit cyclophilin D and prevent MPTP formation[154] and is commonly used as an immunosuppressant. In *mdx* mice, cyclosporine A prevented force drop following exercise and reduced creatine kinase[155]. A small initial study in DMD boys showed that eight weeks of cyclosporin treatment elicited significant improvement in tetanic force and maximum voluntary contraction in TA muscles within two weeks of treatment[156]. However, a later study in a DMD myoblast transfer study showed no effect on strength[157], and side effects associated with long-term immunosupression have dissuaded further investigation of this drug for use in DMD. To overcome this issue, Debio-25 (also known as alisporovir), a non-immunosupressive analogue of cyclosporine, has recently been produced. In *mdx* mice, Debio-025 has been shown to have similar efficacy to cyclosporine A[158] and was reported to be more effective than prednisone in a side-by-side study in reducing fibrosis and infiltration of activated macrophages[159]. Debio-025 had a positive safety profile in clinical trials for hepatitis C, and Debiopharm International SA and Solid Biosciences have announced a collaboration to explore the use of Debio-025 in DMD[160].

7. Countering oxidative stress

Inflammation, fibrosis, and Ca^{2+} dysregulation in dystrophic muscle are associated with production of reactive oxygen species (ROS), which lead to oxidative stress[161, 162]. The influx of Ca^{2+} through the dystrophin-deficient sarcolemma is thought to enhance ROS production by the mitochondria, which stimulates stretch-activated Ca^{2+} channels and further exacerbates Ca^{2+}-induced mitochondrial dysfunction and necrosis[126, 163]. ROS are also generated by membrane-bound NADPH oxidase (NOX)[164], and activate NF-κB signaling to trigger the inflammatory response[165] and TGF-β signaling (see sections 2, 3, and 5). Thus, various antioxidant therapies have been investigated to decrease ROS in dystrophic muscle.

Coenzyme Q10 binds the inner mitochondrial membrane and functions as an electron acceptor in the respiratory chain that not only decreases oxidative stress, but also modulates the MPTP (see section 6.4) and could decrease mitochondrial Ca^{2+} accumulation[166]. Coenzyme Q10 has been reported to increase muscle strength 8.5% in a small trial of DMD boys[167]. Idebenone, a synthetic derivative of coenzyme Q10, was found to correct cardiac diastolic dysfunction, reduce inflammation and fibrosis, and improve voluntary running in *mdx* mice following a long-term 10-month treatment protocol[168]. Idebenone has recently shown promising results in DMD boys, and a phase III double-blind clinical trial in DMD patients on glucocorticoids to assess its efficacy in delaying the loss of respiratory function is in development[169].

L-Arginine, a substrate of nitric oxide synthase, has been shown to reduce contraction-induced damage and myonecrosis in *mdx* mice suggesting partial restoration of Ca^{2+}

handing[170]. In addition, L-Arginine decreases inflammation and enhances muscle regeneration, and increases NF-kB in *mdx* muscle fibers[171]. L-Arginine has also demonstrated efficacy in combination with other pharmacological therapies. When combined with deflazacort, L-Arginine prevented exercise-induced damage and induced a persistent functional improvement in distance run in *mdx* mice. Recently, a small cohort of DMD boys aged 7–10 treated with L-Arginine and metformin for 16 weeks showed significantly increased mitochondrial protein expression and reduced in oxidative stress[172]. Other supplements investigated to improve muscle strength by providing metabolic support include creatine and glutamine, which have been shown to increase grip strength[173] and decrease protein degradation[174], respectively, in DMD boys. However, a CINRG phase II/III trial of creatine and glutamine in ambulant DMD boys did not show any statistically significantly effect on muscle strength[175].

N-Acetylcysteine is an antioxidant that can directly scavenge ROS and was found to reduce creatine kinase by 88% and reduce stretch-induced damage in *mdx* mice[176]. It was also able to prevent *mdx* mice against exercise-induced damage and necrosis after only one week of treatment, improve Ca^{2+} handling, and decrease nuclear NF-κB[177, 178]. Despite promising preclinical data, N-Acetylcysteine has yet to be tested in DMD patients, but is currently recruiting participants for a clinical study of RyR1-related congenital myopathy[179].

