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# 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

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## Introduction

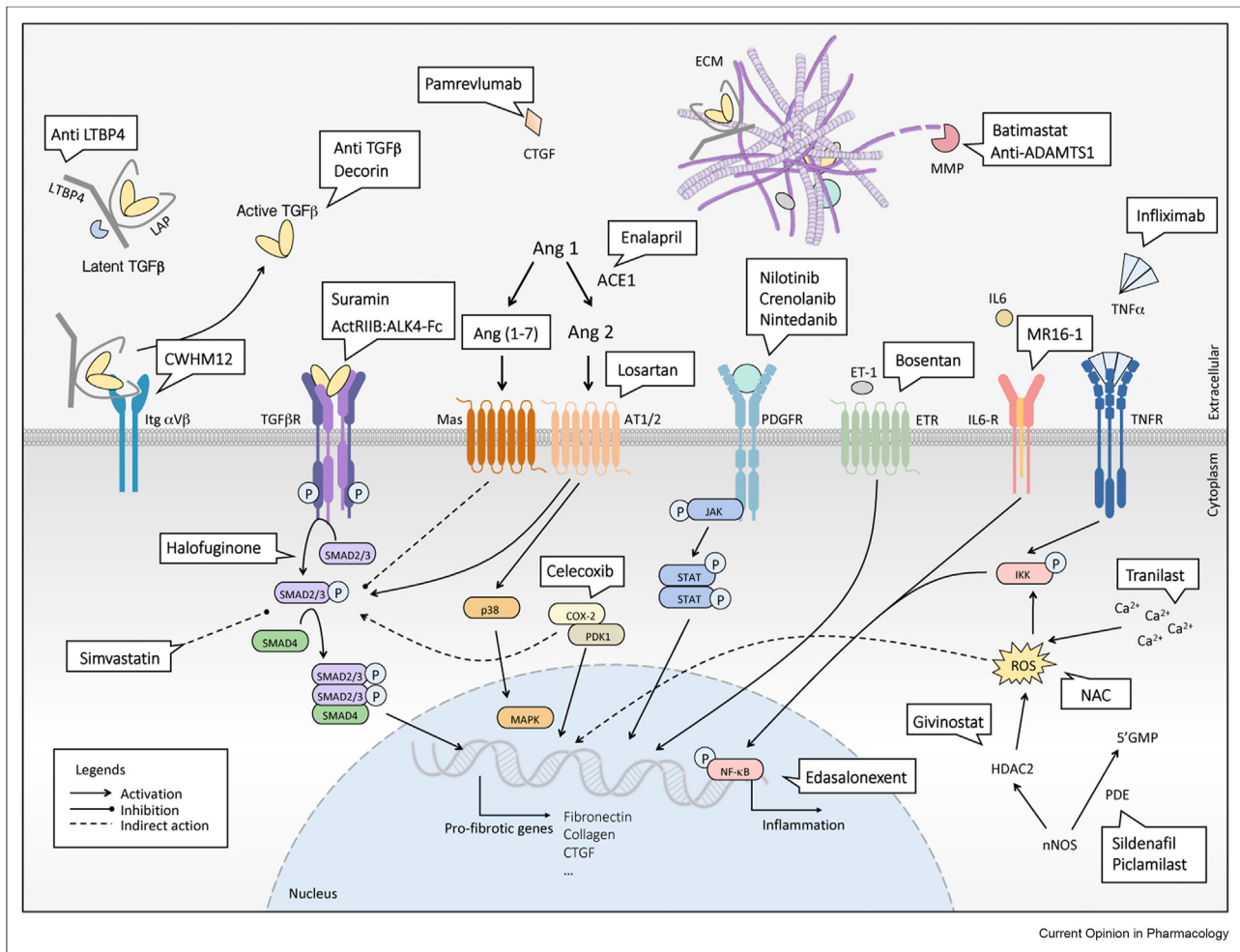
Fibrosis, defined as an excessive accumulation of extracellular matrix (ECM) components such as collagens, proteoglycans or fibronectin has been described in several organs, such as liver, lung, skin, heart as well as in skeletal muscle [1]. In damaged or diseased skeletal muscle, the orchestration of 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. TGF $\beta$ , Transforming growth factor beta; CTGF, connective tissue growth factor; LTBP4, latent TGF $\beta$ -binding protein 4; LAP, latency-associated peptide; TGF $\beta$ R, TGF $\beta$  receptor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; ET-1, endothelin 1; ETR, endothelin receptor; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; 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; NF $\kappa$ B, 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 TGF $\beta$  pathway in muscular disorders leads to the activation of FAPs and an excessive ECM deposition. Several antifibrotic strategies target directly TGF $\beta$ . For example, decorin –a small extracellular proteoglycan that interacts with TGF $\beta$  and ECM proteins- or anti-TGF $\beta$  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 TGF $\beta$ R1 with the antagonist suramin demonstrated an attenuation of fibrosis in the mdx diaphragm and limb muscles [16,17]. However, TGF $\beta$ R1 activation triggers several other non-canonical

pathways involved in many different biological processes [18] including regulation of the immune system. The TGF $\beta$  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 TGF $\beta$  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 TGF $\beta$  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>ppmd</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 –

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### 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.

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

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