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Annu Rev Pharmacol Toxicol. Author manuscript; available in PMC 2019 August 20.

Published in final edited form as:
Annu Rev Pharmacol Toxicol. 2019 January 06; 59: 315–339. doi:10.1146/annurev-pharmtox-010818-021041.

Muscle Wasting Diseases: Novel Targets and Treatments

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Abstract

Adequate skeletal muscle plasticity is an essential element for our well-being. Inversely, compromised muscle function can drastically affect quality of life, morbidity and mortality. Surprisingly however, skeletal muscle remains one of the most under-medicated organs. Accordingly, interventions in muscle diseases are scarce, not only in neuromuscular dystrophies, but also in highly prevalent secondary wasting pathologies such as sarcopenia and cachexia. Even in other diseases that exhibit a well-established risk correlation with a sedentary life-style and exercise, e.g. type 2 diabetes or cardiovascular pathologies, current treatments are mostly targeted on non-muscle tissues. In recent years, a renewed focus on skeletal muscle has led to the discovery of various novel drug targets and the design of new pharmacological approaches. This review will provide an overview of the current knowledge of the key mechanisms involved in muscle wasting conditions, and novel pharmacological avenues that could ameliorate muscle diseases.

Keywords

muscle wasting; atrophy; proteostasis; muscular dystrophy; gene therapy; exon-skipping

Introduction

Muscle wasting is observed in a variety of pathologies such as cancer, chronic kidney disease, heart failure, chronic obstructive pulmonary disease (COPD) as well as after prolonged inactivity or during aging. Reduced muscle mass and function is associated with a

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higher morbidity and mortality as well as reduced quality of life (1; 2). For example, loss of muscle mass is associated with a poorer prognosis and reduced response to therapy in cancer patients. Inversely, exercise exerts powerful effects on the prevention and treatment of many diseases (1; 2). Besides muscle wasting secondary to a pathology, there are several inherited muscular dystrophies characterized by muscle weakness. The etiology and mechanisms of these diseases are diverse and different to secondary muscle wasting. Even though the prevalence of people suffering from any kind of muscle weakness is high, there is no available treatment for muscle wasting and muscle remains an under-medicated organ. This review will provide an overview of the mechanistic aspects of muscle wasting, and summarize novel experimental and clinical pharmacological avenues aimed at skeletal muscle.

Muscle plasticity in different pathological conditions: key players and mechanisms

Skeletal muscle is a plastic tissue that constantly adapts its size and function according to external and internal stimuli. For example, mechanical loading, hormones, cytokines and nutrients shift the balance between protein synthesis and degradation and thereby determine muscle fiber size and contractile function (3). An imbalance in proteostasis is observed in several catabolic conditions such as sarcopenia, cachexia and disuse leading to muscle atrophy. Understanding the molecular mechanisms that regulate protein synthesis or degradation is therefore essential for the identification of potential targets for pharmacological stimulation or inhibition to counteract muscle wasting (Fig. 1). In this section, we will provide an overview of the important mechanisms regulating skeletal muscle proteostasis.

The mammalian target of rapamycin (mTOR), found in two distinct complexes in almost every cell, is a key regulatory nexus of protein synthesis and degradation. Binding of insulin or insulin-like growth factor 1 (IGF-1) to its receptor IGF-1R recruits insulin receptor substrate 1 (IRS-1), which subsequently leads to the activation of the phosphoinositide 3-kinase (PI3K) – Akt/protein kinase B (PKB) – mTOR pathway (3). In addition to IGF-1, growth hormone (GH) also induces PI3K-Akt signaling through the JAK2-mediated activation of IRS-1 (4). mTOR complex 1 (mTORC1), molecularly characterized by the protein raptor, controls protein synthesis mainly by phosphorylating the two downstream effectors S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) (3). In addition to the activation of mTOR and the ensuing stimulation of protein synthesis, Akt also negatively affects muscle atrophy (5). Specifically, Akt-induced phosphorylation of forkhead box O (FoxO) results in its nuclear exclusion and thereby prevents the induction of the E3 ubiquitin ligases muscle atrophy F-box protein (MAFbx) and muscle RING finger-containing protein 1 (MuRF1) (5). Besides the insulin and GH signaling pathways, mTORC1 is also activated by amino acids, in particular branched-chain amino acids (BCAA) such as leucine or its metabolite β-hydroxy-β-methylbutyrate (HMB) in an Akt-independent manner (6), as well as other anabolic stimuli including activation of β_2-adrenoceptors (β_2-AR). Stimulation of these guanine nucleotide-binding G protein-coupled receptors (GPCRs) activates the PI3K – Akt signaling pathway via the $G\beta\gamma$ dimer