The antioxidant and anti-inflammatory properties of melatonin have led to its investigation as a DMD treatment. In *mdx5cv* mice, 10 days of melatonin treatment increased twitch force and tetanic force, decreased creatine kinase, and improved muscle redox status[180]. In a DMD clinical trial, 3 months of melatonin treatment decreased creatine kinase and reduced other hyperoxidative and inflammatory markers[181], suggesting it may slow the degenerative process.

Green tea is an abundant source of free radical scavenging polyphenols, the predominant being epigallocatechin gallate (EGCg). Preclinical studies of EGCg in *mdx* mice showed improved histology, decreased necrosis, and improved muscle function[182–184]. Early treatment with green tea extract also decreased dystrophic muscle pathology, potentially by regulating NF-κB activity in regenerating fibers[185]. An advantage of EGCg is its availability and low potential for toxicity. A double-blind, placebo-controlled phase II/III of EGCg in 5–10 year old DMD boys to assess safety and efficacy is ongoing[186].

As mentioned, ROS produced by NOX triggers the manifestation of dystrophic pathology, making NOX inhibition an attractive strategy. Diapocynin is a synthesized dimer of the NOX inhibitor apocynin, which unlike apocynin and other NOX inhibitors[187], does not promote ROS production in some cell types. In dystrophic myotubes, diapocynin inhibited ROS production and reduced intracellular Ca^{2+} overload through stretch-activated and store-operated channels. Diapocynin also prevented force loss after eccentric contractions and reduced membrane damage in *mdx* muscle[188]. Given the role of NOX in promoting oxidative stress, it would be of interest to assess diapocynin or another potent, efficacious, and selective NOX inhibitor in DMD patients.

8. Phoshodiesterase inhibition

Neuronal nitric oxide synthase (nNOS) is a DAPC-associated protein that produces nitric oxide (NO), a key signaling molecule in regulation of skeletal muscle excitation-contraction coupling[189], myogenesis, and muscle repair. NO stimulates the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), stimulating vasodilation. In healthy muscle, nNOS is enriched at the sarcolemma in fast twitch muscle fibers, but in *mdx* and DMD muscle, nNOS is mislocalized and its activity decreases by 80%[105, 190, 191], implicating aberrant NO signaling in contributing to dystrophic pathology. Accordingly, mouse studies validate negative effects of low NO-induced ischemia on skeletal muscle contraction and fatigue[192, 193]. The therapeutic effects of restoring NO levels have been demonstrated by transgenic overexpression of nNOS in *mdx* skeletal and cardiac muscle, which corrected elevated serum creatine kinase, fibrosis, and macrophage infiltration[194, 195]. Further investigation of this pathway revealed that overexpression of guanylyl cyclase in the heart to enhance cGMP signaling without increased NO production were sufficient to elicit beneficial effects in dystrophic cardiomyopathy[196]. Therefore, pharmacological approaches to enhance cGMP levels have been pursued, specifically through inhibiting the activity of GMP-hydrolyzing phosphodiesterases (PDEs).

Pentoxifylline is a non-specific PDE inhibitor used clinically for the treatment of intermittent claudication[197] and acts to suppress inflammation by inhibiting synthesis of pro-inflammatory cytokines and reactive oxygen species[77]. In *mdx* mice, pentoxifylline was shown to counteract hyperactive voltage-independent Ca^{2+} channels in myofibers[77], increase tetanic tension in the diaphragm, and reduce creatine kinase[198]. Given these therapeutic effects, but without positive effects on fibrosis[77], it is thought to act through PDE5 inhibition. Despite being well tolerated in adult populations, the liquid formulation of pentoxifylline caused intolerable gastrointestinal effects in DMD boys, requiring premature conclusion of the 2011 CINRG clinical study[199]. Thus, its efficacy in these subjects could not be assessed, and alternative routes of administration have been investigated. In a 12-month double-blind study of 64 DMD boys, treatment with a slow-release pentoxifylline formulation was better tolerated, but did not improve or halt the deterioration of muscle strength and function[200].

