



Pharmacological advances for treatment in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a lethal, X-linked muscle-wasting disease caused by lack of dystrophin, essential for muscle fibre integrity. Despite extensive pre-clinical studies, development of an effective treatment has proved challenging. More recently, significant progress has been made with the first drug approval using a genetic approach and the application of pharmacological agents which slow the progression of the disease. Drug development for DMD has mainly used two strategies: (1) the restoration of dystrophin expression or the expression of the compensatory utrophin protein as an efficient surrogate, and (2) the mitigation of secondary downstream pathological mechanisms. This review details current most promising pharmacological approaches and clinical trials aiming to tackle the pathogenesis of this multifaceted disorder.

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Introduction

Duchenne muscular dystrophy is an X-linked recessive progressive wasting disorder caused by loss of function mutations in the dystrophin gene [1^{••}]. DMD affects 1 in 5000 male births [2^{••}] and is generally diagnosed between 2 and 5 years of age as motor developmental delay and abnormal gait, weakened proximal muscles and calf muscle pseudohypertrophy become apparent. Progressive muscle degeneration leads to loss of ambulation at 8–12 years with premature death at 20–30 years due to respiratory and cardiac complications [3].

The DMD gene, consisting of 79 exons, spanning 2.3 Mb of genomic DNA, is the largest known gene in humans [4] and

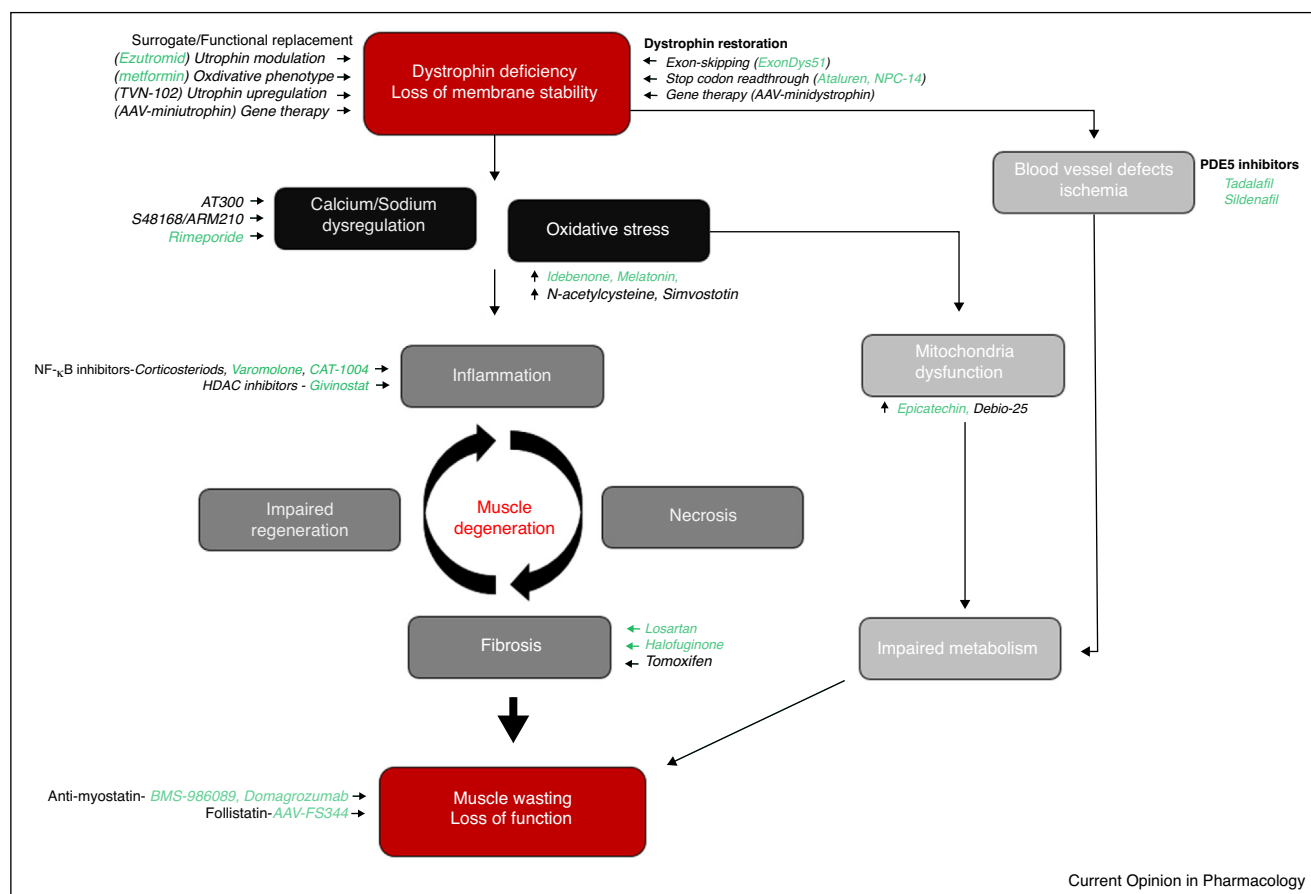
shows one of the highest spontaneous mutation rates. About 68% of the mutations are ‘out of frame’ deletions disrupting the translational reading frame resulting in loss of dystrophin. ‘In-frame’ mutations result in truncated but semi-functional dystrophin proteins which lead to Becker muscular dystrophy (BMD, MIM #300376), a clinically milder disease [5]. Duplications represent 11% and small mutations 20% of DMD cases respectively [2^{••}].