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(7). In addition, β_2-agonists might also promote muscle hypertrophy via the Gα- cAMP – PKA signaling pathway (7; 8). The PKA-mediated phosphorylation of the cAMP response element-binding protein (CREB) induces the transcription of the orphan nuclear receptor NOR-1 which inhibits myostatin (MSTN) expression and thereby contributing to muscle growth (9–11). Accordingly, phosphodiesterases (PDEs), the enzymes that hydrolyze and thereby degrade cAMP, can be a target to interfere with cAMP-dependent modulation of muscle hypertrophy (7).

In contrast to mTOR with its potent positive effect on protein synthesis, MSTN, also known as growth and differentiation factor 8 (GDF-8), is an important negative regulator of muscle mass (12). Upon binding to the receptor complex consisting of activin receptor type-IIB (ActRIIB) and activin receptor-like kinase 4 (ALK4) or ALK5, phosphorylation of the transcription factors SMAD2 and SMAD3 is stimulated and downstream signaling activated (13; 14). A reduction in Akt signaling and activation of FoxO are major targets of MSTN signaling, which thereby negatively regulates protein synthesis and stimulates protein degradation (15). Moreover, recruitment of activated SMAD3 to the SMAD-binding element in the promoter region of MuRF1 and MAFbx synergistically increases transcriptional activity of FoxO (16). In addition to MSTN, GDF11, GDF15 and activin likewise signal through the same receptor complex and are involved in the negative regulation of muscle mass (17; 18). Interestingly, GDF15 also promotes muscle wasting by negatively affecting food intake (19; 20). Thus, elevated GDF15 serum levels in patients with cancer, chronic kidney disease or COPD could contribute to the development of cachexia by reducing appetite as well as inducing muscle-inherent catabolic pathways (19; 21).

In various catabolic conditions associated with muscle wasting such as cancer, aging, denervation and glucocorticoid treatment, the expression of the effector proteins MAFbx and MuRF1 are highly elevated (22). MuRF1 and MAFbx have a distinct role in inducing muscle atrophy: MuRF1 is primarily involved in the proteolysis of sarcomeric proteins and enzymes regulating metabolic processes by ubiquitination of myosin heavy chains (MyHC) and enzymes involved in the generation of ATP, respectively (3; 22). MAFbx on the other hand downregulates protein synthesis and muscle fiber differentiation by mainly targeting the eukaryotic initiation factor 3 subunit f (elF3-f) and the myogenic regulatory transcription factor myogenic differentiation 1 (MyoD) (3; 22). Accordingly, MAFbx and MuRF1 are potential targets to blunt fiber atrophy, as evidenced by the amelioration of denervation-induced muscle mass loss by skeletal muscle-specific deletion of either MAFbx or MuRF1 (23). Numerous upstream signals induce the transcription of these E3 ubiquitin ligases and thereby promote muscle atrophy. For example, in conditions with reduced Akt signaling, dephosphorylated FoxO translocates into the nucleus and promotes transcription of MAFbx and MuRF1 (5; 24). Furthermore, in patients receiving glucocorticoid therapy, but also in sepsis or cancer, chronically elevated glucocorticoid levels promote the translocation of glucocorticoid receptor (GR) into the nucleus leading to increased transcription of various target genes including FoxO1/3, kruppel-like factor-15 (KLF15), MAFbx, and MuRF1 (25). Once induced by the GR, KLF15 can promote gene expression of MAFbx and MuRF1 directly as well as by upregulating FoxO (25). Moreover, KLF15-mediated regulation of branched-chain aminotransferase 2 (BCAT2) reduces mTOR activity and thereby shifts proteostasis towards degradation in a multi-pronged manner (25). Accordingly, GR-induced

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alterations in protein synthesis and degradation can be restored by BCAA-mediated stimulation of mTOR activity, which in turn inhibits GR signaling (25).

