Chapter

The Potential Benefits of Drug-Repositioning in Muscular Dystrophies

Ioana Lambrescu, Emilia Manole, Laura Cristina Ceafalan and Gisela Gaina

Abstract

Muscular dystrophies (MDs) are a complex group of rare neuromuscular disorders caused by genetic mutations that progressively weaken the muscles, resulting in an increasing level of disability. The underlying cause of these conditions consists of mutations in the genes in charge of a person's muscle composition and functionality. MD has no cure, but medications and therapy can help control symptoms and slow the disease's progression. Effective treatments have yet to be developed, despite the identification of the genetic origins and a thorough knowledge of the pathophysiological alterations that these illnesses induce. In this scenario, there is an urgent need for novel therapeutic options for these severe illnesses, and drug repositioning might be one feasible answer. In other words, drug repositioning/repurposing is an accelerated method of developing novel pharmaceuticals since the new indication is based on previously accessible safety, pharmacokinetic, and manufacturing data. This is particularly crucial for individuals with life-threatening illnesses such as MDs, who cannot wait for a conventional medication development cycle. This chapter aims to review the challenges and opportunities of drug-repositioning in a variety of MDs to establish novel treatment approaches for these incurable diseases.

Keywords: muscular dystrophies, drug-repositioning, drug-repurposing, novel therapies

1. Introduction

Muscular dystrophies (MDs) are a diverse array of hereditary muscle disorders defined by gradual weakening in the affected muscles [1]. Even among people with the same condition and genetic abnormalities, there may be differences in the age at which symptoms first appear, the severity of those symptoms, the rate at which they advance, the prognosis, and the most effective treatment [2]. In terms of epidemiology, each form of MD is rather uncommon, but these conditions account for a significant portion of the individuals who suffer from neuromuscular impairment [2].

Over the past decades, significant advancements have been achieved in the treatment of people who suffer from MD. This progress has been made possible

by worldwide cooperation, increasing comprehension of the fundamental genetic mechanisms, and clinical consensus standards [3]. Therapeutic advancements have also expanded, first using mutation and gene-directed techniques, leading to commercially accessible medications targeting particular Duchenne muscular dystrophy (DMD) mutations [3]. Although there is real enthusiasm in the therapeutic area, it is crucial to remember that, since MDs are degenerative conditions, finding a permanent solution will be exceedingly difficult. Therefore, there is an urgent need to find and use innovative pharmacological treatments to enhance the clinical care of MD patients.

Drug repositioning, also known as pharmacological repurposing, is a process that may be used to find innovative therapeutic agents from the current drug molecules that the FDA has authorized for use in clinical settings [4]. Costs for novel treatments may be a significant barrier for researchers and patients in general and with rare illnesses in particular. This difficulty may be significantly addressed by experimenting with the usage of chemicals that were initially intended for other situations.

On average, the success rate of developing a new drug is only 2.01%. According to a report by the Eastern Research Group (ERG), it takes 10 to 15 years to generate a new therapeutic molecule [5]. Furthermore, traditional drug development processes generally include five phases, while drug repurposing only requires four (**Figure 1**) [6]. Researchers currently only require 1–2 years to uncover novel therapeutic targets, while it takes an average of 8 years to produce a repositioned medicine, thanks to the rapid growth of bioinformatics [7].

