



**HAL**  
open science

## Update on anti-fibrotic pharmacotherapies in skeletal muscle disease

Laura Muraine, Mona Bensalah, Gillian Butler-Browne, Anne Bigot,  
Capucine Trollet, Vincent Mouly, Elisa Negrone

► **To cite this version:**

Laura Muraine, Mona Bensalah, Gillian Butler-Browne, Anne Bigot, Capucine Trollet, et al.. Update on anti-fibrotic pharmacotherapies in skeletal muscle disease. *Current Opinion in Pharmacology*, 2022, 68, pp.102332. 10.1016/j.coph.2022.102332 . hal-03997340

**HAL Id: hal-03997340**

**<https://hal.science/hal-03997340>**

Submitted on 20 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Update on anti-fibrotic pharmacotherapies in skeletal muscle disease

Laura Muraine, Mona Bensalah, Gillian Butler-Browne, Anne Bigot, Capucine Trollet, Vincent Mouly and Elisa Negroni

## Abstract

Fibrosis, defined as an excessive accumulation of extracellular matrix, is the end point of a defective regenerative process, unresolved inflammation and/or chronic damage. Numerous muscle disorders (MD) are characterized by high levels of fibrosis associated with muscle wasting and weakness. Fibrosis alters muscle homeostasis/regeneration and fiber environment and may interfere with gene and cell therapies. Slowing down or reversing fibrosis is a crucial therapeutic goal to maintain muscle identity in the context of therapies. Several pathways are implicated in the modulation of the fibrotic progression and multiple therapeutic compounds targeting fibrogenic signals have been tested in MDs, mostly in the context of Duchenne Muscular Dystrophy. In this review, we present an up-to-date overview of pharmacotherapies that have been tested to reduce fibrosis in the skeletal muscle.

## Addresses

Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, Paris, France

Corresponding authors: Mouly, Vincent ([vincent.mouly@upmc.fr](mailto:vincent.mouly@upmc.fr)); Negroni, Elisa ([elisa.negroni@upmc.fr](mailto:elisa.negroni@upmc.fr))

Current Opinion in Pharmacology 2023, 68:102332

This review comes from a themed issue on **Musculoskeletal**

Edited by **Doriana Sandona** and **Vincenzo Sorrentino**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online xxx

<https://doi.org/10.1016/j.coph.2022.102332>

1471-4892/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## Introduction

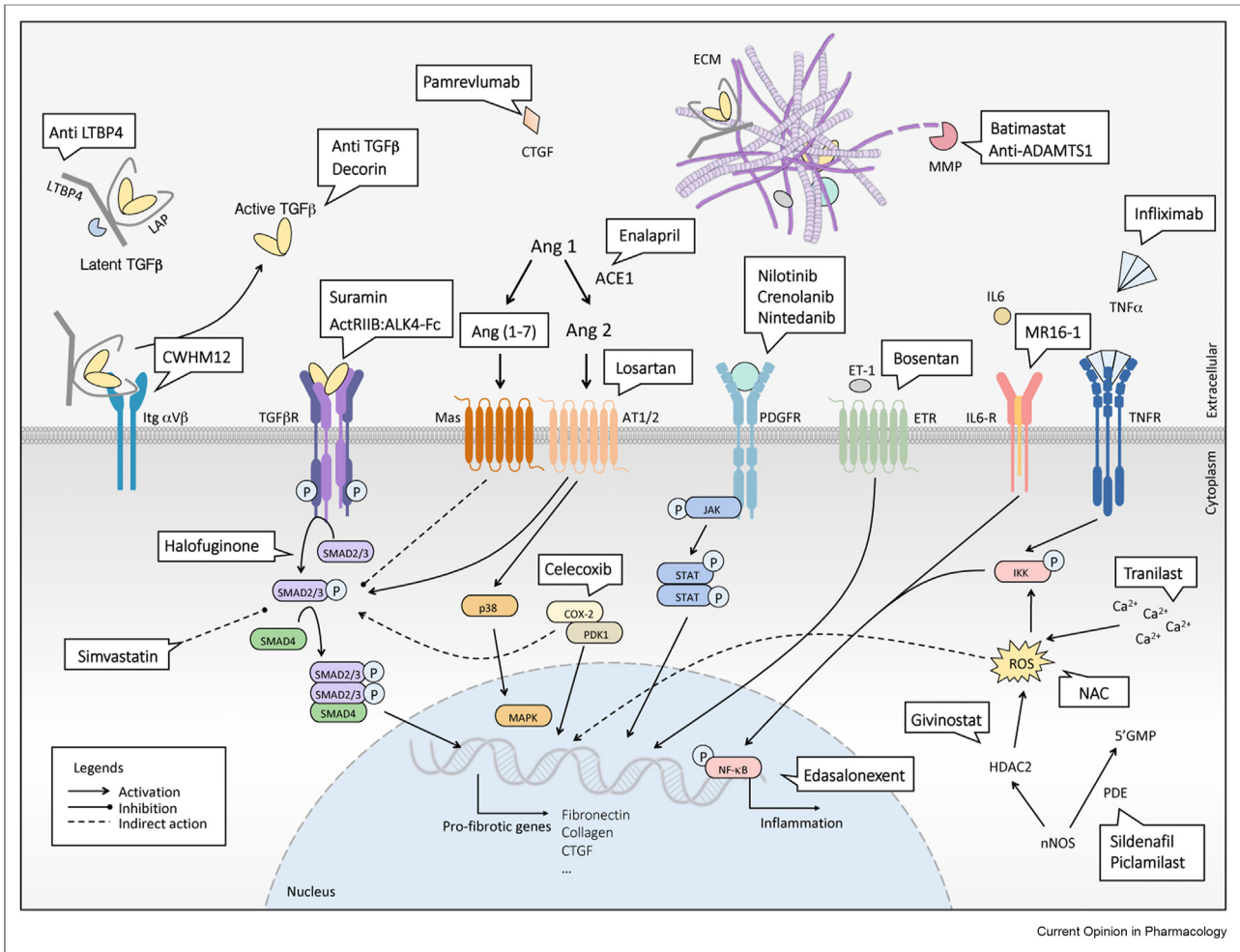
Fibrosis, defined as an excessive accumulation of extracellular matrix (ECM) components such as collagens, proteoglycans or fibronectin has been described in several organs, such as liver, lung, skin, heart as well as in skeletal muscle [1]. In damaged or diseased skeletal muscle, the orchestration of several cell populations is required to restore muscle homeostasis [2]. This orchestration

involves a sequence of events: a phase of inflammation that enables the removal of damaged fibers, followed by the activation and differentiation of the satellite cells (muscle resident stem cells) and the remodeling of the ECM by fibroblast-like cells also called fibro-adipogenic progenitors (FAPs) [3]. Since their discovery, FAPs have been considered as an essential regulator of muscle regeneration and one of the key actors in fibrosis [4]. In skeletal muscle, fibrosis modifies the whole environment, creating an imbalance in both the cell populations involved and in the availability of soluble factors within the matrix, which in turn impacts muscle homeostasis and regeneration, and affects the muscle stem cell niche [5]. Several muscle disorders (MD) are characterized by high levels of fibrosis associated with muscle wasting and weakness, and among them, Duchenne muscular dystrophy (DMD) has been extensively studied [6,7]. Numerous actors of fibrosis have been identified including transforming growth factor  $\beta$  (TGF $\beta$ ), connective tissue growth factor (CTGF), matrix metalloproteinases (MMPs), abnormal level of intracellular calcium associated with aberrant activation of the Renin angiotensin system (RAS) [8], oxidative stress and inflammation [1,9]. The multifactorial causes of muscle fibrosis and the numerous cell types potentially involved make it a complex process to target. However, a growing number of pharmacological agents have been tested in both preclinical and clinical trials and have shown evidence that targeting fibrosis can ameliorate muscle function. Here, we propose an up-to-date review on the pharmacological anti-fibrotic strategies developed for skeletal muscle at both preclinical and clinical stages.