There are also several PDE5 inhibitors currently used clinically including sildenafil (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®), which prevent cGMP breakdown and raise its concentration in muscle, promoting vasodilation[201]. Given that these PDE inhibitors are readily available, have minimal side effects, and have been tested in adult and pediatric subjects, they are an attractive treatment approach and have shown efficacy in preclinical studies. Sildenafil prevented onset of cardiomyopathy in young *mdx* mice, reversed cardiac dysfunction in older *mdx mice* following 3 months of treatment[202], and reduced respiratory muscle weakness and fibrosis[203]. In addition, both sildenafil and tadalafil protect against *mdx* contraction-induced injury and reduce serum CK[196, 204].

The success of the *mdx* studies led to clinical trials of sildenafil and tadalafil in DMD and BMD patients. A study of 10 DMD boys ages 8–13 and 10 healthy males treated with single doses of either tadalafil or sildenafil alleviated ischemia in a dose-dependent manner, and

normalized exercise-induced increase in skeletal muscle blood flow, which is attenuated in DMD boys[205]. However, in a long-term study, 6-month treatment with sildenafil in DMD and BMD adults did not yield positive outcomes, with 29% of subjects actually experiencing worsening cardiac function, though it should be noted that the sample size in this study was relatively small (N=15)[206]. Most recently, Eli Lilly implemented a large phase 3 double blind clinical trial of tadalafil treatment in over 300 DMD patients. Unfortunately, tadalafil treatment did not elicit efficacy in the 6-minute walk distance or other assessments of motor function through 48 weeks of treatment, and the study was ended prematurely[207]. However, there is evidence that suggests that tadalafil treatment improves blood flow during exercise[208], suggesting exercise may be necessary to observe an effect. In addition, it should be noted that the subjects in this study were older, ages 7 to 14, and there is precedence for investigating potential benefits of PDE inhibitors in younger DMD boys in future clinical studies. New evidence just published showed that GRMD dogs treated with tadalafil prior to detectable cardiomyopathy exhibited improved cardiac and skeletal muscle histopathological features, decreased TRPC6 levels, and delayed cardiomyopathy[209].

9. Conclusion

Several potential targets for pharmacologic DMD therapies and the efficacy of relevant drugs tested in preclinical and clinical studies have been presented in this review (Table 1). The number of drugs under investigation is extensive, but while many of these therapies elicit meaningful benefits in the *mdx* mouse and GRMD dog models, this is not always recapitulated in human disease. Although the development of genetic-based therapies targeting *DMD* gene mutations is also a promising more direct approach, it has faced many hurdles in obtaining FDA approval and is not currently tailored to all patients. Therefore, pharmacological approaches that are applicable to all patients and may more quickly and easily enter the clinical setting are still an important area of DMD research.

10. Expert opinion

Absence of dystrophin from the muscle sarcolemma triggers a number of pathological downstream events including inflammation, fibrosis, and necrosis. DMD is a multifaceted disease, and will likely require a multifaceted approach from several angles to address the many features of its pathology. Theoretically, restoration of dystrophin and the DAPC to the muscle sarcolemma would prevent further muscle damage and slow disease progression. Promising genetic-based approaches to achieve this include exon skipping and gene therapy, which have the potential to restore dystrophin in many tissues including the heart. However, they face challenges such as tailoring to specific *DMD* mutations and achieving widespread delivery, respectively, and would not necessarily reverse advanced pathology in older patients with little muscle mass remaining. The pharmacological therapeutics discussed above have potential to improve advanced pathology and would be applicable to all patients regardless of mutation, and would play a critical role in a combinatorial treatment approach for DMD.

Despite the benefits, there are also several challenges for pharmacologic treatment of DMD that must be considered. Given that corticosteroids are the only standing DMD drug therapy

and that most patients are on a prednisone or deflazacort regimen, it is important to ensure candidate drugs act synergistically with them. With any combinatorial treatment approach, there is always the potential for decreased efficacy or side effects from multiple drug interference. As mentioned above regarding prednisolone, underlying glucocorticoid treatment may mask potential benefits from a candidate drug being tested. Significant improvement in quality of life would be achieved with a corticosteroid alternative that decreases inflammation without the side effects as a new standing DMD therapy. There are also meaningful benefits to be gained with an effective antifibrotic agent to decrease the connective tissue replacing force-generating muscle, an agent that promotes muscle growth, reducing oxidative stress, or by enhancing blood flow to optimize function of the muscle that remains.