Dystrophin is a 427 kDa cytoplasmic protein, which is a vital component of the dystrophin-associated protein complex (DAPC) at the sarcolemma, connecting the internal cytoskeleton to the surrounding extracellular matrix. Dystrophin provides structural stability to the skeletal muscle, maintains strength and flexibility and protects the sarcolemma from contraction-induced injury [1^{••}]. Absence of dystrophin and subsequent loss of the DAPC leads to progressive defects including perturbation of the calcium homeostasis, activation of proteases and pro-inflammatory cytokines, and mitochondrial dysfunction resulting in continual influx of inflammation, fibrosis, repeated cycles of necrosis and altered regeneration, with impaired vascular adaptation (Figure 1). The myofibres become more susceptible to contraction-induced injury, which results in premature death, muscle wasting and fatty tissue replacement [6].

Despite exhaustive clinical management and corticosteroid treatment, there is currently no effective treatment for DMD, although considerable progress has been made recently in genetic approaches [7,8]. However, only one exon skipping drug, Exondys51 (Sarepta Therapeutics), has been given conditional approval by the U.S. Food and Drug Administration (FDA) [9]. Translarna (PTC Therapeutics) for reading through of stop codons has received conditional approval from the European Medicines Agency (EMA) but not FDA. These drugs are mutation specific and effects on heart muscle have not been reported. Thus, a therapy which targets all limb, respiratory and cardiac muscles and applicable to all DMD patients is urgently needed.

Current pharmacological intervention for DMD can be categorized into two groups: (1) strategies targeting the primary defect and (2) approaches to mitigate secondary and downstream pathological mechanisms. In this review, we summarize the recent most promising pharmacological therapies for DMD that have been tested in clinical trials or are efficient in preclinical models.

Figure 1



Pathophysiological consequences of the dystrophin deficiency and current therapeutic intervention investigated.

Loss of dystrophin and consequential loss of the DAPC enhances sarcolemma susceptibility to contraction-induced damage. Sarcolemmal lesions and possibly leaky Ca^{2+} channels increase calcium influx into dystrophic fibres. This leads to protease activation and free radical formation via cytosolic and mitochondrial sources triggering muscle degeneration with chronic inflammation. In parallel, defects in blood vessels trigger an ischemia and mitochondrial dysfunction which results in impaired ATP production and metabolic function. Drugs in green are currently being tested in clinical trials.

Pharmacological approaches that target the primary defect and aim to reconstruct the DAPC

Read-through of premature termination codons

Nonsense mutations generating stop codons leading to premature translational termination occur in 11% of the DMD cases [2^{••}]. Aminoglycoside antibiotics such as gentamicin promote the insertion of alternative amino acids at the site of the mutated codon and demonstrated inconsistent increased dystrophin production and renal and otic toxicities in DMD trials [10]. Translarna (formerly Ataluren, PTC124/PTC Therapeutics) is a first-in-class compound promoting nonsense read through. Following pre-clinical studies in *mdx* mice [11], a mouse model for DMD [1^{••}], translarna was shown to be well-tolerated in patients. A Phase 2b trial demonstrated a slower disease progression and a non-significant improvement in a six minute walk test (6MWT) and served as the basis for EMA approval in July

2014. Unfortunately, a confirmatory Phase 3 clinical trial in 228 ambulatory DMD patients demonstrated a non-significant benefit in the 6MWT. Although PTC Therapeutics recently decided to discontinue current clinical development of ataluren in cystic fibrosis, the FDA has granted orphan drug designation to translarna and discussions are in progress for approval for DMD. Another read-through compound arbekacin sulfate NPC-14 acting as a protein 30S ribosomal subunit inhibitor is in a phase 2 trial in Japan. Other compounds such as the nonaminoglycoside RTC13/RTC14 [12] and the gentamicin derivatives NB74 and NB84 show increased read-through efficacy and reduced toxicity in *mdx* myotubes [13] but have not yet been tested in patients.

Utrophin

Utrophin is a structural and functional autosomal paralogue of dystrophin which shares 80% of homology with dystrophin and has functional redundancy [14].

Ubiquitously expressed, utrophin is found abundantly in lung, kidney, liver, spleen, brain with lower levels in adult in skeletal muscle and heart [15]. During human skeletal muscle development, utrophin is highly expressed *in utero* and is progressively replaced at the sarcolemma by dystrophin [16]. In adult skeletal muscles, utrophin is enriched at the neuromuscular and myotendinous junctions [17]. Two promoters, A and B have been reported where expression of utrophin A at the synapse and myotendinous junctions [15,18]. Utrophin A is also found at the sarcolemma in regenerating myofibres as a part of the repair process [19] and utrophin B is limited to endothelial cells and blood vessels [15]. In *mdx* muscles, utrophin, found in dystrophin-negative fibres, is increased (1.8-fold over normal levels) as a part of the natural repair process. This repair mechanism also occurs in DMD patients.

Utrophin was proposed to act as a surrogate to compensate for the lack of dystrophin in DMD and the generation of transgenic mice overexpressing utrophin supports this view [20]. These transgenic mice also show that the continuous localization of utrophin along the sarcolemma is key and that low increases of utrophin (1.5-fold) can be beneficial. The high level of utrophin observed in the transgenic mice is significantly less than the normal levels found in kidney and liver [20] and is not toxic in a broad range of murine tissues.