## Targeting TGF $\beta$ or downstream effectors

The TGF $\beta$  pathway has been established as a key player in the development of fibrosis in almost all tissues [10] and the overexpression of TGF $\beta$ 1 in skeletal muscle is a hallmark of many MDs [11]. TGF $\beta$  is produced (mainly by macrophages and FAPs within muscle) in its latent form and is kept inactive by binding to latent TGF $\beta$  binding proteins (LTBPs) (Figure 1). TGF $\beta$  binds to TGF $\beta$ R1 and operates through the phosphorylation of Smad2/3. The further recruitment of Smad4 allows the complex to translocate to the nucleus and regulate the transcription of fibrogenic factors, such as collagen I,

Figure 1



**Key signaling pathways regulating fibrosis in the skeletal muscle.** In speech, bubbles are mentioned molecules with anti-fibrotic potential. TGFβ, Transforming growth factor beta; CTGF, connective tissue growth factor; LTBP4, latent TGFβ-binding protein 4; LAP, latency-associated peptide; TGFβR, TGFβ receptor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; ET-1, endothelin 1; ETR, endothelin receptor; TNFα, tumor necrosis factor α; TNFR, tumor necrosis factor receptor; ECM, extracellular matrix; MMP, matrix metalloproteinase; Ang (1–7), angiotensin 1–7; Ang 1, angiotensin 1; Ang 2, angiotensin 2; AT1/2, angiotensin 2 type 1/2 receptor; ACE1, angiotensin-converting enzyme 1; ROS, reactive oxygen species; NAC, N acetyl cysteine; HDAC2, histone deacetylase 2; nNOS, nitric oxide synthase; PDE, phosphodiesterase; itg, integrin; NFκB, nuclear factor-kappa B; IL6, interleukin 6; IL6-R, interleukin 6 receptor.

fibronectin, alpha-smooth muscle actin and CTGF [10] (Figure 1). The dysregulation of the TGFβ pathway in muscular disorders leads to the activation of FAPs and an excessive ECM deposition. Several antifibrotic strategies target directly TGFβ. For example, decorin -a small extracellular proteoglycan that interacts with TGFβ and ECM proteins- or anti-TGFβ antibodies, modulate its activity [12,13] (Table 1). Both approaches have been shown to reduce fibrosis in the diaphragm of the mdx mouse model of DMD [14,15]. Other strategies targeting TGFβR1 with the antagonist suramin demonstrated an attenuation of fibrosis in the mdx diaphragm and limb muscles [16,17]. However, TGFβR1 activation triggers several other non-canonical

pathways involved in many different biological processes [18] including regulation of the immune system. The TGFβ pathway has also been shown to be implicated in regulating the pace of fusion of myoblasts in regenerating muscles [19]. Indeed, given the central role of TGFβ and the broad action on many cell types, its direct targeting is unlikely to be a viable therapeutic strategy [20].

Strategies targeting downstream effectors of the TGFβ pathway ensure a more selective effect and avoid off-target effects. For example, halofuginone, that inhibits Smad3 phosphorylation, preventing its entrance into the nucleus, reduced the increase of fibrosis in the diaphragm

Table 1

## Overview of studies using pharmacotherapies to target fibrosis in skeletal muscle.

Compound	CT (DMD)	FDA approved	Mechanism of action	Model	Pathology	Effect	Ref
<b>TGF<math>\beta</math> pathway modulators</b>							
Decorin			TGF $\beta$ 1 ligand binder	mdx mice	DMD	Decreased collagen I levels	(Gosselin <i>et al.</i> , 2004)
Anti-TGF $\beta$ antibody			TGF $\beta$ 1 ligand binder	mdx mice	DMD	Decreased total and endomysial connective tissue	(Andreetta <i>et al.</i> , 2006)
Suramin		x	TGF $\beta$ 1 receptor antagonist	mdx mice	DMD	Decreased fibrosis in skeletal muscle but not heart, decreased CK levels	(Taniguti <i>et al.</i> , 2011)
				mdx mice	DMD	Decreased cardiac and skeletal muscle fibrosis	(De Oliveira Moreira <i>et al.</i> , 2013)
Halofuginone (HT-100)	x		Smad3 phosphorylation inhibitor	mdx mice	DMD	Decreased collagen levels	(Turgeman <i>et al.</i> , 2008)
				mdx mice	DMD	Decreased fibrosis and increased fiber diameter	(Mordechay <i>et al.</i> , 2021)
				dysf $-/-$ mice	Dysferlinopathy	Decreased fibrosis and p Smad3 levels	(Barzilai-Tutsch <i>et al.</i> , 2020)
ActRIIB:ALK4Fc			TGF $\beta$ receptor inhibitor	Adult and aged mdx mice	DMD	Decreased fibrosis, improved PMO treatment	(Li <i>et al.</i> , 2021)
LTBP4 antibody			LTBP4 inhibitor	mdx mice	DMD	Decreased fibrosis, improved respiratory functions	(Demonbreun <i>et al.</i> , 2021)
CWHM12			$\alpha_v$ integrin inhibitor	Adult mice	muscle injury	Decreased cardiac and skeletal muscle fibrosis	(Murray <i>et al.</i> , 2017)
<b>CTGF inhibitor</b>							
Pamrevlumab (FG-3019)	x	x	CTGF inhibitor	mdx mice	DMD	Decreased fibrosis and CK levels, improved satellite cells engraftment	(Morales <i>et al.</i> , 2013)
				Rat + exercise	muscle injury	Decreased fibrosis, TGF $\beta$ and macrophages, improvement of mean voluntary grasp force	(Barbe <i>et al.</i> , 2019)
				Rat + exercise	muscle injury	Reduction of established fibrosis, improved motor function	(Barbe <i>et al.</i> , 2020)
				hSOD1 <sup>G93A</sup> mice	ALS	Decreased fibronectin expression, improved locomotor function and NMJ	(Gonzalez <i>et al.</i> , 2018)

(continued on next page)

Table 1 (continued)

Compound	CT (DMD)	FDA approved	Mechanism of action	Model	Pathology	Effect	Ref
<b>ECM remodeling blockers</b>							
Batimastat (BB-94)			MMPs inhibitor	mdx mice	DMD	Reduction of fibrosis and centronucleated fibers	(Kumar et al., 2010)
				Dysf <sup>pmmd</sup> mice/ murine FAP cells	LGMD2B	Reduction of adipogenic replacement, no effect on fibrosis and FAPs proliferation	(Hogarth et al., 2019)
ADAMTS1 antibodies			ADAMTS1 inhibitor	mdx mice	DMD	Decreased fibrosis, improved muscle strength	(Wang et al., 2021)
<b>Renin-Angiotensin pathway modulators</b>							
Losartan	x	x	AT1 inhibitor	mdx mice	DMD	Decreased cardiac fibrosis but not skeletal muscle	(Spurney et al., 2011)
				mdx mice	DMD	Decreased collagen and fibronectin deposition	(Cabello-Verrugio et al., 2012)
				Dysf <sup>fm1Kcam</sup> mice	LGMD2B	Increased fat and fibrosis, exacerbated muscle wasting and damage	(White et al., 2019)
Enalapril	x	x	ACE inhibitor	mdx mice + exercise	DMD	Decreased fibrosis, improved muscle strength	(Morales et al., 2013) bis
Angiotensin 1-7				mdx mice rat + exercise	DMD Muscle injury	Decreased fibrosis Reduction of collagen levels	(Acuña et al., 2014) (Totou et al., 2021)
				CD-1 mice+ radiation	Radiation-induced fibrosis	Decreased interstitial and perivascular fibrosis, decreased levels of TGFβ and CTGF	(Willey et al., 2016)
<b>Tyrosine kinase blockers</b>							
Nilotinib		x	Tyrosine kinase inhibitor	Adult mice	DMD	Decreased FAPs survival and collagen deposition	(Lemos et al., 2015)
				murine primary myoblasts		Increased myoblasts proliferation, impairment of myogenesis	(Contreras et al., 2018)
Crenolanib			Tyrosine kinase inhibitor	mdx mice	DMD	Decreased fibrosis in skeletal muscle and heart, enhanced regeneration	(Ieronimakis et al., 2016)
Nintedanib		x	Tyrosine kinase inhibitor	mdx mice/ human fibroblasts	DMD	Decreased fibroblasts proliferation and secretion, decreased fibrosis in mdx mice	(Piñol-Jurado et al., 2018)

Table 1 (continued)