With so many targets now known and so many potential drugs that appear promising in animal models, the number of clinical trials is growing rapidly. As we have discussed in this review, therapies that were highly effective in animal models have not necessarily been effective or well tolerated in human patients. Therefore, in this era of DMD therapeutics, the field must be especially thorough and selective in deciding which among the many options move forward to human trials (additional reviews[210–212]). DMD research has a growing number of clinical endpoints that includes ambulation (i.e. 6-minute walk test), ventilatory capacity, strength (i.e. gross motor, grip), MRI, and pharmacodynamic measures such as serum biomarkers to determine drug efficacy. It will be critical to carefully consider multiple outcomes as to not prematurely exclude a candidate that may elicit more subtle but meaningful benefits and may not necessarily translate into improved ambulation. Several drugs under investigation have been tested thoroughly in other diseases, which may hasten their approval for repurposing in DMD therapy.

Research of dystrophin-deficient animal models has greatly advanced our understanding of the pathophysiological mechanisms underlying dystrophic pathology, and has revealed viable DMD therapeutic targets beyond the primary genetic defect. In this review, we have presented several well-defined downstream targets for pharmacologic therapies for DMD and the efficacy of relevant therapeutics in ongoing preclinical and clinical trials. While there are certainly challenges associated with delivery, tolerance, and consistent efficacy, there are more potential treatments than ever before, some of which may have significant impact on disease progression.

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Reference annotations

* Of interest

** Of considerable interest

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Article highlights

- The standing treatment for DMD is corticosteroid therapy, which has been shown to slow disease progression, but is associated with significant side effects.
- Genetic interventions including exon skipping and gene therapy to restore dystrophin expression are promising strategies, but face the challenges of tailoring for specific *DMD* mutations and achieving widespread delivery, respectively.
- Pharmacological approaches are being investigated to address pathophysiological features of DMD including muscle fiber degeneration, inflammation, fibrosis, Ca^{2+} dysregulation, and oxidative stress.
- These include targeting the NF-κB pathway, myostatin, TGF-β signaling, Ca^{2+} channels, reactive oxygen species, and phosphodiesterases. Several of these interventions involve repurposing drugs already used clinically for other diseases to treat DMD.
- Drugs targeting the downstream pathology of DMD would be applicable to all patients irrespective of genetic mutation, and would play a significant role as part of a combinatorial treatment approach with genetic and palliative therapies.

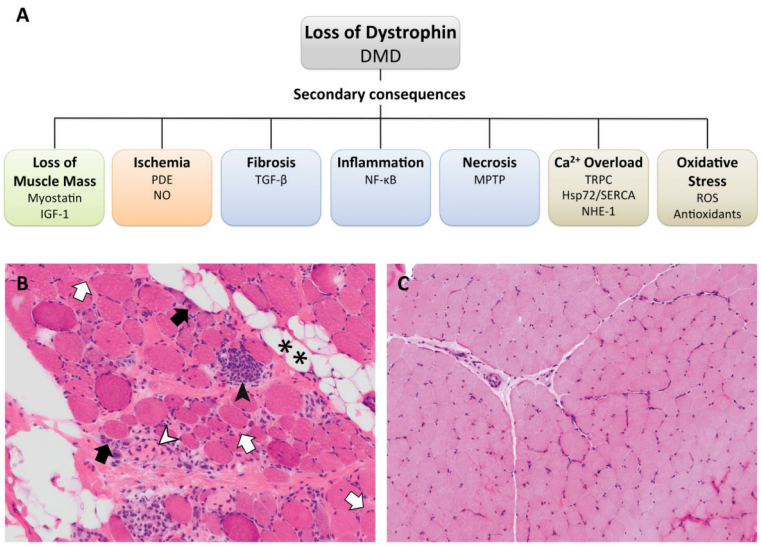

Figure 1.