The nuclear factor κB (NF-κB) is another important transcription factor regulating the expression of the E3 ligases besides FoxO (26). In the context of elevated systemic inflammation (i.e. cancer), muscle atrophy is promoted by the canonical or non-canonical NF-κB signaling pathways (26). In response to binding of tumor necrosis factor α (TNFα) or interleukin 1 (IL-1) to their receptors TNFR1 and IL-1R, respectively, a series of events lead to the phosphorylation and subsequent proteosomal degradation of inhibitor of κB (IκB) by the IκB kinases (IKKα and IKKβ)/NF-κB essential modulator (NEMO/IKKγ) complex (27). This allows the translocation of NF-κB into the nucleus and the induction of gene transcription, including that of MuRF1 (27). TNF-like weak inducer of apoptosis (TWEAK) binding to the receptor fibroblast growth factor inducible 14 (Fn14) likewise increases NF-κB signaling (28). TWEAK/Fn14-induced muscle atrophy however is context-dependent, as TWEAK-signaling is for example increased in response to denervation, but not in response to glucocorticoids (29). Denervation massively increases Fn14 levels rather than TWEAK expression itself (29). TWEAK also regulates muscle mass by inhibiting Akt, which results in reduced protein synthesis as well as indirect promotion of the transcription of E3 ligases (28). In contrast to the activation of NF-κB in response to TWEAK, TNFα or IL-1, IL-6-induced muscle atrophy is primarily mediated by signal transducer and activators of transcription 3 (STAT3) signaling. The binding of IL-6 to the IL-6R/glycoprotein 130 (gp130) complex activates Janus kinases (JAKs), which in turn phosphorylate STAT3, resulting in its nuclear translocation and transcription of target genes such as the suppressor of cytokine signaling 3 (SOCS3) (30). SOCS3 disrupts IGF-1 signaling by inhibiting IRS-1 and thereby impairs protein synthesis (31). IL-6 and JAK signaling are therefore also promising targets to reduce inflammation-induced muscle wasting (32).

In heart failure, chronic kidney disease and other conditions leading to increased antiotensin II (AngII) levels, MuRF1 expression is induced by NF-κB signaling as well as the transcription factor EF (TFEB) (31; 33). Moreover, AngII also disrupts Akt – mTOR signaling by phosphorylating IRS-1 and thereby reducing PI3K activity (34). Besides the prominent effect of AngII on muscle protein turnover, AngII reduces food intake, which furthermore contributes to the development of cachexia (31).

To summarize, a complex network of numerous signals stimulating different pathways lead to muscle atrophy. In many muscle pathologies, e.g. in cancer cachexia, the origin of muscle wasting is multi-factorial, including systemic inflammation, reduced food intake and diminished physical activity, which hamper the treatment of these diseases and accordingly, no approaches are currently available to counteract this type of muscle wasting (35). However, several drugs are being tested that interfere with one or more of the described pathways involved in muscle proteostasis (Fig. 1). In the following section, the different classes of drugs and the current state of knowledge are summarized.

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Novel pharmacological interventions to improve muscle function