Compound	CT (DMD)	FDA approved	Mechanism of action	Model	Pathology	Effect	Ref
<b>Inflammation modulators</b>							
Infliximab (Remicade)		x	TNF $\alpha$ inhibitor	mdx mice	DMD	Decreased collagen I levels after low dose long term treatment but cardiotoxicity	(Ermolova <i>et al.</i> , 2014)
Edasalonexent (CAT1004)	x	x	NF $\kappa$ B inhibitor	mdx mice/ GRMD dogs	DMD	Decreased fibrosis	(Hammers <i>et al.</i> , 2016)
IL-6R antibody (MR16-1)		x	Interleukin-6 receptor antibody	dKO mice	DMD	Reduced fibrosis in quadriceps, no effect on cardiac muscle	(Wada <i>et al.</i> , 2017)
PKC $\theta$ inhibitor (C20)			Protein kinase $\theta$ inhibitor	mdx mice	DMD	Decreased fibrosis in the diaphragm and inflammatory infiltrates	(Lozanoska-Ochser <i>et al.</i> , 2018)
N acetyl cysteine (NAC)		x	Antioxydant	mdx mice	DMD	Decreased fibrosis and inflammatory infiltrates	(Burns <i>et al.</i> , 2019)
<b>Other molecules</b>							
Celecoxib		x	COX2 inhibitor	Adult mice	Muscle injury	Decreased fibrosis, inhibition of myofibroblasts differentiation	(Chen <i>et al.</i> , 2021)
Simvastatin		x	HMG Coa reductase inhibitor	mdx mice	DMD	Decreased fibrosis and inflammation in aged mice	(Whitehead <i>et al.</i> , 2015)
	mdx mice			DMD	Prevention of cardiac fibrosis after long-term treatment	(Kim <i>et al.</i> , 2019)	
	mdx mice			DMD	Failed to decrease fibrosis or inflammation	(Verhaart <i>et al.</i> , 2021)	
Sildenafil	x	x	Phosphodiesterase inhibitor	mdx mice	DMD	Decreased fibrosis, TGF $\beta$ , TNF $\alpha$ and MMP13 levels	(Percival <i>et al.</i> , 2012)
Piclamilast			Phosphodiesterase inhibitor	mdx mice	DMD	Decreased collagen I and fibronectin levels	(Nio <i>et al.</i> , 2017)
Bosentan			Endothelin receptor inhibitor	human primary cells (FAPs)	OPMD	Decreased proliferation and secretion, improved myoblasts fusion	(Bensalah <i>et al.</i> , 2022)
Tranilast		x	Ca <sup>2+</sup> channel inhibitor	mdx mice	DMD	Decreased fibrosis	(Swiderski <i>et al.</i> , 2014)
Givinostat	x	x	HDAC inhibitor	mdx mice	DMD	Decreased fibrosis, fat deposition and inflammation	(Consalvi <i>et al.</i> , 2013)
				mdx mice	DMD	Decreased fibrosis in skeletal muscle but not heart	(Licandro <i>et al.</i> , 2021)

CT: clinical trial, AT1: angiotensin II type 1 receptor, ALS: amyotrophic lateral sclerosis, FAPs: fibro adipogenic progenitors, OPMD : oculopharyngeal muscular dystrophy, DMD: duchenne muscular dystrophy, LGMD2B : limb-girdle muscular dystrophy type 2B, ACE : angiotensin-converting enzyme, NMJ : neuromuscular junction, HDAC : histone deacetylase, NF $\kappa$ B : nuclear factor-kappa B, TNF $\alpha$ : tumor necrosis factor alpha, TGF $\beta$  : transforming growth factor beta, MMP : matrix metalloproteinase, CTGF : connective tissue growth factor, LTBP4: latent TGF $\beta$  binding protein 4, ADAMTS1 : a disintegrin and metalloproteinase with thrombospondin motifs 1, PMO : phosphorodiamidate morpholino oligomer, dKO : dystrophin/utrophin double knock out, CK: creatine kinase.

of aged mdx mice [21,22] as well as in a mouse model of dysferlinopathy [23]. Recently, the use of a novel Fc fusion protein ActRIIB:ALK4-Fc inhibited the interaction between the receptors ActRIIB and ALK4. This dual selectivity allows for a blocking downstream of the TGF $\beta$ /activin pathway without impacting the BMP pathway, involved in vascularization. ActRIIB:ALK4-Fc treatment decreased collagen deposition, partially reversing the fibrotic phenotype in the injured muscle of the mdx mouse [24].

In dystrophic mice and DMD patients, LTBP4 contains polymorphisms that alter its proteolytic stability [25]. The blocking of the LTBP4 hinge region, more prone to cleavage, allows a better stabilization of its protective effect on TGF $\beta$ . LTBP4 antibody treatment improved muscle fibrosis and increased muscle force production in mdx mice, suggesting that targeting LTBP4 could be a good strategy to locally reduce TGF $\beta$  in tissue [26]. Binding to  $\alpha_V$  integrin is one of the modes of activation of TGF $\beta$  (Figure 1). The blockade of  $\alpha_V$  integrins by the small molecule CWHM12 attenuates established fibrosis with inhibition of collagen I expression in PDGFR $\beta$ + mesenchymal cells. This small molecule targeting all the  $\alpha_V$  integrins has potent effect in several tissues [27]. These strategies would allow for a more precise targeting of the TGF $\beta$  signaling but it is necessary to study first the implication of the precise downstream cascades for each fibrotic disease.

### Blocking CTGF/CCN2

Connective tissue growth factor (CTGF or CCN2) is another essential player in fibrosis, present in the ECM and overexpressed in MDs. No canonical receptor has been described yet, but CTGF can bind to integrins, tyrosine kinase receptors, ECM proteins and several cytokines [28]. CTGF plays a role in the modulation of ECM and has been shown to interact with several profibrotic molecules including TGF $\beta$  (potentializing its effect), angiotensin as well as endothelin [28]. Those interactions make CTGF a regulator of multiple signaling pathways.

In the skeletal muscle, pamrevlumab (FG3019), a CTGF blocking antibody, has been shown to decrease fibrosis and reduce the number of activated fibroblasts and macrophages in mdx mice [29]. A 6-weeks treatment with pamrevlumab reduced the early progression of fibrosis [30] as well as reversed established fibrosis in a rat model of chronic overuse [31]. Improvement was also observed in a murine model for amyotrophic lateral sclerosis (ALS) characterized by increased number of FAPs and fibrosis [32] highlighting a potential broad effect in MDs. A phase 2 clinical trial in DMD is ongoing (NCT02606136) and a phase 3 has just started (NCT04632940).

### Inhibiting matrix metalloproteinases (MMPs)

The ECM is in constant remodeling and the main enzymes implicated in its degradation are matrix metalloproteinases (MMPs). Within ECM, MMPs also cleave, and therefore modulate, a large number of chemokines and cytokines. Elevated levels of MMPs were reported in MDs including inflammatory myopathies [33]. Batimastat (BB-94), a pan inhibitor of MMPs reduced fibrosis in the mdx muscles [34] and inhibited the adipogenesis of FAPs in dysferlin deficient mice [35] showing an interesting dual role of this molecule that could act on two sides of the muscle diseases spectrum; decreasing fibrosis and reducing the adipogenesis replacement. However, first generation of MMPs inhibitors like batimastat have been shown to trigger adverse effects after chronic treatments in clinical trials for cancer [36], probably due to their broad effects on other biological processes. The most recent generation of MMPs inhibitors with higher specificity for single MMP led to several clinical trials in a variety of diseases and could be translated in the future to skeletal muscle [37].

More recently, another member of the metalloproteinase family, ADAMTS1, was found upregulated in mdx mice and in the serum of DMD patients, increasing with age. A treatment with an antibody directed against ADAMTS1 decreased fibrosis and increased muscle strength in the gastrocnemius of the mdx mouse [38], while the exact mechanism is not yet really understood.

As the role of MMPs is context dependent and their beneficial or detrimental action can vary through the course of the disease, further studies are needed to clearly identify specific MMPs implicated in each disease before clinical trials can be conducted.

### Blocking the renin-angiotensin system

In the renin-angiotensin system (RAS), angiotensin I is converted into angiotensin II by angiotensin converting enzyme (ACE) which signals via two receptors, AT1 and AT2 (Figure 1). The RAS has been shown to be activated in dystrophic muscles and contributes to fibrosis notably via the regulation of TGF $\beta$  and CTGF activity in skeletal muscle cells [8,39].

Losartan, an angiotensin II type 1 (AT1) receptor blocker has been shown to decrease cardiac and skeletal muscle fibrosis in the mdx mouse [40,41]. A randomized 1-year human clinical trial showed that losartan treatment significantly improved the cardiac function in DMD patients with cardiomyopathy, but skeletal muscle fibrosis was not analyzed [42]. Subsequent studies in a limb girdle muscular dystrophy (LGMD) type 2B mouse model showed contradictory results since its administration aggravated quadriceps muscle damage [43]

suggesting a different effect of losartan on signaling in dysferlinopathic mice. Enalapril—an inhibitor of the ACE—diminished ECM production in sedentary and exercised mdx mice and reduced the CTGF expression and its profibrotic activity, without affecting TGF $\beta$  signaling when losartan did. Its action, upstream of losartan activity, directly affects the availability of Ang-II and promotes the synthesis of Ang1-7, which has opposite effects [44]. Indeed, Ang 1–7 has been shown to diminish fibrosis in mdx mice via an inhibition of the TGF $\beta$ -smad dependent pathway through the Mas receptor [45], the “protective” arm of the RAS pathway (Fig. 1). Recently, Ang1-7 has been shown to prevent fibrosis in rats after eccentric exercise [46] and in a mouse model of radiation-induced fibrosis. It therefore could act as a radioprotectant against skeletal muscle fibrosis [47].