Pathological features of DMD muscle. (a) Secondary consequences of dystrophin loss and their respective therapeutic targets grouped by closely related aspects of disease pathology. (b) Quadriceps muscle biopsy from a 6-year old DMD patient with scattered inflammatory cells in the endomysium and other dystrophic characteristics. **, fatty infiltration; white arrows, endomysial fibrosis; black arrows, regenerating fibers; white arrowhead, necrosis; black arrowhead, myophagocytosis. (c) Quadriceps muscle biopsy from a 7-year old with no identified neuromuscular disorder. Hematoxylin and eosin staining, 20x.

Table 1

Status of pharmacological therapies evaluated for DMD

Target/Class	Drug	Mode of action	Therapeutic effects	Status reached in DMD research	Refs.
Corticosteroid	Prednisone, Deflazacort, Prednisolone	Widespread, NF-κB inhibition	↓ inflammation	Standing long-term DMD therapy	14, 15
	Vamorolone (prev. known as VBP15)	Novel glucocorticoid analogue that inhibits NF-κB	↓ inflammation, ↑ grip strength in *mdx* without side effects	DMD Phase II ongoing	23, 24
NF-κB inhibition	NBD (NEMO binding domain)	Prevents assembly of the IKK catalytic complex	↓ necrosis, ↓ inflammation, ↑ regeneration in *mdx* and GRMD	Non-human primates due to toxicity in GRMD	31–33
	CAT-1004	NF-κB inhibitor	Improved diaphragm muscle function in GRMD	DMD Phase II	34–36
	Infliximab (Remicade®)	Antibody to human TNF-α	↓ skeletal and cardiac fibrosis in *mdx*	Preclinical	37
Myostatin inhibition	MYO-029	High-affinity myostatin binding antibody	Ineffective but well tolerated in BMD, LGMD and FSHD	BMD, LGMD and FSHD Phase II	50
	ACE-031	Recombinant pseudo ActIIB receptor	↑ muscle mass, ↑ whole body pulling force in *mdx*	DMD Phase II	51–53
	PF-06252616	Anti-myostatin monoclonal antibody	↑ muscle mass in *mdx*	DMD Phase II ongoing	54
	ACE-083	Locally-acting myostatin trap	↑ muscle mass and safe in Phase 1 trials in post-menopausal women	FSHD Phase II planned by Acceleron	55
	BMS-986089	Anti-myostatin antibody	↑ muscle mass in Phase I trial	DMD Phase II	56, 57
IGF-1	Increlex	Recombinant IGF-1	No effect on motor function following 6 months of treatment in DMD	DMD Phase II	63
Promoting muscle mass	Albuterol	β2-agonist, increases cAMP	↑ lean body mass in DMD/BMD	DMD/BMD Phase II	67, 68
	Urocortin	Increases cAMP	↑ diaphragm force in *mdx*	Preclinical	69, 70
	DT-200 (prev. known as GLPG0492)	Androgen receptor modulator	↑ running, ↑ diaphragm force in *mdx*	DMD Phase I	74, 75
TGF-β inhibition	Losartan	Blocks angiotensin II receptor activation	↓ cardiac and diaphragm fibrosis, ↑ grip strength in *mdx*, inconclusive in DMD	DMD Phase II	84–87
	Pirfenidone	Suppress expression of TFG-β	↓ cardiac contractility in *mdx*	Preclinical	89–92