The use of small compounds which act through the promoter to modulate utrophin levels is thus a viable strategy for the therapy of DMD. Such small molecules have been shown to prevent pathology in the *mdx* mouse [21,22,23**]. Summit Therapeutics have completed Phase 1a and 1b clinical trials with ezutromid (formally known as SMT C1100) showing it to be well tolerated [24**,25]. Summit Therapeutics is currently carrying out a Phase 2 open-label clinical trial. Other molecules have been developed in the same chemical series as ezutromid and show efficacy in *mdx* mice [23**] validating the ezutromid drug series and this approach to utrophin modulation. Others drugs as AICAR [22], nabumetone, heregulin and resveratrol [26] were previously also described to modulate the utrophin promoter and to be efficient in *mdx* mice but no clinical trials for DMD are reported.

Utrophin expression is under transcriptional, as well as post-transcriptional control via several mechanisms and associated pharmacological agents to increase utrophin expression have been explored. Heregulin increases utrophin levels through epigenetic regulation of the utrophin-A promoter via activation of mitogen- and stress-activated protein kinase (MSK1/2) and phosphorylated histone H3 in an extracellular signal-regulated kinase (ERK)-dependent manner [27]. Utrophin can also be increased by promotion of the slow oxidative phenotype. In slow fibre

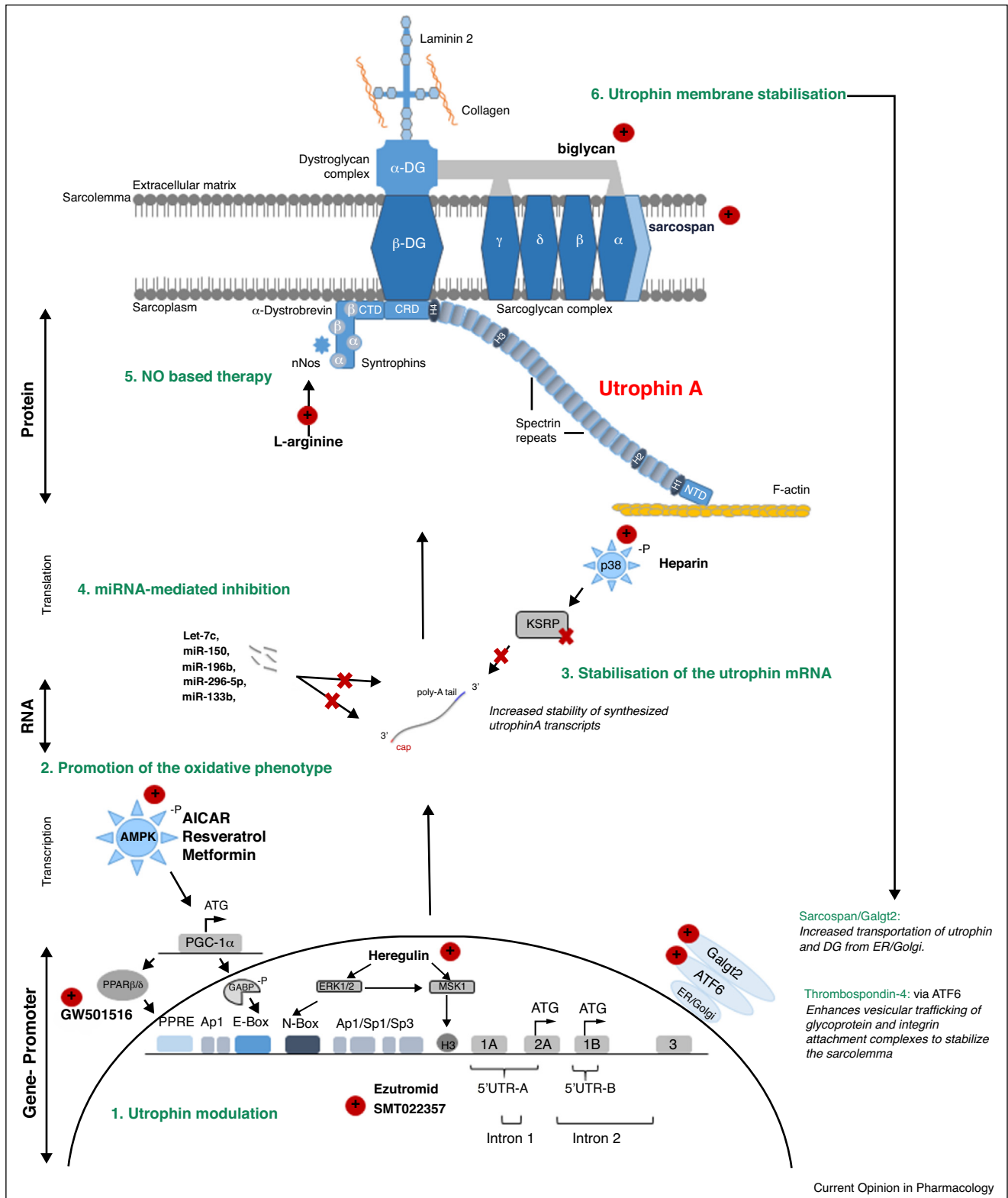
type I muscle, levels of utrophin are 3–4 fold higher than in fast fibre type II due to transcriptional and post-transcriptional modulation, controlled in part by the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α)/estrogen related receptor alpha (ERR α)/nuclear respiratory factor 1 (NRF-1)/MTF α axis. Treatment with AICAR targeting peroxisome-proliferator-activated receptor beta/delta (PPAR- β/δ), 5' adenosine monophosphate activated protein kinase (AMPK) and sirtuin 1 (SIRT1) acting on the PGC-1 α levels results in an upregulation of utrophin and functional benefits [28]. Other AMPK activators such as resveratrol [29], quercetin [30] and metformin [31] have also been described. PPAR- β/δ agonists such as GW501516, also promote the oxidative phenotype and stimulate utrophin A expression [27]. By activation of p38 MAP Kinase, heparin mediates a sequestration of the RNA-binding protein KSRP by the regulatory protein 14-3-3 resulting in post-transcriptional stabilisation of the utrophin mRNA [32**].