### Inhibiting tyrosine kinase

Aberrant activation of Tyrosine kinase receptors, such as the platelet derived growth factor receptors (PDGFR), impacts homeostasis and leads to fibrosis through the activation of fibroblasts [48]. PDGFR $\alpha$  is predominantly expressed in FAPs, and one of its ligands, PDGF-AA, has been shown as profibrotic in MDs [49]. Nilotinib is a tyrosine kinase inhibitor (TKI) that targets several tyrosine kinases including PDGFR [50]: it induces the apoptosis of murine FAPs and therefore decreases collagen I deposition in mdx where fibrosis is induced by chronic damage [51]. However, off-target effects on myoblasts were also described [52] stressing the fact that read-out drug assays should take into account potential detrimental effects on muscle cell differentiation. Some cardiovascular adverse events have also been reported [53]. Nintedanib, already clinically tested in idiopathic pulmonary fibrosis, reduced the proliferation and ECM secretion of human fibroblasts and reduced skeletal muscle fibrosis in mdx mice [54], however the potential effect of inhibition on the vascular endothelial growth factor receptor (VEGFR) needs to be considered before going to clinical trials in young DMD patients. Crenolanib, a more selective PDGFR $\alpha$  inhibitor, reduced fibrosis in the diaphragm of mdx mice [55]. This treatment could be safer than TKI with a broad spectrum. Recently, several TKIs, including nilotinib, sorafenib, masitinib were tested on murine TGF $\beta$ -treated FAPs [56]. *In vitro*, Sorafenib presented a dose dependent reduction in the number of COL1A1-positive FAPs while masitinib was effective only at high doses. *In vivo*, none of these drugs were able to clearly decrease fibrosis in the diaphragm of mdx:utr +/- mice. These discrepancies could be explained by the effect of those drugs on other skeletal muscle cell actors including immune and muscle cells.

### Targeting muscle inflammation

Persistent inflammation is strongly linked to fibrosis in pathological regeneration processes.

It has been reported that dystrophic muscles show over-expression of pro-inflammatory cytokines including TNF $\alpha$  and Interleukin-6 (IL-6). TNF $\alpha$  is released predominantly by infiltrating macrophages and its long-term secretion activates the NF- $\kappa$ B signaling cascade leading to a chronic inflammation [57,58]. The targeting of TNF $\alpha$  by the antibody Infliximab significantly reduced fibrosis and increased muscle strength in mdx mice, but had a negative effect on cardiac function [59]. Several trials with infliximab in other diseases (sepsis, multiple sclerosis) have been suspended due to increased severity of the symptoms after treatment. Those results highlight the difficulty to block inflammatory mediators without risking negative effects on the immune system.

Edasalonexent (CAT-1004) and CAT-1041, NF- $\kappa$ B inhibitors, decreased fibrosis in skeletal muscle of mdx mice and in GRMD dogs [60]. However, although well tolerated [61], a phase 3 clinical trial (NCT03703882) on < 8 years DMD patients did not show any significant effect on muscle [62].

The MR16-1 antibody, targeting IL-6 receptor, inhibits the activated IL-6 signaling pathway resulting in a reduced fibrosis in quadriceps of dKO mice, a more severe model of DMD [63], at advanced stages. It had, however no effect on cardiac muscle. Already approved for children with systemic juvenile idiopathic arthritis, this treatment could potentially be used in DMD [64].

Early activation of T cells plays a critical role in promoting fibrosis early in the disease pathology. The inhibition of protein kinase  $\theta$  (PKC- $\theta$ ), a key effector of T cells activation, reduced inflammation, necrosis and fibrosis in the diaphragm [65] and maintained muscle performance [66] of mdx mouse. The advantage of this strategy is the specific targeting of activated effector T cells without causing a global immune suppression. However, a study of the long-term effect of this inhibition needs to be considered.

A close relationship between inflammation and oxidative stress has been demonstrated in MDs [67]. Targeting oxidative stress with N-acetyl-cysteine (NAC), an antioxidant, reduced fibrosis and immune cell infiltration in the diaphragm of mdx mice [68]. NAC treatment also reduced TNF $\alpha$  levels in the diaphragm of mdx mice [69]. However, side effects of NAC treatment in mdx mice included loss of muscle mass and the suppression of a gain in body weight. Cyclooxygenase-2 (COX2), the enzyme that synthesizes prostaglandin, regulates inflammation and oxidative stress. It is found increased in activated fibroblasts. Celecoxib, a COX2 inhibitor, prevented myofibroblast differentiation and reduced fibrosis formation in a skeletal muscle injury mouse model, via several pathways including canonical and non-canonical TGF $\beta$  pathway [70].



### Molecules targeting other pathways

Statins (HMG Coa reductase inhibitors) in addition to their lipid lowering purposes, have pleiotropic effects especially on redox signaling, ultimately reducing oxidative stress [71]. Although statins have been considered to be toxic to skeletal muscle [72], Simvastatin showed a functional improvement in DMD with a reduction of fibrosis in mdx mice diaphragm [73] as well as in heart [74]. However more recent work did not confirm these effects on fibrosis in the mdx mouse diaphragm [75].

In DMD, the absence of dystrophin delocalizes and downregulates Nitric oxide (NO) synthase, attenuating the NO pathway [76]. This delocalization of nNOs is found in several other myopathies [77]. Phosphodiesterase inhibitors like Sildenafil can amplify this signaling and was found to slow down the establishment of fibrosis in the diaphragm of mdx mouse [78]. However, the beneficial effects of this treatment in the mdx mice [79] was not confirmed in a clinical trial conducted in DMD and BMD adults, with no improvement of cardiac function [80]. Piclamilast, another phosphodiesterase inhibitor, decreased fibrosis in the gastrocnemius of mdx mice [81]. Although it is still unclear which cell type is targeted by those compounds, phosphodiesterase inhibitors have also been shown to exert an anti-inflammatory effect by suppressing TNF $\alpha$  secretion, as well as decreasing levels of pro fibrotic MMPs [78].

This deficient NO pathway is linked in DMD with an increase of Histone deacetylase HDAC2 [82]. Thus, epigenetic regulators, such as givinostat, an HDAC inhibitor significantly decreased fibrosis in several muscles of the mdx and the more severe D2-mdx mouse models of DMD [83,84]. The safety and efficacy of this compound is currently being assessed in a phase 3 clinical trial in ambulant patients with DMD (NCT02851797).

Endothelin (ET-1), a vasoconstrictor peptide, has been shown to play a fundamental role in fibrosis, contributing to fibrotic disorders in several tissues [85]. Among others, TGF $\beta$  and AngII have been described to increase ET-1 expression, while NO decreased it [86]. Recently, we demonstrated the potential of bosentan, an inhibitor of ET-1 receptors, to block the secretion and proliferation of human FAPs isolated from oculopharyngeal muscular dystrophy (OPMD) affected muscles. This compound reversed the negative effect of the FAPs on the differentiation of myoblasts [87] and could represent a new candidate to decrease fibrosis in MDs. Further studies should determine if this drug, already used in several pathologies, could be beneficial in MDs.

Tranilast, a current anti-allergic agent and a Ca<sup>2+</sup> channel inhibitor is able to block the increased influx of Ca<sup>2+</sup> observed in DMD and other muscular dystrophies

which also play a role in oxidative stress and leads to muscle dysfunction [88].

Tranilast reduced fibrosis in the diaphragm of mdx mice [89] as well as in a hamster model of LGMD [90].

### Conclusion

So far, there is no treatment to effectively eradicate fibrosis, therefore identifying compounds to reduce or prevent fibrosis in the skeletal muscle is crucial. Anti-fibrotic therapies have a great potential to improve the evolution of MDs as they can help reverse pre-existing fibrosis while preserving the skeletal muscle structure and function. A growing number of pharmaceutical compounds targeting fibrosis are currently emerging and more of them are being tested in the skeletal muscle. As the onset and development of MDs are multifactorial, anti-fibrotic strategies include several targets, including cellular actors like FAPs and immune cells or muscle fibers themselves. Fibrosis is a complex process involving several cellular actors and interconnected pathways mediating multiple biological processes. Thus, drug selectivity is crucial to avoid unwanted side effects, but it requires a better understanding of the cell actors involved in its development and the exact signaling cascades impacting them throughout different diseases. Combined therapies targeting several signaling pathways or different aspects of the pathologies might also be a good option to have a better therapeutic impact in MDs at advanced stages of fibrosis. On the look-out for anti-fibrotic strategies, the selection of the animal models for MDs and in particular fibrosis is paramount, as these do not always recapitulate the severe characteristics of human MDs. As an example, the benchmarked DMD model mdx is known to develop significantly less fibrosis than DMD patients, and confined to the diaphragm. Moreover, DMD has been the most studied MD, consequently more attention has been focused on fibrosis associated with dystrophin deficiency to the detriment of other MDs. However, the course of these diseases and the level and establishment of fibrosis are different. More work is therefore needed to characterize fibrosis and to identify and analyze the effect of anti-fibrotic therapies in different MDs. In addition, among all the studies performed so far, few have studied the effect of anti-fibrotic compounds on human cells. Human FAPs from several MDs have now been described and antifibrotic strategies should be tested on those cells to investigate their potential in future human clinical trials.