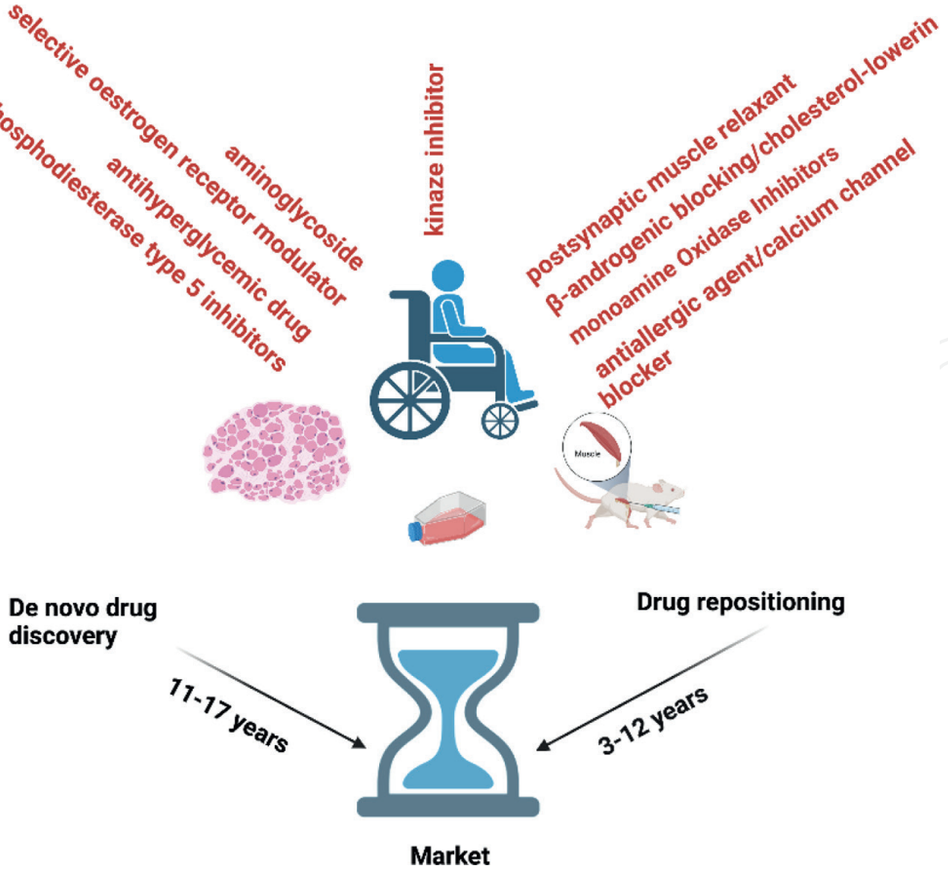

Figure 1.
Approaches for drug repurposing.

This chapter focuses on the most promising options for repositioning medications for three of the most prevalent forms of MDs: both in preclinical investigations and clinical trials.

2. DMD/BMD: showing old molecules how to accomplish new things

The X-linked muscle disorders known as dystrophinopathies include the muscular dystrophies Duchenne (DMD), Becker (BMD), and DMD-associated dilated cardiomyopathy (D). DMD, an X-linked recessive condition that mostly affects men, is characterized clinically by gradual muscular weakening and deterioration that initially affects proximal muscles [8]. The dystrophin gene (*DMD* gene), which is located on chromosome Xp21.2 and encodes for the dystrophin protein via its 79 exons, is the cause of DMD and BMD depending on the mutation [9]. Dystrophin is an essential component of the protein complex that via the cell membrane binds the cytoskeleton of a muscle fiber to the surrounding extracellular matrix, stabilizing it during muscle contraction [8]. A number of potentially useful therapy techniques have been created and studied using DMD animal models. Nevertheless, the results of clinical trials have been far less spectacular. Currently, there are no treatments that can reverse dystrophinopathies underlying etiology. Conventional therapies employing corticosteroids aim to relieve symptoms, but their long-term administration is linked with substantial side effects [10]. Regarding the concept of targeted therapy several approaches have been developed for restoring dystrophin, each customized to a specific type of mutation. Stop-codon read-through, exon skipping, vector-mediated gene therapy, and the emerging CRISPR/Cas9 gene editing are all promising strategies. However, in the context of these therapies, the initial enthusiasm is overshadowed by all the questions regarding treatment effect, safety, and financial burden [11]. Therefore, drug repositioning could be a cost and time-effective approach when it comes to a rare disease such as muscular dystrophy.

2.1 Targeting Utrophin a via repurposed drugs

Dystrophin's primary purpose in terms of its functional role is to forge a connection between the internal cytoskeletal actin network and the extracellular matrix. Consequently, this will ensure that the sarcolemma of muscle fibers retains its structural integrity [12, 13]. Utrophin, a paralogue of dystrophin, is a protein highly expressed in developing muscle [14]. To complete the connection from the cytoskeleton through the membrane and into the extracellular matrix, utrophin interacts with the dystrophin-associated protein complex [14]. Therefore, techniques based on targeting dystrophin or utrophin may be applied together in dystrophic muscles. There are two full transcription forms for utrophin. The neuromuscular junction (NMJ), tendon, choroid plexus, pia mater, and glomerulus all express utrophin A, whereas endothelial cells produce utrophin B [15]. Muscle-specific trans-factor known as eukaryotic elongation factor 1A2 (eEF1A2) was discovered to interact with utrophin A's 5'UTR, which is why eEF1A2 targeting might be a possible therapeutic approach for DMD patients [16].