Anti-inflammatory drugs

Several pro-inflammatory cytokines are often elevated in plasma of patients with muscle wasting conditions and hence, anti-inflammatory drugs are attractive candidates to treat muscle wasting, albeit with mixed success. For example, direct inhibition of NF-κB signaling was effective to prevent denervation- or tumor-induced muscle mass loss in mice (26). Likewise, treatment of tumor-bearing mice with monoclonal antibody against Fn14 successfully prolonged survival and prevented loss of body weight, muscle and fat mass (36). Somewhat less pronounced, treatment with soluble TNFα receptor or IL-6 receptor antibodies ameliorated the cachectic phenotype, but could not completely reverse muscle wasting in animals (37; 38). Similarly, even though an early study showed beneficial effects of TNFα inhibitor (Thalidomide) on weight and muscle loss in cancer patients (39), several other clinical trials testing the effects of TNFα inhibitors or soluble TNFα receptors failed to show an improvement in appetite, body weight or muscle volume (see Table 1) (40; 41). Inhibition of IL-1α signaling by an IL1α-specific humanized monoclonal antibody (Xilonix, MABp1) increased lean body mass (LBM), however no control group was included in the study (42). Further studies are now ongoing and recruiting colorectal and pancreatic cancer patients (NCT02138422, NCT03207724). The humanized IL-6 monoclonal antibody ALD518 had no effect on LBM, but improved fatigue, which was most likely caused by the beneficial effect on anemia (43; 44). Whether a block in downstream signaling of IL-6 by the JAK1/2 inhibitor Ruxolitinib ameliorates muscle wasting in cancer patients remains to be investigated (NCT02072057). Likewise, pharmacological targeting of TWEAK action has so far only been tested in healthy volunteers regarding muscle plasticity. Due to the key role in the synthesis of prostanoids and prostaglandins, inhibition of cyclooxygenases (COXs) can potently affect systemic inflammation (45). Accordingly, plasma levels of IL-6 and IL-1 were significantly reduced and muscle mass increased upon treatment of aged rats with COX inhibitors (45). Furthermore, the COX2 blocker Meloxicam reduced rheumatoid arthritis-induced muscle mass loss in rats, together with a marked reduction in MAFbx and MuRF1 expression (46). This drug has so far not been tested in human patients in the context of muscle wasting. The COX2 inhibitor Celebrex elicited mixed results, with no effect on LBM in one study and a positive outcome on LBM and grip strength, as well as reduced serum TNFα in cancer patients in a second trial (47; 48).

Interfering with MSTN/ActRII signaling

MSTN powerfully reduces muscle mass and therefore, MSTN and the related signaling pathways mediated by the ActRII are promising avenues to counteract muscle wasting. Earlier trials aimed at exclusively blocking MSTN action have shown little clinical efficacy (49). In contrast, novel strategies that target the MSTN receptor and therefore additionally reduce the activity of other ligands seem more promising. For example, blocking MSTN and activin A signaling by administration of soluble ActRIIB preserved body weight and muscle mass and dramatically extended survival in tumor-bearing mice (50). This striking effect on cachexia and survival was achieved without affecting tumor mass or systemic inflammation (50). Several drugs inhibiting ActRIIB signaling are currently being tested in clinical trials (Table 1). At this point, there are two main strategies to target MSTN signaling: first, by

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directly neutralizing MSTN with a humanized MSTN antibody (LY2495655 or REGN1033) and second by blocking ActRII with a soluble ActRIIB (ACE-031 or ACE-083) or ActRII antibody (Bimagrumab/BMY338). LY2495655 treatment resulted in a mixed outcome by slightly increasing appendicular LBM and gait speed in elderly subjects (51). However, despite the gain in muscle mass, grip strength was not affected (51). In contrast, in pancreatic cancer patients, LY2495655 improved muscle mass as well a grip strength (52). Similarly, REGN1033 increased LBM with no effect on muscle strength and function in patients with sarcopenia (53). Even though blocking ActRII using BMY338 massively increased muscle mass in mice and prevented dexamethasone-induced muscle atrophy (54), the results in humans were less pronounced (55). LBM, muscle mass and 6-minutes-walk test seem to improve after 8 weeks of treatment in patients with body myositis, however no significant differences were observed after 24 weeks of treatment (55). Moreover, no beneficial effects on these parameters were reported in cancer patients, whereas in patients with COPD, muscle volume increased with no effect on functional measures, which is similar to the effects of BMY338 in patients with sarcopenia (56). Thus, so far, mainly muscle mass could be improved with these treatments with less consistent effects on muscle strength and other functional parameters. Nevertheless, several additional clinical trials are ongoing (NCT02152761, NCT02333331, NCT02468674).

Similar to the positive outcome of anti-MSTN treatment in animal models, GDF15 blockage in tumor-bearing mice prevented cachexia and massively prolonged survival (20). Besides its effect on skeletal muscle, GDF15 also signals through the glial cell-derived neurotrophic factor (GDNF) receptor alpha-like (GFRAL) in the brainstem to control food intake (57). To leverage these effects, a trial is starting in 2018 testing a monoclonal antibody against the GDF15 receptor GFRAL (NGM120) for the treatment of anorexia and cachexia in cancer patients (NTC03392116). To our knowledge, a direct targeting of GDF15 has so far not been tested in humans.