### Author contributions

Laura Muraine: Writing – original draft, Conceptualization, Writing – review & editing, Visualization, Mona Bensalah: Writing – original draft, Conceptualization, Writing – review & editing, Gillian Butler-Browne: Writing – review & editing, Vincent Mouly: Writing –

review & editing, Supervision, Project administration, Funding acquisition. Anne Bigot: Writing – review & editing, Conceptualization, Supervision. Capucine Trollet: Writing – review & editing, Conceptualization, Supervision, Project administration, Funding acquisition, Visualization, Elisa Negroni: Writing – review & editing, Conceptualization, Supervision, Project administration, Funding acquisition, Visualization

### Conflict of interest statement

LM, MB, AB, EN and CT are inventors on a patent on targeting endothelin to treat fibrosis. GBB and VM have declared that no conflicts of interest exist.

### Acknowledgments

We acknowledge funding from the Association Française contre les Myopathies (AFM Telethon), Inserm, Sorbonne Université, Fondation pour la Recherche Médicale (FRM; EQUIPE FRM EQU201903007784).

### References

Papers of particular interest, published within the period of review, have been highlighted as:

- \* of special interest
- \*\* of outstanding interest

1. Henderson NC, Rieder F, Wynn TA: **Fibrosis: from mechanisms to medicines.** *Nature* 2020, **587**:555–566, <https://doi.org/10.1038/s41586-020-2938-9>.
2. Baghdadi MB, Tajbakhsh S: **Regulation and phylogeny of skeletal muscle regeneration.** *Dev Biol* 2018, **433**:200–209, <https://doi.org/10.1016/j.ydbio.2017.07.026>.
3. Schmidt M, Schüller SC, Hüttner SS, von Eyss B, von Maltzahn J: **Adult stem cells at work: regenerating skeletal muscle.** *Cell Mol Life Sci* 2019, <https://doi.org/10.1007/s00018-019-03093-6>.
4. Molina T, Fabre P, Dumont NA: **Fibro-adipogenic progenitors in skeletal muscle homeostasis, regeneration and diseases.** *Open Biol* 2021, **11**, 210110, <https://doi.org/10.1098/rsob.210110>.
5. Mahdy MAA: *Skeletal muscle fibrosis: an overview, cell and tissue research.* 2018, <https://doi.org/10.1007/s00441-018-2955-2>.
6. Cordova G, Negroni E, Cabello-Verrugio C, Mouly V, Trollet C: **Combined therapies for duchenne muscular dystrophy to optimize treatment efficacy.** *Front Genet* 2018, **9**:114, <https://doi.org/10.3389/fgene.2018.00114>.
7. Serrano AL, Muñoz-Cánoves P: **Fibrosis development in early-onset muscular dystrophies: mechanisms and translational implications.** *Semin Cell Dev Biol* 2017, **64**:181–190, <https://doi.org/10.1016/j.semcdb.2016.09.013>.
8. Sun G, Haginoya K, Dai H, Chiba Y, Uematsu M, Hino-Fukuyo N, Onuma A, Iinuma K, Tsuchiya S: **Intramuscular renin-angiotensin system is activated in human muscular dystrophy.** *J Neurol Sci* 2009, **280**:40–48, <https://doi.org/10.1016/j.jns.2009.01.020>.
9. Grounds MD, Terrill JR, Al-Mshhdani BA, Duong MN, Radley-Crabb HG, Arthur PG: **Biomarkers for Duchenne muscular dystrophy: myonecrosis, inflammation and oxidative stress.** *Dis Model Mech* 2020, **13**, dmm043638, <https://doi.org/10.1242/dmm.043638>.
10. Meng X, Nikolic-Paterson DJ, Lan HY: **Tgf- $\beta$ : the master regulator of fibrosis.** *Nat Rev Nephrol* 2016, **12**:325–338, <https://doi.org/10.1038/nrneph.2016.48>.
11. Smith LR, Barton ER: **Regulation of fibrosis in muscular dystrophy.** *Matrix Biol* 2018, **68–69**:602–615, <https://doi.org/10.1016/j.matbio.2018.01.014>.
12. Iozzo RV: **The biology of the small leucine-rich proteoglycans.** *J Biol Chem* 1999, **274**:18843–18846, <https://doi.org/10.1074/jbc.274.27.18843>.
13. A. Hildebrand, W.A. Border, E. Ruoslahti, Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor, (n.d.) 8.
14. Andreetta F, Bernasconi P, Baggi F, Ferro P, Oliva L, Arnoldi E, Cornelio F, Mantegazza R, Confalonieri P: **Immunomodulation of TGF- $\beta$ 1 in mdx mouse inhibits connective tissue proliferation in diaphragm but increases inflammatory response: implications for antifibrotic therapy.** *J Neuroimmunol* 2006:10.
15. Gosselin LE, Williams JE, Deering M, Brazeau D, Koury S, Martinez DA: **Localization and early time course of TGF- $\beta$ 1 mRNA expression in dystrophic muscle.** *Muscle Nerve* 2004, **30**:645–653, <https://doi.org/10.1002/mus.20150>.
16. De Oliveira Moreira D, Pereira JA, Taniguti APT, Matsumura CY, Ramos LAF, Areas MA, Neto HS, Marques MJ: **Suramin attenuates dystrophin-deficient cardiomyopathy in the mdx mouse model of duchenne muscular dystrophy: suramin in mdx Mouse Heart.** *Muscle Nerve* 2013, **48**:911–919, <https://doi.org/10.1002/mus.23858>.
17. Taniguti APT, Perteille A, Matsumura CY, Neto HS, Marques MJ: **Prevention of muscle fibrosis and myonecrosis in mdx mice by suramin, a TGF- $\beta$ 1 blocker: suramin and Antifibrosis Therapy in mdx Mice.** *Muscle Nerve* 2011, **43**:82–87, <https://doi.org/10.1002/mus.21869>.
18. Zhang YE: **Non-Smad pathways in TGF- $\beta$  signaling.** *Cell Res* 2009, **19**:128–139, <https://doi.org/10.1038/cr.2008.328>.
19. Girardi F, Taleb A, Ebrahimi M, Datye A, Gamage DG, Peccate C, Giordani L, Millay DP, Gilbert PM, Cadot B, Le Grand F: **TGF $\beta$  signaling curbs cell fusion and muscle regeneration.** *Nat Commun* 2021, **12**:750, <https://doi.org/10.1038/s41467-020-20289-8>.
20. Györfi AH, Matei A-E, Distler JHW: **Targeting TGF- $\beta$  signaling for the treatment of fibrosis.** *Matrix Biol* 2018:68–69, <https://doi.org/10.1016/j.matbio.2017.12.016>. 8–27.
21. Mordechay S, Smullen S, Evans P, Genin O, Pines M, Halevy O: **Differential effects of halofuginone enantiomers on muscle fibrosis and histopathology in duchenne muscular dystrophy.** *IJMS* 2021, **22**:7063, <https://doi.org/10.3390/ijms22137063>.  
This study analyzes the effect of two enantiomers of Halofuginone. Both enantiomers have differential effects and the +/- enantiomer surpasses the racemic form of Halofuginone in improving histopathology and fibrosis mdx mice.
22. Turgeman T, Hagai Y, Huebner K, Jassal DS, Anderson JE, Genin O, Nagler A, Halevy O, Pines M: **Prevention of muscle fibrosis and improvement in muscle performance in the mdx mouse by halofuginone.** *Neuromuscul Disord* 2008, **18**: 857–868, <https://doi.org/10.1016/j.nmd.2008.06.386>.
23. Barzilai-Tutsch H, Genin O, Pines M, Halevy O: **Early pathological signs in young dysf mice are improved by halofuginone.** *Neuromuscul Disord* 2020, **30**:472–482, <https://doi.org/10.1016/j.nmd.2020.04.001>.  
Study on the effect of pSmad3 inhibitor halofuginone in dysferlinopathies. Demonstrate a better ability of the myoblasts to form mature myofibers, an effect on decreasing fibrosis and an improvement of muscle histopathology and motor coordination in dysf-/- mice.
24. Li J, Fredericks M, Cannell M, Wang K, Sako D, Maguire MC, Grenha R, Liharska K, Krishnan L, Bloom T, Belcheva EP, Martinez PA, Castonguay R, Keates S, Alexander MJ, Choi H, Grinberg AV, Pearsall RS, Oh P, Kumar R, Suragani RNVS: **ActRIIB:ALK4-Fc alleviates muscle dysfunction and comorbidities in murine models of neuromuscular disorders.** *J Clin Invest* 2021, **131**, e138634, <https://doi.org/10.1172/JCI138634>.  
Demonstrate the effect of a new fusion protein targeting TGF $\beta$  super-family ligands with novel selectivity, shown to alleviate acute fibrosis in mdx and improves NMJ in ALS model.
25. Flanigan KM, Ceko E, Lamar K-M, Kaminoh Y, Dunn DM, Mendell JR, King WM, Pestronk A, Florence JM, Mathews KD, Finkel RS, Swoboda KJ, Gappmaier E, Howard MT, Day JW, McDonald C, McNally EM, Weiss RB: **LTBP4 genotype predicts age of ambulatory loss in duchenne muscular dystrophy:**