In 2020, Peladeau and colleagues published an interesting study that aimed to identify FDA-approved drugs that acted on the eEF1A2-utrophin A pathway in *mdx* mice [17]. The *in vitro* and *in vivo* experiments focused on five leads: Acarbose, Betaxolol, Labetalol, Pravastatin, and Telbivudine. The authors found that the

beta-androgenic blocking medication *Betaxolol* and the cholesterol-lowering medication *Pravastatin* were the most effective activators of both eEF1A2 and utrophin through its 5′UTR internal ribosome entry site. This observation was based on a 7-day drug treatment of transgenic mice harboring the bicistronic reporter construct containing the utrophin 5'UTR. Furthermore, muscle strength was increased, and both muscle fiber shape and sarcolemma integrity were improved after *mdx* mice were treated with these medicines for 4 weeks [17].

2.2 The monoamine oxidase inhibitors

Although the pathophysiological basis for DMD is yet unknown, oxidative stress and mitochondrial dysfunction are thought to be major contributors to the development of muscle injury [18–20]. In dystrophic muscles, the mitochondrial enzyme known as monoamine oxidase (MAO) is a key generator of reactive oxygen species (ROS). This mitochondrial enzyme has been researched extensively in the central nervous system [21]. In 2010, a group conducted by Menazza demonstrated that oxidative changes of myofibrillar proteins and cell death, which result in a notable decrease in contractile performance, are significantly influenced by MAO-dependent reactive oxygen species (ROS) buildup [21]. *Pargyline*, an inhibitor of both MAO isoforms, was introduced in the US and the UK in 1963 as an antihypertensive drug. This compound was also administered to dystrophic animals, resulting in a reduction in tropomyosin oxidation and an improvement in disease phenotype [21]. Nevertheless, Pargyline's clinical use has been halted due to its considerable adverse effects [22].

Safinamide is a potent and specific inhibitor of MAO-B, which is approved for the treatment of mid to late-stage fluctuating Parkinson's disease [23]. Since intracellular signaling requires a small amount of ROS, the specificity of MAO inhibition is a crucial element [24]. In 2018, Vitiello and colleagues analyzed the impact of safinamide on the skeletal muscle of *mdx* mice and cultured muscle cells obtained from DMD patients. Even after a brief (1 week) course of therapy, reducing MAO-B had a beneficial impact *in vivo*, indicating a mechanism lacking significant tissue remodeling. The reduction of reactive oxygen species (ROS) levels in the fibers of treated animals and the oxidative status of a critical component of the contractile apparatus (tropomyosin) has been confirmed by analysis of muscle sections taken from animals that were given safinamide. Given that *in vitro* cultures, the dystrophin gene is expressed in myotubes but not in myoblasts, the in vitro experiments showed that increased susceptibility to oxidative stress in dystrophic cells appeared to be independent of dystrophin expression [24].

The observation that MAO catalyzes catecholamine removal proves the potential benefit of MAO treatment in DMD [21]. Chronic diseases can be associated with an increased level of catecholamines and DMD patients make no exception to this rule, as an excess of urine catecholamine has been documented in connection to age and disease progression [25]. All of this information, along with the benefit of using MAO-B inhibitors to prevent the hypertensive crisis that MAO-A inhibitors might cause, makes safinamide a therapy with a safe profile capable of increasing muscle performance [24].

2.3 The selective estrogen receptor modulator

Tamoxifen, a selective estrogen receptor modulator, has been commonly used to treat breast cancer for decades [26]. Estrogen receptor alpha (ER), the target via

which tamoxifen operates, is present in both skeletal and cardiac muscle [27, 28]. Numerous studies have shown that tamoxifen protects against contraction-induced membrane damage, controls calcium influx, reduces oxidative stress, and prevents fibrosis [29–32], which is why it was hypothesized that DMD patients could benefit from this drug. In 2013, Dorchies and colleagues published an interesting study that assessed the effect of tamoxifen on dystrophic muscle structure and function [33]. Compared to previous research on normal rodents, the effects observed in the current study were attained with tissue levels of TAM and its primary metabolites that were significantly smaller. A daily dose of 10 mg/kg/day delivered to *mdx*^{5Cv} mice for 15 months enhanced whole-body force, causing a change toward a slower phenotype [33].