Other anti-catabolic drugs

Application of an angiotensin converting enzyme (ACE) inhibitor resulting in reduced AngII signaling preserved grip strength in tumor-bearing mice, but was not able to prevent loss of muscle mass (58). In patients with COPD, the ACE inhibitor Fosinopril did not affect muscle volume, strength or expression levels of E3 ligases in quadriceps muscle (59). In old mice, the AngII inhibitor Losartan prevented immobilization-induced muscle loss (60). However, no results have so far been published on the trial with Losartan in elderly subjects (NCT01989793).

Stimulation of protein synthesis

Instead of inhibition of protein degradation, stimulation of protein synthesis is another strategy to beneficially affect proteostasis and thereby counteract muscle wasting. For example, increasing muscle mass by testosterone treatment is well documented although its exact mechanism of action is still not fully elucidated (61; 62). Although the effects of testosterone on protein synthesis by binding to the androgen receptor (AR) may be muscle specific and more pronounced in perineal muscles, it is likely that AR signaling also induces IGF-1 transcription as well as activates the Akt – mTOR pathway by interacting with PI3K

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in limb muscles (63–65). However, the potential therapeutic anabolic application of testosterone on muscle tissue is marred by a wide number of unwanted and adverse effects. Therefore, tissue-specific non-steroid selective androgen receptor modulators (SARM), which retain anabolic, but not androgenic and other unwanted properties, are of great interest in treating muscle wasting (66). The treatment of healthy men, sarcopenic elderly or cancer patients with SARMs (LGD-4033, MK-0773 or Enobosarm) increased LBM, albeit without enhancing strength (67–70). Various SARMs are currently being tested in clinical trials (NCT03359473, NCT02463032, NCT02499497).

Although β_2-agonists such as Clenbuterol or Formoterol can reduce cancer- or denervation-induced muscle atrophy in rats (71; 72), clinical trials using β_2-agonists to treat muscle wasting are scarce. In cancer patients, APD209 (a combination of Megestrol, an orexigenic progestin with anti-androgen activity, and Formoterol) slightly increased muscle mass with no effect in strength (73). However, only 7 (out of 14) patients finished the study and no control group was included. Espindolol (a non-selective β-blocker with central serotonin 1A receptor and partial β2 receptor agonist effects) on the other hand increased LBM and grip strength in cancer patients, but could not improve in functional parameters (74).

Ghrelin exerts its effect on muscle wasting by several means such as stimulating appetite as well as the release of GH which in turn increases liver IGF-1 production (75). Ghrelin also has an anti-atrophic effect by stimulating Akt signaling independent of the GH/IGF-1-axis (76). Although ghrelin receptor agonists or synthetic ghrelin analogs such as Anamorelin and SUN11031 increased body weight and LBM, grip strength and other functional parameters were not increased in cachectic cancer patients (77–80). To complement the multimodal approach in cancer cachexia, numerous clinical trials aimed at orexigenic central effects have been executed, e.g. with Megestrol or cannabinoids, exhibiting only minor effects on muscle mass or functional parameters (81), even though several trials are still ongoing (NCT02359123, NCT02802540, NCT03245658). Similarly, nutritional interventions are being considered, for example the use of BCAAs, HMB or L-carnitine to mitigate muscle wasting (81). Even though some studies indicated that these nutrients provide some relief in cancer cachexia (81), more clinical studies are needed to quantify efficacy.

Inherited and sporadic muscular dystrophies

Besides secondary pathologies such as cachexia or sarcopenia, muscular dystrophies are a diverse group of diseases associated with often lethal muscle wasting. Even though some of these diseases are sporadic, many muscular dystrophies are caused by mutations of a particular gene leading to a truncated or dysfunctional protein. There is a large variability in disease etiology, onset, primarily affected muscles, severity as well as the involvement of respiratory and cardiac muscles (82). Accordingly, muscular dystrophies are divided into different subtypes such as congenital muscular dystrophy (CMD), Duchenne or Becker muscular dystrophy (DMD or BMD, respectively) or limb-girdle muscular dystrophy (LGMD) based on gene mutation and/or phenotype (83). The different subtypes of muscular dystrophies sharing a similar phenotype can be caused by mutations in distinct proteins with a similar cellular function (82). Even though almost every organelle and cellular structures,