Another interesting novel therapeutic avenue is the regulation by miRNAs as let-7c, miR-150, miR-196b, miR-296-5p, miR-133b which have been reported previously to repress utrophin mRNA expression [33]. Upregulation of utrophin is also stimulated by NO synthase (nNOS) substrate L-arginine enhancing production of nitric oxide (NO) which inhibits the proteolytic activity of calpain [34]. Finally, there are also agents which act through utrophin stabilisation at the sarcolemma: biglycan, a small Leucine Rich Proteoglycan (SLRP) directs the assembly of a utrophin associated complex, including nNOS, resulting in an increase of utrophin leading to significant improvement of muscle function in *mdx* mice [35]. Over expression of sarcospan leads to activation of Akt and increased levels of CT GalNAc transferase (Galgt2) leading to α -dystroglycan (α -DG) glycosylation. This improves cell surface expression of utrophin by increasing transportation of utrophin- α -DG from endoplasmic reticulum/Golgi membranes [36]. Overexpression CT-GalNAc transferase is known to stabilise the utrophin protein complex [37]. Thrombospondin-4 selectively enhances vesicular trafficking of dystrophin-glycoprotein and integrin attachment complexes to stabilise sarcolemmal protein as utrophin [38]. Agents to promote utrophin expression via these mechanisms, could be used together as their effects would be predicted to be additive (see Figure 2 and Table 1).

Pharmacological approaches that target the secondary pathology down-stream of the dystrophin deficiency

Many pharmacological approaches to DMD target secondary pathology downstream of the dystrophin deficiency such as calcium dysregulation, oxidative stress and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway, mitochondria

Figure 2



Therapeutic strategies and mechanisms to enhance utrophin-associated protein complex (UAPC) at the sarcolemma. Utrophin consists of an actin-binding N-terminal domain (NTD), hinge domains (H1-H4) and a cysteine-rich domain (CRD) adjacent to a carboxy-terminal domain (CTD). Spectrin repeats (R1-R24) make up the rod domain. Utrophin shares 80% sequence homology to dystrophin and is an efficient surrogate to compensate for the lack of dystrophin in dystrophic muscle. Different pathways have been targeted to increase utrophin

dysfunction, fibrosis, muscle wasting and muscle ischemia. Here, we focus on some of the most advanced strategies.

Calcium dysregulation

Dystrophin deficiency results in membrane tears and leaky Ca^{2+} release channels which compromise the intracellular Ca^{2+} homeostasis leading to chronic inflammation, cycles of degeneration/regeneration and fibrosis [39]. AT-300 (Akashi Therapeutics) is a peptide which blocks mechanosensitive Ca^{2+} channels resulting in modest benefits in mdx mice [40]. Plans for clinical trials are in progress. Following post-translational modifications of the ryanodine receptor subtype 1 (RyR1), the intracellular calcium calstabin-RyR channel complex is dissociated in dystrophic muscles. Acting as a stabiliser, treatment with the small drug ARM210/S48168 (ARMGO Pharma) results in functional improvement in mdx mice, notably in the diaphragm (Capogrosso *et al.*, abstract in Neuro-muscul Disord. G.P.90 2014, 24:9–10). In dystrophin deficient tissue, activity of the Na^+/H^+ exchanger type-1 (NEH-1) is increased resulting in Na^+ overload which may contribute to the Ca^{2+} excess. A phase 1 trial is currently recruiting to study Rimeporide, a (NHE-1) inhibitor with anti-fibrotic and anti-inflammatory properties in mdx skeletal and cardiac muscles. Others strategies such as the hsp72 inducer BGP-15 are being investigated to rescue calcium homeostasis (Figure 1, Table 2).

Oxidative stress/ROS

Influx of Ca^{2+} enhances production of reactive oxygen species (ROS) leading to elevated oxidative stress [41^{*}]. ROS exacerbates Ca^{2+} dysregulation, induces mitochondria dysfunction and activates NF- κ B and TGF- β pathways triggering inflammation. Initial results in DMD boys treated with Coenzyme Q10 [42], a hydrophobic antioxidant acting as an electron acceptor molecule for complexes I (NADH) and II (SDH) of the respiratory chain and binding the inner mitochondrial membrane, paved the way for the study of Raxone/idebenone, a synthetic derivative of Coenzyme Q10. Developed by Santhera Pharmaceuticals, this antioxidant stimulating mitochondrial electron reflux has been reported to be cardioprotective and to improve exercise performance in the mdx mouse. A recent phase 3 clinical trial demonstrated reduced loss of respiratory function in DMD patients [43^{**}]. Various antioxidant therapies such as melatonin or *N*-acetylcysteine are currently being investigated (Table 2). Recently, simvastatin showed great promise in the mdx mice [44^{*}]. While lowering LDL cholesterol, simvastatin decreases oxidative stress by reduction of nicotinamide adenine dinucleotide phosphate-oxidase 2

(NOX2) levels, one major source of ROS. Whereas simvastatin is a common statin medication, used in children, it is essential to remember that muscle-related side effects occur with statin use and that this repurposed drug is under early investigation for DMD.