- LTBP4 Genotype in DMD.** *Ann Neurol* 2013, **73**:481–488, <https://doi.org/10.1002/ana.23819>.
26. Demonbreun AR, Fallon KS, Oosterbaan CC, Vaught LA, Reiser NL, Bogdanovic E, Velez MP, Salamone IM, Page PGT, Hadhazy M, Quattrocchi M, Barefield DY, Wood LD, Gonzalez JP, Morris C, McNally EM: **Anti-latent TGF $\beta$  binding protein 4 antibody improves muscle function and reduces muscle fibrosis in muscular dystrophy.** *Sci Transl Med* 2021, **13**, <https://doi.org/10.1126/scitranslmed.abf0376>. eabf0376.
- Evaluation of the efficacy of LTBP4 antibodies in mdx mice as well as mdx mice expressing both human and mouse LTBP4. Demonstrates a reduction of fibrosis and enhanced muscle performance.
27. Murray IR, Gonzalez ZN, Baily J, Dobie R, Wallace RJ, Mackinnon AC, Smith JR, Greenhalgh SN, Thompson AI, Conroy KP, Griggs DW, Ruminski PG, Gray GA, Singh M, Campbell MA, Kendall TJ, Dai J, Li Y, Iredale JP, Simpson H, Huard J, Péault B, Henderson NC:  **$\alpha$ v integrins on mesenchymal cells regulate skeletal and cardiac muscle fibrosis.** *Nat Commun* 2017, **8**, <https://doi.org/10.1038/s41467-017-01097-z>.
28. Rebolledo DL, Lipson KE, Brandan E: **Driving fibrosis in neuromuscular diseases: role and regulation of Connective tissue growth factor (CCN2/CTGF).** *Matrix Biol* 2021, **11**, 100059, <https://doi.org/10.1016/j.mbplus.2021.100059>.
29. Morales MG, Gutierrez J, Cabello-Verrugio C, Cabrera D, Lipson KE, Goldschmeding R, Brandan E: **Reducing CTGF/CCN2 slows down mdx muscle dystrophy and improves cell therapy.** *Hum Mol Genet* 2013, **22**:4938–4951, <https://doi.org/10.1093/hmg/ddt352>.
30. Barbe MF, Hilliard BA, Delany SP, Iannarone VJ, Harris MY, Amin M, Cruz GE, Barreto-Cruz Y, Tran N, Day EP, Hobson LJ, Assari S, Popoff SN: **Blocking CCN2 reduces progression of sensorimotor declines and fibrosis in a rat model of chronic repetitive overuse.** *J Orthop Res* 2019, **37**:2004–2018, <https://doi.org/10.1002/jor.24337>.
31. Barbe MF, Hilliard BA, Amin M, Harris MY, Hobson LJ, Cruz GE, Popoff SN: **Blocking CTGF/CCN2 reduces established skeletal muscle fibrosis in a rat model of overuse injury.** *Faseb J* 2020, **34**:6554–6569, <https://doi.org/10.1096/fj.202000240RR>.
32. Gonzalez D, Rebolledo DL, Correa LM, Court FA, Cerpa W, Lipson KE, van Zundert B, Brandan E: **The inhibition of CTGF/CCN2 activity improves muscle and locomotor function in a murine ALS model.** *Hum Mol Genet* 2018, **27**:2913–2926, <https://doi.org/10.1093/hmg/ddy204>.
33. Alameddine HS, Morgan JE: **Matrix metalloproteinases and tissue inhibitor of metalloproteinases in inflammation and fibrosis of skeletal muscles.** *JND* 2016, **3**:455–473, <https://doi.org/10.3233/JND-160183>.
34. Kumar A, Bhatnagar S, Kumar A: **Matrix metalloproteinase inhibitor Batimastat alleviates pathology and improves skeletal muscle function in dystrophin-deficient mdx mice.** *Am J Pathol* 2010, **177**:248–260, <https://doi.org/10.2353/ajpath.2010.091176>.
35. Hogarth MW, Defour A, Lazarski C, Gallardo E, Manera JD, Partridge TA, Nagaraju K, Jaiswal JK: **Fibroadipogenic progenitors are responsible for muscle loss in limb girdle muscular dystrophy 2B.** *Nat Commun* 2019, **10**:2430, <https://doi.org/10.1038/s41467-019-10438-z>.
36. Vandenbroucke RE, Libert C: **Is there new hope for therapeutic matrix metalloproteinase inhibition?** *Nat Rev Drug Discov* 2014, **13**:904–927, <https://doi.org/10.1038/nrd4390>.
37. Fields GB: **The rebirth of matrix metalloproteinase inhibitors: moving beyond the dogma.** *Cells* 2019, **8**:984, <https://doi.org/10.3390/cells8090984>.
38. Wang Y, Xiao Y, Zheng Y, Yang L, Wang D: **An anti-ADAMTS1 treatment relieved muscle dysfunction and fibrosis in dystrophic mice.** *Life Sci* 2021, **281**, 119756, <https://doi.org/10.1016/j.lfs.2021.119756>.
- This study demonstrates a correlation of the levels of ADAMTS1 with age in DMD patients. The treatment with ADAMTS1 antibodies improved the function of dystrophic muscles and decreased fibrosis in the gastrocnemius of mdx mice.
39. Morales MG, Vazquez Y, Acuña MJ, Rivera JC, Simon F, Salas JD, Álvarez Ruf J, Brandan E, Cabello-Verrugio C: **Angiotensin II-induced pro-fibrotic effects require p38MAPK activity and transforming growth factor beta 1 expression in skeletal muscle cells.** *Int J Biochem Cell Biol* 2012, **44**:1993–2002, <https://doi.org/10.1016/j.biocel.2012.07.028>.
40. Cabello-Verrugio C, Morales MG, Cabrera D, Vio CP, Brandan E: **Angiotensin II receptor type 1 blockade decreases CTGF/CCN2-mediated damage and fibrosis in normal and dystrophic skeletal muscles.** *J Cell Mol Med* 2012, **16**:752–764, <https://doi.org/10.1111/j.1582-4934.2011.01354.x>.
41. Spurney CF, Sali A, Guerron AD, Iantorno M, Yu Q, Gordish-Dressman H, Rayavarapu S, van der Meulen J, Hoffman EP, Nagaraju K: **Losartan decreases cardiac muscle fibrosis and improves cardiac function in dystrophin-deficient mdx mice.** *J Cardiovasc Pharmacol Therapeut* 2011, **16**:87–95, <https://doi.org/10.1177/1074248410381757>.
42. Allen HD, Flanigan KM, Thrush PT, Dvorchik I, Yin H, Canter C, Connolly AM, Parrish M, McDonald CM, Braunlin E, Colan SD, Day J, Darras B, Mendell JR: **A randomized, double-blind trial of lisinopril and losartan for the treatment of cardiomyopathy in duchenne muscular dystrophy.** *PLoS Curr* 2013, **5**, <https://doi.org/10.1371/currents.md.2cc69a1dae4be7dfe2bcb420024ea865>. ecurrents.md.2cc69a1dae4be7dfe2bcb420024ea865.
43. White Z, Milad N, Tehrani AY, Chen WW-H, Donen G, Sellers SL, Bernatchez P: **Angiotensin II receptor blocker losartan exacerbates muscle damage and exhibits weak blood pressure-lowering activity in a dysferlin-null model of Limb-Girdle muscular dystrophy type 2B.** *PLoS One* 2019, **14**, e0220903, <https://doi.org/10.1371/journal.pone.0220903>.
44. Morales MG, Cabrera D, Céspedes C, Vio CP, Vazquez Y, Brandan E, Cabello-Verrugio C: **Inhibition of the angiotensin-converting enzyme decreases skeletal muscle fibrosis in dystrophic mice by a diminution in the expression and activity of connective tissue growth factor (CTGF/CCN-2).** *Cell Tissue Res* 2013, **353**:173–187, <https://doi.org/10.1007/s00441-013-1642-6>.
45. Acuña MJ, Pessina P, Olguin H, Cabrera D, Vio CP, Bader M, Muñoz-Canoves P, Santos RA, Cabello-Verrugio C, Brandan E: **Restoration of muscle strength in dystrophic muscle by angiotensin-1-7 through inhibition of TGF- $\beta$  signalling.** *Hum Mol Genet* 2014, **23**:1237–1249, <https://doi.org/10.1093/hmg/ddt514>.
46. Totou NL, Moura SS, Martins Júnior F de AD, Sousa FB, Coelho DB, Oliveira EC, dos Santos RAS, Becker LK, Lima WG: **Oral administration of angiotensin-(1–7) decreases muscle damage and prevents the fibrosis in rats after eccentric exercise.** *Exp Physiol* 2021, **106**:1710–1719, <https://doi.org/10.1113/EP089308>.
47. Willey JS, Bracey DN, Gallagher PE, Tallant EA, Wiggins WF, Callahan MF, Smith TL, Emory CL: **Angiotensin-(1-7) attenuates skeletal muscle fibrosis and stiffening in a mouse model of extremity sarcoma radiation therapy.** *J Bone Joint Surg* 2016, **98**:48–55, <https://doi.org/10.2106/JBJS.O.00545>.
48. Beyer C, Distler JHW: **Tyrosine kinase signaling in fibrotic disorders.** *Biochim Biophys Acta (BBA) - Mol Basis Dis* 2013, **1832**:897–904, <https://doi.org/10.1016/j.bbadis.2012.06.008>.
49. Zhao Y, Haginoya K, Sun G, Dai H, Onuma A, Iinuma K: **Platelet-derived growth factor and its receptors are related to the progression of human muscular dystrophy: an immunohistochemical study.** *J Pathol* 2003, **201**:149–159, <https://doi.org/10.1002/path.1414>.
50. Blay J-Y, von Mehren M: **Nilotinib: a novel, selective tyrosine kinase inhibitor.** *Semin Oncol* 2011, **38**:S3–S9, <https://doi.org/10.1053/j.seminoncol.2011.01.016>.
51. Lemos DR, Babaeijandaghi F, Low M, Chang C-K, Lee ST, Fiore D, Zhang R-H, Natarajan A, Nedospasov SA, Rossi FMV: **Nilotinib reduces muscle fibrosis in chronic muscle injury by promoting TNF-mediated apoptosis of fibro/adipogenic progenitors.** *Nat Med* 2015, **21**:786–794, <https://doi.org/10.1038/nm.3869>.