The main DMD charity in the UK published a statement in July 2022 on preliminary data from the tamoxifen-DMD clinical case–control trial. The collaborating parties were disappointed to conclude that although patients receiving tamoxifen showed less disease progression, the differences between the tamoxifen and placebo group did not reach statistical significance [34]. For more DMD clinical trials please refer to **Table 1**.

2.4 Dantrolene—Then and now

Dantrolene sodium is a postsynaptic muscle relaxant that inhibits calcium release from the sarcoplasmic reticulum, reducing excitation-contraction coupling in muscle cells [35]. Since 1991, researchers have examined how this compound affects DMD, finding that it significantly lowers CK levels during the first year of treatment compared to age-matched historical controls [35]. Dantrolene is the agent of choice for treating and preventing malignant hyperthermia, a condition triggered by general anesthesia [36].

Exon skipping is a novel therapy that employs an antisense oligonucleotide (ASOs) customized to the patient's DNA mutation to target particular exons for exclusion from mRNA. As a result, the out-of-frame DMD mutation is converted to in-frame deletions, which might result in a partly functioning dystrophin protein [37]. Although 30% of patients may benefit from the existing exon skipping, most of the research on these therapies has focused on low-level dystrophin restoration (less than 6%) [38]. Due to differences in increased muscular function, across clinical studies small molecules were used to augment the effect of exon skipping. Dantolene's safety profile is already known due to its use in patients with DMD and malignant hyperthermia. This information, in conjunction with the fact that this drug reduced CK levels in both *mdx* mice and humans, raised the question of whether dantrolene can modulate exon skipping [35, 39]. Kendall and colleagues tried to answer this question by administering ASOs and dantrolene to *mdx* mice. The authors observed that DMD-directed ASOs and dantrolene cooperate to enhance targeted DMD exon skipping probably by interacting with specific molecular targets that subtly influence splicing activity. A further benefit of dantrolene is that it is effective independent of the specific ASO sequence, as seen by the enhancement of exon skipping activity for human exons 50 and 51 and mouse exon 23 [37]. In 2019, the group conducted by Berthelemy looked into the effects of dantrolene on skipping exons 44 and 45 in cultured myotubes from DMD patients' inducible directly reprogrammable myotubes (iDRMs) and induced pluripotent stem cells (iPSCs) [40]. In both exon 44 and 45 skip-amendable DMD cell models, the administration of dantrolene with the suitable ASOs raises the level of skipped mRNA compared with ASO alone. In patient-derived

Compound	Group	Original indication	Preclinical studies	Clinical trials
[Tamoxifen]	estrogen receptor modulator	breast cancer	10.1093/hmg/ddn151 10.1093/hmg/ddy258 10.1016/j.nmd.2021.09.003 10.1038/s41536-022-00214-x 10.1016/j.ajpath.2012.10.018 10.1085/jgp.202213081 10.1038/s41598-020-67,372-0 10.1089/scd.2016.0136 10.1038/mtm.2014.25 10.1371/journal.pone.0016184	NCT02835079 NCT03354039
Statins	HMG-CoA reductase inhibitor	reduce blood levels of low-density lipoprotein (LDL) cholesterol	10.3390/ijms23042016 10.1186/s13395-021-00273-3 10.3233/JND-200524 10.1038/s41467-020-15,971-w 10.14814/phy2.14018 10.1073/pnas.1509536112	—
Pargyline	Monoamine oxidase inhibitors	moderate to severe hypertension	10.1093/hmg/ddq339 10.1016/j.freeradbiomed.2014.07.006 10.3389/fphys.2018.01087 10.1038/nrn1883	—
Safinamide		Parkinson's disease		
Dantrolene sodium	postsynaptic muscle relaxant	malignant hyperthermia	10.1016/j.xcrm.2021.100298 10.1016/j.omtn.2019.09.020 10.1016/j.omnt.2018.02.002 10.1007/978-1-4939-8651-4_19 10.1126/scitranslmed.3005054	—
Metformin	biguanide	type 2 diabetes mellitus	10.15252/embr.202153955 10.1080/21655979.2021.1967029 10.3389/fphys.2021.642908 10.3389/fcell.2020.609493 10.1016/j.bcp.2018.04.022 10.1002/mus.24692	NCT02516085 NCT01995032
Tranilast			10.1186/1755-1536-7-1	10.1186/s13023-022-02352-3* 10.2169/internalmedicine.8651-16**