Mitochondrial dysfunction

Excessively elevated intracellular Ca^{2+} in DMD leads to mitochondrial dysfunction [45] impairing Ca^{2+} buffering from myofibres and organelles resulting in lower ATP production and increase of ROS. Mediated by cyclophilin D, formation of large mitochondrial permeability transition pores (MPTP) also cause permeabilisation of mitochondria. To overcome this defect, Alisporovir/Debio-25, an analogue of cyclosporine shown to inhibit cyclophilin D and prevent MPTP formation, was produced. Treatment in mdx demonstrated a greater efficacy than prednisone in reducing inflammation and macrophage infiltration in mdx mice [46]. Another strategy is Epicatechin (Cardero Therapeutics), a flavonoid released in response to exercise. Mimicking the effects of certain exercise regimens, Epicatechin promotes an oxidative phenotype through the NO/AMPK/SIRT1/PGC-1 α pathway and results in mitochondrial biogenesis, reduction of oxidative stress, and an increase of utrophin and follistatin. Following promising results in seven BMD patients (NCT01856868), Cardero Therapeutics is currently recruiting for a Phase 1/2 trial in non-ambulatory DMD boys. Mitobridge is also developing small molecules which improve mitochondrial function (Table 2).

NF- κ B pathways—anti-inflammatory agents

Following Ca^{2+} level increases leading to fibre necrosis, dystrophic muscles are infiltrated by T cells, macrophages and neutrophils. This inflammatory response is mediated by activation of the NF- κ B pro-inflammatory pathway and elevated levels of interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1) [47]. Currently, anti-inflammatory glucocorticoids such as prednisone and deflazacort, recently approved [48], are the current standard of care for DMD. Primarily acting through inhibition of the NF- κ B pathway, steroids prolong ambulation by 2–5 years and modestly improve muscle strength and cardiopulmonary function but are often associated with significant side effects [49]. An alternative steroid, Vamorolone (VBP-15/Reveragen) has been developed without these side effects. Preclinical studies in mdx mice showed reduction of inflammation and an increased strength without the immunotoxicity [50]. Vanoroalone is progressing to a phase 2a trial. Catabasis has developed another promising oral NF- κ B modulator, the conjugate of salicylate and docosahexaenoic Edasalonexent (CAT-1004).

(Figure 2 Legend Continued) expression such as (1) utrophin modulation (ezetromid, heregulin), (2) promotion of the oxidative phenotype (AICAR, resveratrol, metformin, GW501516), (3) increasing the stability of existing mRNAs (heparin), (4) miRs-mediated inhibition, (5) NO-based therapy (L-arginine) and (6) membrane stabilisation/association of utrophin at the sarcolemma (biglycan).

Table 1

Status of the main and most promising therapeutic strategies targeting the primary defect of Duchenne

Drug name	Company	Mechanism of action	Chemistry	Delivery route	Percentage of applicable patients	Current stage	Results to date	Clinical trial	References
Read-through of premature termination codons									
Gentamicin	Nationwide Children's Hospital	Stop codon read-through	Antibiotic	IV	11%	Phase 1 (Conclude)	Preclinical (<i>mdx</i>):/ Clinical: Inconsistent dystrophin restoration, ↓ CK level, risk of renal failure and irreversible ototoxicity	NCT00451074 NCT00005574	[10]
Translarna (Ataluren)	PTC Therapeutics	Stop codon read-through	Small molecule	Oral	11%	Approved (UE)	Preclinical (<i>mdx</i>): ↑ dystrophin in skeletal and cardiac muscle over 2–8 weeks of treatment Clinical: Well tolerated, slowed loss of walking ability in DMD patients in 6MWT	NCT01557400	[11]
NPC-14 (Arbekacin Sulfate)	Kobe University	Stop codon read-through	Small molecule	Oral	11%	Phase 2 (Ongoing)		NCT01918384	
RTC13/RTC14		Stop codon read-through	Small molecule	Oral	11%	Pre-clinical	Preclinical (<i>mdx</i>): Partially restore dystrophin protein, ↓ CK levels, ↑ muscle strenght and function		[12]
NB74/NB84		Stop codon read-through	Small molecule	Oral	11%	Pre-clinical	Preclinical (cells): Eeduced cell toxicit and superior readthrough efficiency than those of gentamicin		[13]
Utrophin upregulation									
Ezutromid	Summit Therapeutics	Modulation	Small molecule	Oral	100%	Phase 2	Preclinical (<i>mdx</i>): ↑ Utrophin (2x), ↑ Membrane stability, ↓ Regeneration, ↓ Inflammation, ↑ Muscle function Clinical (Phase 1): Safe and well-tolerated in adults and DMD patients	NCT02056808 NCT02383511 NCT02858362	[21,24**,25]
SMT022357	Summit Therapeutics	Modulation	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (2.5x), ↑ Membrane stability, ↓ Regeneration, ↓ Inflammation, ↑ Muscle function		[23**]
Heregulin	UtroDMD Alliance	Modulation	Peptide	IP	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (2.7x), ↑ Membrane stability, ↓ Regeneration, ↓ Necrosis, ↑ Muscle function		[26,27]
AICAR		↑ oxidative phenotype	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (1.25x), ↑ Membrane stability, ↑ Mitochondria function, ↑ Muscle function		[22,28]
Resveratrol		↑ oxidative phenotype	Small compound	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (1.5x), ↓ Inflammation		[26,29]
Quertecin		↑ oxidative phenotype	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (1.4x), ↑ Membrane stability, ↓ cardiac pathology, protect respiratory function		[30]
Metformin	University Hospital, Basel, Switzerland	↑ oxidative phenotype	Small molecule	Oral	100%	Phase 1	Preclinical (<i>mdx</i>): ↑ Utrophin (1.5x)	NCT01995032 NCT02516085	[31]
GW501516		↑ oxidative phenotype (PPAR-β/δ agonist)	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (1.5x), ↑ Membrane stability, ↑ Muscle function		[27]