52. Contreras O, Villarreal M, Brandan E: **Nilotinib impairs skeletal myogenesis by increasing myoblast proliferation.** *Skeletal Muscle* 2018, **8**:5, <https://doi.org/10.1186/s13395-018-0150-5>.
53. Moslehi JJ, Deininger M: **Tyrosine kinase inhibitor-associated cardiovascular toxicity in chronic myeloid leukemia.** *J Clin Orthod* 2015, **33**:4210–4218, <https://doi.org/10.1200/JCO.2015.62.4718>.
54. Piñol-Jurado P, Suárez-Calvet X, Fernández-Simón E, Gallardo E, de la Oliva N, Martínez-Muriana A, Gómez-Gálvez P, Escudero LM, Pérez-Peiró M, Wollin L, de Luna N, Navarro X, Illa I, Díaz-Manera J: **Nintedanib decreases muscle fibrosis and improves muscle function in a murine model of dystrophinopathy.** *Cell Death Dis* 2018, **9**, <https://doi.org/10.1038/s41419-018-0792-6>.
55. Ieronimakis N, Hays A, Prasad A, Janebodin K, Duffield JS, Reyes M: **PDGFR $\alpha$  signalling promotes fibrogenic responses in collagen-producing cells in Duchenne muscular dystrophy.** *J Pathol* 2016, **240**:410–424, <https://doi.org/10.1002/path.4801>.
56. Theret M, Low M, Rempel L, Li FF, Tung LW, Contreras O, Chang C-K, Wu A, Soliman H, Rossi FMV: **In vitro assessment of anti-fibrotic drug activity does not predict in vivo efficacy in murine models of Duchenne muscular dystrophy.** *Life Sci* 2021, **279**, 119482, <https://doi.org/10.1016/j.lfs.2021.119482>.
57. Kuru S, Inukai A, Kato T, Liang Y, Kimura S, Sobue G: **Expression of tumor necrosis factor- $\alpha$  in regenerating muscle fibers in inflammatory and non-inflammatory myopathies.** *Acta Neuropathol* 2003, **105**:217–224, <https://doi.org/10.1007/s00401-002-0635-4>.
58. Messina S, Vita GL, Aguenouz M, Sframeli M, Romeo S, Rodolico C, Vita G: **Activation of NF-kappaB pathway in Duchenne muscular dystrophy: relation to age.** *Acta Myol* 2011, **30**:16–23.
59. Ermolova NV, Martinez L, Vetrone SA, Jordan MC, Roos KP, Sweeney HL, Spencer MJ: **Long-term administration of the TNF blocking drug Remicade (cV1q) to mdx mice reduces skeletal and cardiac muscle fibrosis, but negatively impacts cardiac function.** *Neuromuscul Disord* 2014, **24**:583–595, <https://doi.org/10.1016/j.nmd.2014.04.006>.
60. Hammers DW, Sleeper MM, Forbes SC, Coker CC, Jirousek MR, Zimmer M, Walter GA, Sweeney HL: **Disease-modifying effects of orally bioavailable NF- $\kappa$ B inhibitors in dystrophin-deficient muscle.** *JCI Insight* 2016, **1**, <https://doi.org/10.1172/jci.insight.90341>.
61. Donovan JM, Zimmer M, Offman E, Grant T, Jirousek M: **A novel NF- $\kappa$ B inhibitor, edasalonexent (CAT-1004), in development as a disease-modifying treatment for patients with duchenne muscular dystrophy: phase 1 safety, pharmacokinetics, and pharmacodynamics in adult subjects.** *J Clin Pharmacol* 2017, **57**:627–639, <https://doi.org/10.1002/jcph.842>.
62. (For the PolarisDMD Study Group), Finkel RS, McDonald CM, Lee Sweeney H, Finanger E, Neil Knierbein E, Wagner KR, Mathews KD, Marks W, Statland J, Nance J, McMillan HJ, McCullagh G, Tian C, Ryan MM, O'Rourke D, Müller-Felber W, Tulinius M, Burnette WB, Nguyen C-T, Vijayakumar K, Johannsen J, Phan HC, Eagle M, MacDougall J, Mancini M, Donovan JM: **A randomized, double-blind, placebo-controlled, global phase 3 study of edasalonexent in pediatric patients with duchenne muscular dystrophy: results of the PolarisDMD trial.** *JND* 2021, **8**:769–784, <https://doi.org/10.3233/JND-210689>.
63. Rodrigues M, Echigoya Y, Fukada S, Yokota T: **Current translational research and murine models for duchenne muscular dystrophy.** *JND* 2016, **3**:29–48, <https://doi.org/10.3233/JND-150113>.
64. Wada E, Tanihata J, Iwamura A, Takeda S, Hayashi YK, Matsuda R: **Treatment with the anti-IL-6 receptor antibody attenuates muscular dystrophy via promoting skeletal muscle regeneration in dystrophin-tutrophin-deficient mice.** *Skeletal Muscle* 2017, **7**:23, <https://doi.org/10.1186/s13395-017-0140-z>.
65. Lozanoska-Ochser B, Benedetti A, Rizzo G, Marrocco V, Di Maggio R, Fiore P, Bouche M: **Targeting early PKC $\theta$ -dependent T-cell infiltration of dystrophic muscle reduces disease severity in a mouse model of muscular dystrophy: targeting early T-cell infiltration ameliorates muscular dystrophy.** *J Pathol* 2018, **244**:323–333, <https://doi.org/10.1002/path.5016>.
66. Marrocco V, Fiore P, Benedetti A, Pisu S, Rizzuto E, Musarò A, Madaro L, Lozanoska-Ochser B, Bouché M: **Pharmacological inhibition of PKC $\theta$  counteracts muscle disease in a mouse model of duchenne muscular dystrophy.** *EBioMedicine* 2017, **16**:150–161, <https://doi.org/10.1016/j.ebiom.2017.01.001>.
67. Petrillo S, Pelosi L, Piemonte F, Travagliani L, Forcina L, Catteruccia M, Petrini S, Verardo M, D'Amico A, Musarò A, Bertini E: **Oxidative stress in Duchenne muscular dystrophy: focus on the NRF2 redox pathway.** *Hum Mol Genet* 2017, **26**:2781–2790, <https://doi.org/10.1093/hmg/ddx173>.
68. Burns DP, Drummond SE, Bolger D, Coiscaud A, Murphy KH, Edge D, O'Halloran KD: **N-Acetylcysteine decreases fibrosis and increases force-generating capacity of mdx diaphragm.** *Antioxidants* 2019, **8**:581, <https://doi.org/10.3390/antiox8120581>.
69. de Senzi Moraes Pinto R, Ferretti R, Moraes LHR, Neto HS, Marques MJ, Minatel E: **N-Acetylcysteine treatment reduces TNF- $\alpha$  levels and myonecrosis in diaphragm muscle of mdx mice.** *Clin Nutr* 2013, **32**:472–475, <https://doi.org/10.1016/j.clnu.2012.06.001>.
70. Chen H, Qian Z, Zhang S, Tang J, Fang L, Jiang F, Ge D, \*\* Chang J, Cao J, Yang L, Cao X: **Silencing COX-2 blocks PDK1/TRAF4-induced AKT activation to inhibit fibrogenesis during skeletal muscle atrophy.** *Redox Biol* 2021, **38**, 101774, <https://doi.org/10.1016/j.redox.2020.101774>.
- Identified COX-2 as a major player of fibrosis and demonstrated the effect of celecoxib on weakening oxidative stress and pro fibrotic kinase pathways, highlight the role of PDK1/Akt in this signaling.
71. Tanaka S, Fukumoto Y, Nochioka K, Minami T, Kudo S, Shiba N, Takai Y, Williams CL, Liao JK, Shimokawa H: **Statins exert the pleiotropic effects through small GTP-binding protein dissociation stimulator upregulation with a resultant Rac1 degradation.** *Arterioscler Thromb Vasc Biol* 2013, **33**:1591–1600, <https://doi.org/10.1161/ATVBAHA.112.300922>.
72. Bouitbir J, Sanvee GM, Panajatovic MV, Singh F, Krähenbühl S: **Mechanisms of statin-associated skeletal muscle-associated symptoms.** *Pharmacol Res* 2020, **154**, 104201, <https://doi.org/10.1016/j.phrs.2019.03.010>.
73. Whitehead NP, Kim MJ, Bible KL, Adams ME, Froehner SC: **A new therapeutic effect of simvastatin revealed by functional improvement in muscular dystrophy.** *Proc Natl Acad Sci USA* 2015, **112**:12864–12869, <https://doi.org/10.1073/pnas.1509536112>.
74. Kim MJ, Bible KL, Regnier M, Adams ME, Froehner SC, Whitehead NP: **Simvastatin provides long-term improvement of left ventricular function and prevents cardiac fibrosis in muscular dystrophy.** *Phys Rep* 2019, **7**, e14018, <https://doi.org/10.14814/phy2.14018>.
75. Verhaart IEC, Cappellari O, Tanganyika-de Winter CL, Plomp JJ, Nnorom S, Wells KE, Hildyard JCW, Bull D, Aartsmas-Rus A, Wells DJ: **Simvastatin treatment does not ameliorate muscle pathophysiology in a mouse model for duchenne muscular dystrophy.** *JND* 2021, **8**:845–863, <https://doi.org/10.3233/JND-200524>.
76. Brenman JE, Chao DS, Xia H, Aldape K, Bredt DS: **Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy.** *Cell* 1995, **82**:743–752, [https://doi.org/10.1016/0092-8674\(95\)90471-9](https://doi.org/10.1016/0092-8674(95)90471-9).
77. Kobayashi YM, Rader EP, Crawford RW, Iyengar NK, Thedens DR, Faulkner JA, Parikh SV, Weiss RM, Chamberlain JS, Moore SA, Campbell KP: **Sarcolemma-localized nNOS is required to maintain activity after mild exercise.** *Nature* 2008, **456**:511–515, <https://doi.org/10.1038/nature07414>.
78. Percival JM, Whitehead NP, Adams ME, Adamo CM, Beavo JA, Froehner SC: **Sildenafil reduces respiratory muscle weakness**