Compound	Group	Original indication	Preclinical studies	Clinical trials
Sildenafil	phosphodiesterase type 5 inhibitors	erectile dysfunction	10.1152/japplphysiol.00664.2018 10.1096/fj.201700249R 10.1093/hmg/ddt579 10.1093/hmg/dds415 10.1002/path.4054 10.1152/ajpheart.00522.2010 10.1073/pnas.1013077107	NCT01580501 NCT01168908 NCT01359670
Tadalafil	phosphodiesterase type 5 inhibitors	erectile dysfunction	10.1161/JAHA.116.003911 10.1002/jcp.25075 10.1371/journal.pone.0000806	NCT01580501 NCT05195775 NCT01359670 NCT01865084 10.1371/journal.pone.0232870 10.1002/mus.26736
Nintedanib	kinase inhibitor	idiopathic pulmonary fibrosis	10.1038/s41419-018-0792-6	—
Sunitinib	kinase inhibitor	gastrointestinal stromal tumor renal cell carcinoma well-differentiated pancreatic neuroendocrine tumors	10.1093/hmg/ddac042 10.1093/hmg/ddz044	—
Zidovudine	antiretroviral	HIV/AIDS	10.1186/s40478-018-0530-4	—
Streptomycin	aminoglycoside	bacterial infections	10.1152/ajpcell.000056.2011 10.1016/j.ajpath.2010.11.027 10.1371/journal.pone.00003644 10.1152/ajpheart.00688.2006 10.1016/j.nmd.2006.07.024 10.1113/jphysiol.2004.075275	—

Compound	Group	Original indication	Preclinical studies	Clinical trials
Gentamicin	aminoglycoside	bacterial infections	10.1073/pnas.2122004119 10.1093/nar/gkab194 10.1093/hmg/dds223 10.1248/bpb.34.712 10.1016/j.ejphar.2009.11.034 10.1111/j.1582-4934.2009.00718.x 10.1096/fj.08-115,618 10.1016/j.nbd.2008.07.009	NCT00451074 NCT00005574

**The study included DMD, BMD, and LGMD patients.*
***Pilot study.*

Table 1.
Examples of repurposed drugs for muscular dystrophies.

iDRM DMD myotube culture, dantrolene increases exon 44 skippings. Even though the improvement is small, it is still important because research shows that even small amounts of dystrophin can affect muscles [40].

2.5 Repositioning cardiological drugs

Dilated cardiomyopathy (DCM) is a severe consequence of DMD and one of the most important predictors of life expectancy [24]. In the context of this causality, it might be argued that therapy for the DCM serves as "repositioning." In DMD patients, **angiotensin-converting enzyme inhibitors** (ACEi) and **ß-blockers** (BBs) (and less often diuretics) are recommended for the reduction of peripheral circulatory resistance, blood volume, hyperadrenergic activation and oxygen consumption [24, 41, 42]. In addition, it is well known that BBs lower the incidence of potentially life-threatening arrhythmias initiated by foci of myocardial fibrosis [42]. Tamoxifen is another medication that has been demonstrated to impact the heart muscle significantly. The group conducted by Dorchies revealed that tamoxifen reduced cardiac fibrosis by 50% and positively impacted the diaphragm. As a result, there was a significant quantity of contractile tissue made accessible for respiratory function in the *mdx*^{5Cv} mouse model [33]. The question of whether or not these treatments qualify as repositioning drugs is less significant when viewed in the context of the fact that cardiovascular and respiratory disorders are unavoidable complications of DMD. It is for this reason that we have devoted a brief subchapter to discussing them.

Calcium is essential for muscular function, but its intracellular accumulation is toxic, triggering apoptosis. As the literature provides extensive research on the function of calcium in muscle injury, it is fair to speculate that calcium antagonists may be useful in DMD [43].

A Cochrane database of systematic reviews investigating the impact of calcium antagonists on muscular power and function in DMD was published by Phillips and Quinlivan in 2008 [44]. The authors conclude that although *verapamil* significantly increased muscular strength, it also caused several cardiac adverse effects [45]. Additionally, no significant differences in efficacy between *diltiazem, nifedipine*, and *flunarizine* were found in the remaining studies [44]. In 2009, Matsumura and colleagues published an interesting study that evaluated the effect of verapamil and diltiazem in *mdx* mice [46]. The dystrophic phenotype of *mdx* mice was improved as shown by a lower serum CK level and reduced muscle deterioration in the diaphragm. Moreover, between the two calcium antagonists, diltiazem seems to protect against muscle degeneration more effectively [46].