Table 1 (Continued)

Drug name	Company	Mechanism of action	Chemistry	Delivery route	Percentage of applicable patients	Current stage	Results to date	Clinical trial	References
Heparin		RNA stabilisation	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (1.5–3x), ↑ Membrane stability, ↓ Regeneration, ↑ Muscle function		[32]
L-Arginine	Massachusetts General Hospital	↑ nNOS activity	Small molecule	Oral	100%	Phase 1	Preclinical (<i>mdx</i>): ↑ Utrophin (1.3–1.9x), ↑ Improved respiratory and muscle function	NCT01388764	[34]
CT-GalNAc transferase		Protein stabilisation	AAV	IV	<100%	Pre-clinical	Preclinical (transgenic- <i>mdx</i>): ↑ Utrophin, ↑ Membrane stability, ↑ Muscle function	NCT02516085	[37]
TV-102/Biglycan	Tivrosan Therapeutics	Protein stabilisation	Small molecule	IP	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↑ Utrophin (1.7–2.5x), ↑ Membrane stability, ↓ Regeneration, ↑ Muscle function		[35]

Treatment reduces muscle fibrosis and improves muscle mass in *mdx* mice and improved the more severe GRMD phenotype [51]. Following a successful phase 1 safety, pharmacokinetics, and pharmacodynamics trial in adult subjects [52], a phase 1/2 trial in 30 ambulatory DMD patients is in progress. Unfortunately, Catabasis recently reported that the drug failed to demonstrate a significant benefit after 12 weeks of treatment in DMD boys [53]. Although all these studies are promising, the complex role of NF-κB in many other tissues such as liver [54] could potentially lead to a negative outcome and highlights the importance of defining appropriate doses.

Histone deacetylase (HDAC) inhibitors

In dystrophin-deficient muscles, delocalization and down-regulation of nNOS leads to deficient S-nitrosylation and constitutive activation of Histone deacetylase 2 (HDAC2) [55]. Interruption of DAPC-NO signalling and hyperactivity of HDAC2 may contribute to the impairment of muscle regeneration and compromise microfibre adaptation to contraction. Therefore, approaches to counter upregulated histone deacetylase 2 (HDAC2) activity in dystrophic muscles with HDAC inhibitors (HDACi) are an interesting avenue. In *mdx* mice, exposure to HDACi result in functional and morphological beneficial benefits and counters the disease progression [56]. Givinostat (Italfarmaco SpA) is a HDAC inhibitor efficient in animal models and currently being tested in a Phase 2 trial. One year of treatment, drug treatment was well-tolerated and resulted in positive histological effects [57**]. Nevertheless, off target effects, toxicity and long term consequences of such treatment on stem cell pool depletion need to be addressed.

TGF-β pathway—anti-fibrotic agents

Fibrosis is defined by excessive deposition of extracellular matrix protein in response to chronic tissue injury and inflammation. The transforming growth factor-β (TGF-β) pathway, which controls in part the fibrosis processes, is a major target of anti-fibrotic therapy. Halofuginone (HT-100/Akashi Therapeutics), showed antifibrotic, anti-inflammatory and muscle regenerative effects in *mdx* mice [58] but the Phase 2 study was suspended after the death of a DMD patient treated with the highest dose (Akashi Therapeutics press release: <http://akashirx.com/news/dosing-and-enrollment-in-ht-100-trial-suspended>). Other anti-fibrotic strategies as losartan/lisinopril and tamoxifen are currently being investigated in clinical studies (Table 2).

Regulators of muscle growth/myostatin inhibitors

Myostatin, as a negative regulator of muscle mass, has been a target for therapeutic intervention with mixed success. Disruption of this highly conserved myokine results in a dramatic increase of muscle mass due to an increase of number and/or size of muscle fibres [59]. Anti-myostatin molecules and/or those which reduce activity of

Table 2

Status of the main and most promising therapeutic strategies acting on downstream pathological mechanisms of Duchenne