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including metabolic pathways, mitochondrial function or the contractile apparatus can be affected and lead to the development of a dystrophic phenotype (84), the majority of mutated proteins belong to the dystrophin-associated glycoprotein complex composed of dystrophin, sarcoglycans and dystroglycans, (82). This complex links the cytoskeleton to the extracellular matrix and thereby provides stability to the muscle fiber against contraction-induced mechanical forces (85). Therefore, a dysfunctional protein leading to impaired stability of the dystrophin-associated glycoprotein complex renders the muscle fiber more vulnerable to contraction- and stretch-induced muscle damage. Small tears in the plasma membrane in response to mechanical stress cause calcium influx into the cytoplasm activating a series of events normally leading to repair and regeneration of the muscle fiber (85). In dystrophic muscles, an impaired regenerative capacity, exacerbated muscle damage and the ensuing dysregulation of signaling pathways however results in degeneration and necrosis of the muscle (85). Histologically, muscle fibers of dystrophic patients are thus often characterized by centrally located nuclei, infiltration of macrophages, fiber necrosis and replacement of muscle tissue by fat and fibrotic tissue (83). Additionally, due to increased muscle damage, serum creatine kinase (CK) levels are elevated and used as a diagnostic marker (83).

Among the inherited muscular dystrophies, DMD is the most common childhood muscle disease affecting approximately 1 in 6000 newborn boys worldwide (86). DMD is caused by a mutation of the dystrophin gene, which is located on the X chromosome (82). A nonsense mutation or in-frame deletion in the dystrophin gene leading to a truncated protein induces a milder form of the disease called BMD (85). Dystrophin plays an important role in linking the cytoskeleton to the dystroglycan complex by N-terminal binding to F-actin and C-terminal binding to β-dystroglycan (85). As the most functional domains of dystrophin are in the N- and C-terminus, deletion of exons located in the central rod-domain of the gene by exon-skipping would not affect the main functionality and could thereby ameliorate DMD (87).

Mutations of sarcolemma-associated proteins such as the sarcoglycans or dysferlin are generally the underlying cause of LGMDs (82). LGMDs can be inherited in a dominant or recessive manner and are named LGMD1 or LGMD2, respectively (82). Defects in extracellular matrix proteins including laminin α2 or collagen VI predominantly result in CMD (82). Interestingly, mutations in genes encoding enzymes involved in the glycosylation of α-dystroglycan can cause LGMDs as well as CMD, highlighting the complexity and diversity of the etiology of muscular dystrophies (82). Even mutations within the same gene can lead to different phenotypes as seen in patients with mutations in dysferlin causing either LGMD2B affecting predominantly proximal muscles or Miyoshi muscular dystrophy affecting distal muscles (88) or the glycosyltransferase fukutin associated with either LGMD2M or CMD (82). Even though a great number of genes of which mutations lead to muscular dystrophies are known, the complete spectrum of the function of these proteins is not fully elucidated. Therefore, the diversity of genes responsible for muscular dystrophies as well as the different manifestation of the disease aggravates the development of therapies for patients. Based on the etiology of these dystrophies, specific genes or even particular mutations have to be targeted in a personalized manner. In the following section, we will provide an overview of the different novel approaches to treat muscular dystrophies.

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Therapeutic strategies to treat muscular dystrophies