Nifedipine, another calcium channel blocker, was the main focus of a 2013 study by Altamirano [47]. The results add to the growing body of data indicating that the calcium level is high in *mdx* muscles and may be regulated by nifedipine. On *mdx* mice, this treatment resulted in a reduction in the basal ATP release from dystrophic fibers and a decreased prooxidative/apoptotic gene expression. Consequently, a reduced muscle injury was observed in *mdx* mice, as shown by a substantial decrease in serum CK and an improvement in muscular strength [47].

2.6 Metformin: A pleiotropic drug

As monotherapy or in combination with other medications, *metformin,* a biguanide, is now one of the most often prescribed drugs in the world for the treatment of type 2 diabetes (T2D) [48]. Metformin has a wide range of molecular mechanisms of action,

which explain why it may be used to treat autoimmune illnesses, prevent cancer, and protect the cardiovascular system [49–51]. Pharmacological actions of metformin include a decrease of glucose and lipid production via inhibition of mitochondrial complex I (NADH: ubiquinone oxidoreductase) and activation of AMP-activated protein kinase (AMPK) [52]. In this context, it was natural for researchers and clinicians to be drawn to the possible use of this medication in MDs.

Metformin's potential to improve muscular fibrosis and strength was shown in mdx mice via non-AMPK-related pathways [53]. Another preclinical study led by Lai and colleagues hypothesized that metformin could downregulate different chemokines in MD mouse models [54]. Thus, in mdx mice, levels of CXCL12 (C–X–C motif chemokine ligand 12) both a glucocorticoid target and a differentially expressed gene and its receptor CXCR4 (C–X–C motif chemokine receptor 4) were increased. As a result of prednisone therapy, their concentration decreased considerably. Furthermore, CXCL12 and CXCR4 expression was similarly shown to be reduced in mdx mice after treatment with metformin, suggesting that this pathway may be an attractive therapeutic target for DMD [54].

As previously discussed, ASOs-mediated exon-skipping is a promising line of treatment for DMD patients. However, a significant obstacle to its therapeutic use is the poor systemic effectiveness, necessitating drugs that enhance ASOs' activity. Based on previous studies that demonstrated an improvement of phosphorodiamidate morpholino oligomer (PMO) delivery to peripheral muscle in mdx mice by intravenous administration of glycine, the group conducted by Lin analyzed the effect of oral glycine and metformin alongside PMO in dystrophin/utrophin double knock-out (DKO) mice [55]. Thus, without any toxicity that could be detected and with a life span extension, the scientists demonstrated improvements in the cardio-respiratory and skeletal systems and a phenotypic rescue in DKO mice [55].

Though widely administered in the T2D adult population it is important to mention that metformin has been tested in children and adolescents with neurogenic defects and muscle disorders [56]. In 2010, Casteels and colleagues reported that metformin is an insulin sensitizer capable of limiting weight gain and visceral adiposity in children with a neurogenic or myogenic motor deficit [56]. Thus, exploring the use of metformin as an additional therapy in a variety of illnesses has reached the clinical context of DMD. In 2019, Hafner and colleagues published the results of a randomized double-blind placebo-controlled parallel-group clinical trial that included 47 ambulant male children aged 6.5 to 10 years with DMD that received treatment with a combination of l-citrulline and metformin. Among ambulant patients with DMD, the co-treatment was not associated with an overall halting of the decline in motor function, although the stable subgroup of patients presented a decrease in motor function impairment [57]. Also, Metformin or L-citrulline supplementation in BMD patients results in notable antidromic changes in the arginine glycine amidinotransferase and guanidinoacetate methyltransferase pathways.

Metformin treatment has also been shown to be useful in congenital muscular dystrophy type 1A [58]. Metformin therapy promotes weight growth and energy efficiency, improves muscular function, and improves skeletal muscle histology in female dy2J/dy2J mice (but not in male dy2J/dy2J mice) [58]. Metformin also improved the mobility and walking abilities of people with myotonic dystrophy [59]. Metformin has also been shown to increase autophagy and provide cardioprotection in a mouse model deficient in δ-sarcoglycan, a protein encoded by the SGCD gene and associated with LGMD R6 (LGMD2F) [60].