Drug name	Company	Mechanism of action	Chemistry	Delivery route	Percentage of applicable patients	Current stage	Results to date	Clinical trial	References
Calcium dysregulation									
Rimeporide	EspeRare Foundation	↓ Na ⁺ /Ca ²⁺ exchangers, Na ⁺ /H ⁺ exchanger	Small molecule	Oral	100%	Phase 1b (Recruiting)	Pre-clinical (<i>mdx</i>): ↓ fibrosis, ↓ inflammation in skeletal muscles (including diaphragm) and cardiac muscles	NCT02710591	
AT-300/GsMTx4	Akashi Therapeutics	Calcium channel inhibitor	Small molecule	Injection	100%	Pre-clinical	Pre-clinical (<i>mdx</i>): Modest results (due to PK/dosing problem)		[40]
ARM210/S48168		Stabilisation RyR 1 complex	Small molecule	Oral	100%	Pre-clinical	Pre-clinical (<i>mdx</i>): ↓ Cytosolic calcium level, ↑ Muscle function, prevent the decline in running performance		
BGP-15		Hsp 72 inducer	Small molecule	Oral	100%	Pre-clinical	Pre-clinical (<i>mdx</i> , <i>mdx;utr</i> ^{-/-}): ↑ Muscle architecture, ↑ Contractile function, ↑ Muscle strength		
Oxidative-stress drugs									
Coenzyme Q10	Santhera Pharmaceuticals	Electron acceptor for NADH and SDH	Small molecule	Oral	100%	Phase 3 (Complete)	Clinical (Phase 3): ↑ Muscle strength	NCT00033189	[42]
Raxone/Idebenone		Antioxidant				Phase 3 (Complete)	Preclinical (<i>mdx</i>): ↓ Cardiac inflammation and fibrosis, ↑ Exercise performance Clinical (Phase 3): ↓ Loss of respiratory function	NCT00308113 NCT00758225 NCT00654784 NCT01027884	[43**]
Melatonin		Antioxidant				Phase 1/2 (Complete)	Preclinical (<i>mdx</i>): ↓ CK levels, ↑ Force, improved muscle redox status Clinical (Phase 1/2): ↓ Oxidative stress, ↓ Inflammation, ↓ CK levels		
N-Acetylcysteine		Antioxidant, ROS scavenger	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↓ CK levels, ↓ Necrosis, ↓ Regeneration, ↑ Utrophin, ↑ Muscle force		
Simvastatin		↓ LDL cholesterol	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↓ Oxidative stress, Inflammation, Fibrosis, ↓ CK levels, ↑ Muscle function (strength, fatigue)		[44*]
Mitochondria drugs									
(+) Epicatechin	Cardero Therapeutics	↑ NO/AMPK/SIRT1/PGC-1 α	Small molecule	Oral	100%	Phase 1/2 (Recruiting)	Preclinical: ↑ Utrophin, ↑ Follistatin, ↑ Mitochondrial biogenesis, ↓ Fibrosis Clinical (Phase 1/2, Becker patients): ↑ Mitochondrial biogenesis, ↑ Utrophin, ↑ Follistatin/Myostatin	NCT02964377	
Debio-025	Debiopharm International SA Mitobridge	Inhibitor of mitochondrial pore, Cyclophilin inhibitor	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↓ Inflammation and macrophage infiltration		[46]
MTB-1			Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): ↓ Inflammation, regeneration and fibrosis, ↑ Voluntary activity and endurance		
NF- κ B pathway									
Vamorolone (VBP-15)	ReveraGen BioPharma	NF- κ B inhibition	Small molecule	Oral	100%	Phase 2 (Recruiting)	Preclinical (<i>mdx</i>): ↓NF- κ B activity, ↓ Inflammation, ↑ Membrane stability, ↑ Specific force, No side effect Clinical (Phase 1): Safe and well-tolerated with a 20 mg/kg/day dose	NCT02415439 NCT02760264 NCT02760277	[50]

Table 2 (Continued)

Drug name	Company	Mechanism of action	Chemistry	Delivery route	Percentage of applicable patients	Current stage	Results to date	Clinical trial	References
Edasalonexent (CAT-1004)	Catabasis Pharmaceuticals	NF- κ B inhibition	Small molecule	Oral	100%	Phases 1/2 (Ongoing)	Preclinical (GRMD): \uparrow Diaphragm function Clinical (Phase 1): Safe and well-tolerated Clinical (Phase 1/2): No significant benefit after 12 weeks of treatment in DMD boys	NCT01440166 NCT02439216	[51–53]
HDAC drug Givinostat	Italfarmaco	HDAC inhibitor	Small molecule	Oral	100%	Phase 1/2 (Ongoing)	Preclinical (<i>mdx</i>): \downarrow Fibrosis and fatty infiltration, \uparrow Membrane stability, \uparrow Endurance performance Clinical (Phase 1/2): Well-tolerated and resulted in positive histological effects	NCT01761292	[56,57**]
Anti-fibrotic/TGF- β drugs Losartan	Nationwide Children's Hospital	Angiotensin II type 1 receptor blocker	Small molecule	Oral	100%	Phase 2 (Complete)	Preclinical (<i>mdx</i>): \downarrow Fibrosis, \downarrow Calcification, \downarrow Regeneration, \downarrow CK level	NCT01982695	
Halofuginone (HT-100)	Akashi Therapeutics	Inhibitor of collagen α 1 and MMP2	Small molecule	Oral	100%	Phase 1/2 (Suspended)	Preclinical (<i>mdx</i>): \downarrow Fibrosis, \downarrow Degeneration/Regeneration, \uparrow Muscle strength Clinical (Phase 1/2): Suspended after the death of a DMD patient treated with the highest dose	NCT01847573 NCT01978366	[58]
F3-3019	Fibrogen	Monoclonal anti-CTGF antibody	Small molecule	Oral	100%	Pre-clinical	Preclinical (<i>mdx</i>): \downarrow Fibrosis, \uparrow Muscle exercise		
Tamoxifen	Hadassah Medical Organization	Inhibition of TGF- β	Small molecule	Oral	100%	Phase 1 (Not yet open)	Preclinical (<i>mdx</i>): \downarrow Fibrosis in diaphragm and heart, \uparrow Muscle function	NCT02835079	
Muscle grow/regeneration drugs Domagrozumab (PF-06252616)	Pfizer	Human anti-myostatin monoclonal antibody	Antibody	IV	100%	Phase 2 (Recruiting)	Preclinical (<i>mdx</i>): \uparrow in body weight, lean mass, muscle weight and grip strength Preclinical (Cynomolgus monkeys): \uparrow Muscle volume Clinical (Phase 1): Generally safe and well tolerated, \uparrow Muscle mass by 6% in healthy subjects	NCT02310763	
BMS-986089	Bristol-Myers Squibb	Human anti-myostatin adnectin	Antibody	SC	100%	Phase 1/2 (Recruiting)	Preclinical (Cynomolgus monkeys): \uparrow Muscle volume Clinical (Phase 1): Generally safe and well tolerated, \uparrow Muscle mass by 5% in healthy subjects	NCT02145234 NCT02515669	
Follistatin	Nationwide Children's Hospital	rAAV1.CMV.huFollistatin344	AAV	IM	<100%	Phase 1/2 (Recruiting)	Preclinical (<i>mdx</i>): \uparrow Muscle mass, \uparrow Muscle force, \downarrow CK levels, Improve muscle histology Preclinical (Cynomolgus monkeys): \uparrow Muscle volume, \uparrow Muscle strength Clinical (Phase 1/2, Becker patients): \uparrow Muscle mass, stabilisation or improvement in 6MWT	NCT02354781	