Target/Class	Drug	Mode of action	Therapeutic effects	Status reached in DMD research	Refs.
	Suramin	Inhibits TGF-β	↓ CK, ↓ skeletal muscle fibrosis, ↓ necrosis in *mdx*	Preclinical	96–98
	Imatinib Mesylate (Gleevec®)	Inhibits TGF-β	↓ CK, ↓ fibrosis, ↑ hind limb grip strength in *mdx*	Preclinical	99–101
	Halofuginone	Impedes Smad 3 binding to DNA, enhances Akt and MAPK/ERK signaling	↓ fibrosis in *mdx*, ↑ muscle strength in DMD	DMD Phase II	102–105
	Tamoxifen	Inhibits TGF-β	↓ cardiac and diaphragm fibrosis, ↑ muscle force in *mdx*	Preclinical	111–113
Calcium regulation	Streptomycin	Non-specific Ca^{2+} channel blocker	↓ muscle degeneration, ↑ muscle force in *mdx*	Preclinical	118, 126
	AT-300 (prev. known as GsMtx4)	Blocks mechanosensitive Ca^{2+} channels	↓ muscle degeneration, ↑ sarcolemmal stability in *mdx*	Preclinical	118, 126, 128–130
	Recombinant Mitsugumin 53	Facilitates membrane repair at sites of injury	↓ muscle pathology in *mdx*	Preclinical	133-, 134
	Poloxamer 188	Membrane sealant	↓ Ca^{2+} overload in *mdx* cardiomyocytes, ↓ myocardial fibrosis in GRMD, ineffective in *mdx* skeletal muscle	Preclinical	135–139
	BGP-15	Induces Hsp72 to enhance SERCA	↑ diaphragm strength in *mdx*, ↑ lifespan in *mdx;utrn*$^{-/-}$	Preclinical	141–143
	Rycal 107 ARM210/S48168	RyR stabilizers	↓ SR Ca^{2+} leakage, ↓ cardiac arrhythmias, ↑ specific force in *mdx*	Preclinical	146
	Rimeporide	NHE-1 inhibitor	↓ Ca^{2+} and Na^{+} in dystrophic myotubes, ↓ muscle degeneration in BIO14.6 hamsters	DMD Phase Ib	150–152
Mitochondria	Cyclosporine A	Immunosupressant, inhibits cyclophilin D	↓ force drop, ↓ CK in *mdx*, variable efficacy in DMD	DMD Phase II/III	154–157
	Debio-025	Inhibits cyclophilin D	↓ fibrosis, ↓ macrophage infiltration	DMD Phase I	158–160
Oxidative stress	Coenzyme Q10	Antioxidant, metabolic support	↑ muscle strength in DMD	DMD Phase III	166. 167
	Idebenone (synthetic CoQ10)	Antioxidant	↓ fibrosis, ↓ inflammation, ↑ voluntary running in *mdx*	DMD Phase III	168, 169
	L-Arginine	Metabolic support	↑ NF-κB, ↑ NF-κB regeneration in *mdx* muscle fibers, ↓ oxidative stress in DMD	DMD Phase I	170–172

Target/Class	Drug	Mode of action	Therapeutic effects	Status reached in DMD research	Refs.
	Creatine	Metabolic support	↓ bone degeneration, ↑ hand grip strength in DMD	DMD Phase III (II/III with glutamine)	173, 175
	Glutamine	Metabolic support	↓ protein degradation in DMD	DMD Phase III (II/III with creatine)	174, 175
	N-Acetylcysteine	Antioxidant, ROS scavenger	↓ CK, ↓ necrosis, ↓ NF-κB in *mdx*	Preclinical	176–178
	Melatonin	Antioxidant	↓ CK, ↓ oxidative stress in *mdx5cv* and DMD	DMD	180, 181
	Green tea extract/Epigallocatechin gallate	Antioxidant	↓ necrosis, ↑ muscle function	DMD Phase II/III	182–186
	Diapocynin	Inhibits ROS production by NOX	↓ ROS production in dystrophic myotubes, ↓ force loss in *mdx*	Preclinical	188
Phosphodiesterase inhibition	Pentoxifylline	Non-specific PDE inhibitor	↑ diaphragm tension, ↓ CK in *mdx*, No effect on muscle strength and function in DMD	DMD Phase II	77, 198–200
	Sildenafil (Viagra®)	PDE5 inhibitor, increases cGMP	↓ cardiomyopathy, ↓ fibrosis in *mdx*, ↓ muscle ischemia, no effect in functional testing in DMD	DMD Phase II	196, 201–206
	Tadalafil (Cialis®)	PDE5 inhibitor, increases cGMP	↓ CK, ↓ contraction-induced injury in *mdx*, ↓ cardiomyopathy, ↓ histopathology in GRMD	DMD Phase III	196, 201, 204, 205, 207–209