2.7 Tranilast: From allergies to Duchenne cardiomyopathy

The life expectancy of DMD patients has improved recently due to advancements in the prevention of respiratory complications, however, there has been a notable increase in advanced cardiomyopathy symptoms [61]. To eventually cure DMD, gene replacement or other correction therapy must also approach the existence of fibrosis [62]. Furthermore, this concept favors DMD patients who develop DCM, marked by inflammation, fibrosis, and necrosis [63]. A compound with anti-fibrotic properties is *tranilast*, an anti-allergic agent and a calcium channel blocker prescribed for over 30 years to adults and children [64]. In mdx mice, improving muscle pathology and motor performance with Ca-handling drugs prevented aberrant intracellular Ca influx through the transient receptor potential cation channel, subfamily V 2 (TRPV2) [65]. The results of a single-arm, open-label, multicenter study on the safety and efficacy of tranilast for heart failure in patients with MDs—of whom the vast majority were DMD patients—were published in 2022 by Matsumura and colleagues [66]. Although there was no significant improvement in cardiac function, the authors concluded that tranilast was safe and effective in inhibiting TRPV2 expression and may represent a viable medication for patients with early heart failure [66].

Even in MD patients with advanced heart failure, tranilast is safe and effective at inhibiting TRPV2 expression.

2.8 Phosphodiesterase type 5 inhibitors

Increased muscle damage and irregular blood flow after muscle contraction—the condition known as functional ischemia—are hallmarks of DMD [67]. Thus, multiple pathways have been studied in the past decades to alleviate the ischemic picture in MDs. A potential novel therapeutic target for DMD is the nitric oxide (NO) - cyclic guanosine monophosphate (cGMP) pathway. *Phosphodiesterase type 5 (PDE5) inhibitors*, which extend the half-life of cGMP, have been shown to improve the function of the limb, respiratory, and cardiac muscles in mdx mice and to increase the lifespan of dystrophin-deficient zebrafish [68–70]. Since their major indication is erectile dysfunction, PDE5 can be considered as repurposed therapy in MDs. There are four major types of PDE5 inhibitors approved by the FDA [71] of which *sildenafil* and *tadalafil* were the most studied outside of their intended use. According to a 2012 study by Percival and colleagues, sildenafil administration for 14 weeks decreased diaphragm muscle weakness and supported normal extracellular matrix organization in mdx animals [72]. However, a placebo-controlled phase II trial (REVERSE DBMD; [73] including patients with Duchenne and Becker MD was terminated earlier due to worsening cardiomyopathy in some of the patients who received sildenafil [72]. As a PDE inhibitor tadalafil is more selective for PDE5 than sildenafil [74], which is why its evaluation advanced to a randomized, placebo-controlled phase III trial [73] that enrolled 331 patients. Nevertheless, tadalafil did not demonstrate efficacy in reducing the decline of walking ability in the treated group [75].

2.9 A new perspective on antibiotics

Since the early 1990s, it has been recognized that some antibiotics may reduce premature termination codons in eukaryotic cells [76]. In animal studies, the antibiotic family known as aminoglycosides has been shown to prevent nonsense mutations [24]. Thus, it was only a matter of time before researchers used this compound in

MDs. Barton-Davis and colleagues reported in 1999 that subcutaneous injections of gentamicin restored dystrophin levels in the skeletal muscles of mdx mice, demonstrating the potential of this family of antibiotics for the first time in *in vivo* [77].

Stretch-activated channels (SACs) are another pathway that has been proposed as being relevant in the pathophysiology of DMD [78]. These channels are permeable to Na^+ and Ca^2 and respond to mechanical stress [79, 80]. Lack of dystrophin increases skeletal muscle SAC activity in mdx mice [81–83]. Therefore, in muscles exhibiting various degrees of the dystrophic phenotype, researchers looked at the transient receptor potential canonical channel 1 (TRPC1) level and the effects of streptomycin, a SAC blocker [78]. In diaphragm and sternomastoid muscles, streptomycin decreased creatine kinase and reduced exercise-induced increases in total calcium and Evans blue dye absorption (a sensitive and early marker of myofiber injury) [78]. However, it must be taken into account when using aminoglycosides, that these drugs present various levels of oto- and/or nephrotoxicity [84, 85].