Up to date, there is no effective treatment available for the vast majority of muscular dystrophies. For example, standard care for DMD patients includes glucocorticoid treatment, which decelerates the decline in muscle strength and function and thereby prolongs ambulation (89). These benefits, which might be based on the anti-inflammatory effect of these compounds (90), are somewhat paradoxical in light of the pro-atrophic action of the GR in muscle, and caveats about potential adverse effects in long-term treatment have been raised (89). In DMD and other muscular dystrophies with cardio-respiratory impairment, therapies also address cardiological as well as respiratory symptoms, the latter by mechanical ventilation (83). Furthermore, various efforts are made to treat the skeletal muscle wasting symptoms of dystrophies in a similar manner as in secondary muscle wasting, including the use of anti-inflammatory drugs, MSTN blockers (PF-06252616/ Domagrozumab, BMS-986089, follistatin by rAAV1.CMV.huFollistin344), anti-oxidants (Raxone/Idebenone), histone deacetylase (HDAC) inhibitors (Givinostat), ACE or PDE5 inhibitors (Tadalafil), or by stimulation of muscle regeneration (91; 92). Other approaches are targeted at the specific genes affected in muscular dystrophies, e.g. replacement of the defective protein by pharmacological upregulation of a protein that compensates for the lost function. In DMD, the induction of utrophin, a functional homolog of dystrophin that normally is only expressed in the subsynaptic region of adult muscle, by small molecules such as Ezutromid/SMTC1100 or the second-generation compound SMT022357, aims at ameliorating disease progression (93; 94). Of these compounds, Ezutromid currently is in phase II trials in DMD patients (NCT02858362). Intriguingly, several compounds boost utrophin gene expression by inducing an oxidative muscle phenotype in pre-clinical models, including the AMP-dependent kinase (AMPK) activator AICAR, resveratrol, metformin and L-Arginine. Of note, the latter two have now entered phase I clinical trials (92).

In muscular dystrophies with known gene mutations, strategies aimed at targeting the primary defect by replacing the mutated gene, premature termination codon (PTC) read-through and exon-skipping have made tremendous progress recently. So far, adeno-associated viral (AAV)-based vectors are the most efficient system to deliver DNA into nuclei of non-dividing cells with a low immunogenicity (95). Although in most cases, AAV is a suitable vehicle for human use and to induce long-term expression of the payload gene, the packaging capacity of the virus is limited to ~4.7 kb (95). However, even though full-length genes might exceed this capacity (i.e. the full-length dystrophin cDNA is 14 kb in size), a smaller, truncated version of the target gene containing the most functional parts of the protein could still exert therapeutic effects by reducing the disease phenotype. In mice or golden retrievers with DMD, transduction of two different truncated forms of dystrophin, mini-dystrophin and micro-dystrophin, ameliorated disease progression and extended lifespan (96–99). Based on these promising pre-clinical data, several phase I/II clinical trials are currently being planned or conducted aiming at treating DMD patients with mini- or micro-dystrophin (see Table 1) (100). Phase I clinical trials of LGMD2C and LGMD2D patients treated with AAV vectors containing either γ-sarcoglycan or α-sarcoglycan, respectively, have been completed or are ongoing. Even though no adverse effects have been reported, detection of the transcript and protein is variable (101–103). Clinical trials are

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currently ongoing to treat LGMD2B patients with AAV vectors containing dysferlin. So far, the clinical trials have only used intramuscular injections as proof-of-concept. In a next step, efficient systemic delivery via intravenous injections, which enables gene transfer in all muscles, is being established and optimized. The validity of this approach is still gaining traction and several pre-clinical trials showed encouraging results. For example, a combination of elevation of a mini-agrin together with specifically designed linker proteins to enhance the polymerization of laminins enhanced muscle function and life expectancy of a mouse model of merosin-deficient CMD (104).

In 10% of DMD patients, nonsense mutations lead to a PTC, resulting in a truncated dystrophin and, depending on the size of the remaining protein, in a milder phenotype of the disease (105). Premature termination can be suppressed by the binding of a read-through agent such as the aminoglycoside antibiotic gentamicin to the ribosome and thereby preventing the recognition of the stop codon, thus allowing full-length translation of the protein (106). However, due to serious oto-nephrotoxic side effects of gentamicin, Ataluren (also known as PTC124/Translarna) with little off-target effects and a better safety profile is currently being pursued as a PTC agent in patients (106; 107). In Phase II and III trials, Ataluren successfully increased dystrophin levels in the majority of treated DMD patients, lead to a reduction in CK levels, but could not robustly improve walking ability (108–110). Thus, while Ataluren is approved by the European Medicine Agency (EMA) under the trade name Translarna, approval by the FDA has so far been declined (111). Several clinical trials with Ataluren are still ongoing and currently recruiting DMD patients (see Table 1). Although other muscular dystrophies could likewise benefit from PTC suppression by Ataluren, for example as suggested in experiments in myotubes from patients with a nonsense mutation in the dysferlin gene, this drug has only been tested in clinical trials in DMD so far (112).