- and fibrosis in the *mdx* mouse model of Duchenne muscular dystrophy: Sildenafil treatment in *mdx* mice.** *J Pathol* 2012, **228**:77–87, <https://doi.org/10.1002/path.4054>.
79. Adamo CM, Dai D-F, Percival JM, Minami E, Willis MS, Patrucco E, Froehner SC, Beavo JA: **Sildenafil reverses cardiac dysfunction in the *mdx* mouse model of Duchenne muscular dystrophy.** *Proc Natl Acad Sci USA* 2010, **107**:19079–19083, <https://doi.org/10.1073/pnas.1013077107>.
  80. Leung DG, Herzka DA, Thompson WR, He B, Bibat G, Tennekoon G, Russell SD, Schuleri KH, Lardo AC, Kass DA, Thompson RE, Judge DP, Wagner KR: **Sildenafil does not improve cardiomyopathy in Duchenne/Becker muscular dystrophy: Sildenafil in DBMD.** *Ann Neurol* 2014, **76**:541–549, <https://doi.org/10.1002/ana.24214>.
  81. Nio Y, Tanaka M, Hirozane Y, Muraki Y, Okawara M, Hazama M, Matsuo T: **Phosphodiesterase 4 inhibitor and phosphodiesterase 5 inhibitor combination therapy has antifibrotic and anti-inflammatory effects in *mdx* mice with Duchenne muscular dystrophy.** *Faseb J* 2017, **31**:5307–5320, <https://doi.org/10.1096/fj.201700249R>.
  82. Colussi C, Mozzetta C, Gurtner A, Illi B, Rosati J, Straino S, Ragone G, Pescatori M, Zaccagnini G, Antonini A, Minetti G, Martelli F, Piaggio G, Gallinari P, Steinkühler C, Clementi E, Dell'Aversana C, Altucci L, Mai A, Capogrossi MC, Puri PL, Gaetano C: **HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment.** *Proc Natl Acad Sci USA* 2008, **105**:19183–19187, <https://doi.org/10.1073/pnas.0805514105>.
  83. Licandro SA, Crippa L, Pomarico R, Perego R, Fossati G, Leoni F, Steinkühler C: **The pan HDAC inhibitor Givinostat improves muscle function and histological parameters in two Duchenne muscular dystrophy murine models expressing different haplotypes of the LTBP4 gene.** *Skeletal Muscle* 2021, **11**:19, <https://doi.org/10.1186/s13395-021-00273-6>.  
Comprehensive study of the effect of givinostat, an HDAC inhibitor on several muscle and heart on two models of DMD mice. Demonstrates a decreased fibrosis and muscle function improvement.
  84. Consalvi S, Mozzetta C, Bettica P, Germani M, Fiorentini F, Del Bene F, Rocchetti M, Leoni F, Monzani V, Mascagni P, Puri PL, Saccone V: **Preclinical studies in the *mdx* mouse model of duchenne muscular dystrophy with the histone deacetylase inhibitor Givinostat.** *Mol Med* 2013, **19**:79–87, <https://doi.org/10.2119/molmed.2013.00011>.
  85. Rodríguez-Pascual F, Busnadiego O, González-Santamaría J: **The profibrotic role of endothelin-1: is the door still open for the treatment of fibrotic diseases?** *Life Sci* 2014, **118**:156–164, <https://doi.org/10.1016/j.lfs.2013.12.024>.
  86. Miyauchi T, Masaki T: **Pathophysiology of endothelin in the cardiovascular system.** *Annu Rev Physiol* 1999, **61**:391–415, <https://doi.org/10.1146/annurev.physiol.61.1.391>.
  87. Bensalah M, Muraine L, Boulinguez A, Giordani L, Albert V, Ythier V, Dhiab J, Oliver A, Hanique V, Gidaro T, Perié S, St-Guily JL, Corneau A, Butler-Browne G, Bigot A, Mouly V, Negroni E, Trollet C: **A negative feedback loop between fibro-adipogenic progenitors and muscle fibres involving endothelin promotes human muscle fibrosis.** 2022:14.  
Study of the impact of the fibro-adipogenic progenitors population in human fibrotic muscles. Identification of the implication of endothelin in human muscle fibrosis, demonstrate that blocking this pathway reverse the negative effect on myoblasts fusion.
  88. Mareedu S, Million ED, Duan D, Babu GJ: **Abnormal calcium handling in duchenne muscular dystrophy: mechanisms and potential therapies.** *Front Physiol* 2021, **12**, 647010, <https://doi.org/10.3389/fphys.2021.647010>.
  89. Swiderski K, Todorov M, Gehrig SM, Naim T, Chee A, Stapleton DI, Koopman R, Lynch GS: **Tranilast administration reduces fibrosis and improves fatigue resistance in muscles of *mdx* dystrophic mice.** *Fibrogenesis Tissue Repair* 2014, **7**:1, <https://doi.org/10.1186/1755-1536-7-1>.
  90. Iwata Y, Katanosaka Y, Shijun Z, Kobayashi Y, Hanada H, Shigekawa M, Wakabayashi S: **Protective effects of Ca<sup>2+</sup> handling drugs against abnormal Ca<sup>2+</sup> homeostasis and cell damage in myopathic skeletal muscle cells.** *Biochem Pharmacol* 2005, **70**:740–751, <https://doi.org/10.1016/j.bcp.2005.05.034>.