In 2003, the group led by Politano published the results of a small study on four patients with DMD. Immunohistochemistry and immunoblotting showed that dystrophin re-expression occurred in muscle biopsies performed after gentamicin treatment in three out of four patients that presented a more permissive UGA stop codon [86]. Seven years later, Malik and colleagues published the results of a multicenter trial that evaluated the safety of gentamicin infusions twice a week for 6 months [87]. Except for one patient who received an incorrectly calculated dosage, no patient showed a decreased renal or hearing function. The study's second goal was to see whether gentamicin improved muscular strength and increased dystrophin binding at the muscle membrane. Dystrophin levels significantly improved ($p = 0.027$) after 6 months of gentamicin treatment, and this was associated with a decrease in serum CK [88].

2.10 Tyrosine kinase inhibitors

Muscle degeneration and poor muscle regeneration brought on by a lack of dystrophin are major characteristics of DMD pathophysiology [89]. Several variables, including a reduction in satellite cell (SC) capacity (the critical precursors to myogenesis), have been linked to this defective regeneration [90]. SC proliferation and self-renewal in response to resistance training and muscle damage depend on the interleukin-6 (IL-6) cytokine's activation of the signal transducer and activator of transcription 3 (STAT3) [91–93]. Sunitinib is a multi-targeted tyrosine kinase inhibitor and a therapeutic option for treating renal cell carcinoma, gastrointestinal stromal tumors, and progressive, well-differentiated, advanced panNETs [24, 94]. Sunitinib was evaluated for its efficacy in the mdx mice model of DMD, in which it demonstrated the ability to induce muscle regeneration via transient STAT3 activation [90]. Furthermore, in 2022, Oliveira-Santos and colleagues published the results of an interesting study that evaluated how long-term sunitinib use affected heart pathology and function in mdx mouse model. The authors concluded that sunitinib increased cardiac electrical performance and reduced ventricular hypertrophy, cardiomyocyte membrane damage, and fibrotic tissue deposition in the heart muscle of mdx animals via lowered STAT3 phosphorylation [95].

Nintedanib is also a tyrosine kinase inhibitor approved for treating idiopathic pulmonary fibrosis (IPF) [96].

Previous studies on primary lung fibroblasts and dermal fibroblasts from patients with IPF and systemic sclerosis have demonstrated the anti-fibrotic activity of nintedanib, thus rendering this compound a potential aid in DMD [97, 98]. A one-month

course of nintedanib therapy in mdx mice reduced skeletal muscle fibrosis and improved muscle function, thus reinforcing the idea that tyrosine kinase inhibitors could have a potential role for clinical exploration in DMD [99].

3. Conclusions

Recent scientific breakthroughs have enhanced diagnostic skills and permitted experimental research into gene therapy and standard pharmacological therapies, eventually leading to successful treatments for many inherited disorders.

Among them, muscular dystrophies and genetic diseases caused by mutations in over 40 genes result in dystrophic alterations on muscle biopsy and cause progressive weakness and degeneration of skeletal muscles. Due to advances in molecular biology techniques and an understanding of the mechanisms underlying these diseases, the genetic defect of most muscular dystrophies can now be precisely determined. Also, specialized therapy to help patients can be provided for some types of dystrophies, while many others are in the advanced clinical development stage. Although advances in the management and care of people with these disorders have slowed disease progression, more research is needed because the patients still face a lack of effective treatments. As a result, new forms of therapies are required. Repurposing existing intensively studied drugs with well-known pharmacokinetic, pharmacodynamic, tolerability, and safety profiles holds promise for timely effective therapies for patients suffering from life-threatening conditions who cannot wait for a traditional drug development cycle. Quickly redirecting these drugs to other directions can improve life expectancy and quality of life in affected patients. The use of this path is accelerated by incentives, guidance, and protection provided by holding an orphan drug designation status. Drug repurposing is a valuable strategy with enormous potential for delivering long-awaited therapies that may be safeguarded by orphan drug designation status, enabling successful, fair-priced commercialization.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Ioana Lambrescu[1,2], Emilia Manole[1,3], Laura Cristina Ceafalan[1,2] and Gisela Gaina[1*]

1 Cell Biology, Neurosciences and Experimental Myology Laboratory, Victor Babes Institute of Pathology, Bucharest, Romania

2 Department of Cellular and Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

3 Pathology Department, Colentina Clinical Hospital, Bucharest, Romania

*Address all correspondence to: gisela.gaina@ivb.ro

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