Table 2 (Continued)

Drug name	Company	Mechanism of action	Chemistry	Delivery route	Percentage of applicable patients	Current stage	Results to date	Clinical trial	References
Muscle ischemia Tadalafil	Eli Lilly and Company	PDE5 inhibitor	Small molecule	Oral	100%	Phase 3 (Complete)	Preclinical (mdx): ↓ Ischemia, ↓ contraction-induced injury Preclinical (GRMD): Delay cardiomyopathy, ↑ Utrophin in cardiac muscle Clinical (Phase 3): No effect in 6MWD, NSAA or other standard measures of ambulatory ability, no effect on upper limb function as measured by the PUL Preclinical (mdx): ↓ Ischemia, ↓ Fibrosis, ↓ Respiratory weakness, ↓ Cardiomyopathy, Clinical (Phase 2): No effect on cardiomyopathy in Becker and Duchenne patients	NCT01865084	[62,63*]
Sildenafil	Cedars-Sinai Medical Center (USA)	PDE5 inhibitor	Small molecule	Oral	100%	Phase 1/2 (Complete)		NCT01359670	

this factor and/or disruption activity of the receptor Activin receptor type IIB have been extensively studied [60]. Initial therapeutic trials revealed disappointing results due to lack of efficacy, interference or potential adverse side effects. A Phase I trial with Domagrozumab/PF-06252616 (Pfizer), a human anti-MSTN monoclonal antibody showed a +6.1% muscle mass after treatment compared to the placebo group. A double blind and multiple ascending Phase 2 study is currently recruiting. BMS-986089 (Bristol Myers Squibb) is currently recruiting for a Phase 2 trial with a human anti-myostatin adnectin. Delivery of follistatin, a potent activin-binding protein inhibiting myostatin activity, by AAV delivery of the gene has completed a successful clinical trial in BMD patients and trials in DMD are planned. Although these drugs are promising, increasing muscle mass may have long term implications on depletion of the satellite cell pool.

Muscle ischemia

The absence of dystrophin leads to mislocalization of nNOS at the sarcolemma and reduction of the major isoforms of nitric oxide which are required to equilibrate muscle oxygenation and protect the exercising muscle against excessive sympathetic vasoconstriction through production of cyclic guanosine monophosphate (cGMP) [61]. Treatment with the phosphodiesterase-5 inhibitor (PDE5i) increases the intracellular cGMP level in vascular smooth muscle cells and leads to vasodilation. Pre-clinical data in mice and dogs show benefits in skeletal and cardiac muscles [62]. PDE5i Tadalafil alleviates muscle ischemia in BMD and DMD patients [63*] but the largest DMD placebo-controlled Phase 3 trial involving 331 patients ages 7–14 showed no functional improvement after 48 weeks. Patients involved in this study may not have been engaged in enough daily leg exercise for tadalafil to impact use-dependent leg muscle injury. Further studies are needed.

Safety and pharmacokinetic/dynamic considerations

The majority of the pharmacological approaches described in this review employ non-selective strategies and act through on key target pathways such as NF-κB, HADAC or ROS. It is therefore essential to consider potential off-target effects in other tissues. Pharmacokinetic and pharmacodynamic considerations [64], in relationship with potential detrimental effects must be carefully assessed in all organs and drug-drug interactions need to be considered if they are used in conjunction with steroids which most boys will be taking. Genetic strategies such as viral delivery of dystrophin minigenes and exon skipping are tissue specific and therefore do not suffer from these issues. Nevertheless, there is little doubt that an effective treatment may well depend on a combination therapy, ideally a genetic approach together with the pharmacological approaches reviewed here.

Concluding remarks

Progressive and numerous pathophysiological consequences of the lack of dystrophin offer several downstream targets for DMD therapy which can be used in all patients whatever their mutation. Many strategies are showing promise in clinical trials. They may have limitations for long term use but used in combination, these drugs may well transform the quality of life for DMD boys in the future.

Conflict of interest

K.E.D. is a shareholder of Summit Therapeutics plc.

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- of special interest
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