The majority of mutations in DMD patients are large deletions (68%) of which 80% are located between exon 45-55 (105). These patients accordingly will not benefit from PTC-based strategies. However, exon-skipping would help in individuals with frame-shift-inducing and other non-PTC mutations by restoring the expression of a truncated dystrophin protein similar to that of BMD patients with an in-frame deletion, hence resulting in a milder phenotype. To induce exon skipping, antisense oligonucleotides (AONs) are delivered to muscle tissue as 2´-O-methyl-phosphorothionate RNA olionucleotides (2'OMe) or phosphorodiamidate morpholino oligonucleotides (PMOs), which then bind to the targeted exon of the pre-mRNA (113–115). By masking the recognition site, spliceosome binding is hindered, leading to the splicing of the intron as well as the targeted exon (91; 115). In DMD, skipping of exon 51 is applicable for 14% of patients representing the largest group, followed by exon 53 and 45 with 10.1% and 9% eligible patients, respectively (105). Of all therapies for DMD, exon skipping is the most advanced and promising so far, with the AON Eteplirsen (also known as AVI-4658/Exondys 51) as the first accelerated FDA-approved representative of this class of drugs (116). Further clinical trials are being performed by Sarepta Therapeutics to further substantiate the clinical benefits in DMD (see Table 1). Treating DMD patients with Eteplirsen partially restores dystrophin expression and, importantly, significantly improves walking ability (114; 117–119). Sarepta Therapeutics has designed other AONs targeting exon 45 (SRP-4045/Casimersen) and 53 (SRP-4053/

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Golodirsen), currently in phase III, and a second-generation exon 51 AON (SRP-5051) in phase I (120). Two other AONs, DS-5141b and NS-065/NCNP-01, targeting exon 45 and 53, respectively, are also in clinical trials. The development of the AONs Drisapersen/Kyndrisa/PRO051/GSK2402968, BMN044/PRO044, BMN045/PRO045 and BMN053/PRO053 by BioMerin Pharmaceutical have been terminated because the phase III trial of Drisapersen failed to improve walking ability (NCT01254019) (121) and FDA approval was accordingly refused (122).

Conclusion and perspectives

To summarize, despite the massive efforts to develop therapies to treat muscle wasting, there are still very few drugs available in a small subset of these pathologies. Even though some aspects of muscle wasting could be ameliorated with some of the tested drugs (i.e. muscle mass), most trials fail to show significant improvement in the defined clinical endpoints, which usually are functional parameters. Therefore, drug discovery will have to go hand-in-hand with the development of sensitive tests to measure clinically relevant functional improvements, which then could help clinical trials to reach their primary goals. In addition, due to the multi-factorial nature of secondary muscle wasting and the large variations between patients, a multipronged approach that includes pharmacological, nutritional and exercise interventions might be necessary, aimed at skeletal muscle and other tissues, e.g. orexigenic effects in the brain. Furthermore, as more mechanistic aspects of muscle wasting are being revealed, novel potential targets are emerging, most of which exhibited promising results in experimental and pre-clinical experiments. For example, elevated parathyroid-hormone-related peptide (PTHrP) levels in models of cancer or kidney failure induces muscle atrophy as well as browning of white adipose tissue and thereby exacerbates energy expenditure (123; 124). Neutralizing PTHrP accordingly prevented cancer cachexia (123; 124). Similarly, the peroxisome proliferator-activated receptor α (PPARα) emerged from a metabolic analysis of liver function in cancer cachexia and pharmacological activation of PPARα by Fenofibrate also blunted muscle wasting in cancer cachexia by systemic remodeling of metabolism (125). Finally, the PPARγ coactivator 1α (PGC-1α), a regulatory nexus of endurance adaptation in skeletal muscle, reduced fiber damage and atrophy, as well as increased contractile function in different animal models of muscle wasting, including DMD (126; 127). Nutritional and pharmacological interventions aimed at these and similar factors could constitute potential therapeutic avenues to treat muscle wasting, e.g. by leveraging exercise-associated mechanisms (see Sidebar) (128–130). However, these factors and the related pharmacological activators will have to stand the test of time in a clinical setting.

Exon-skipping and gene editing are areas were currently more concrete progress is observed in regard to optimization of existing and the development of new treatment strategies. In many cases, exon skipping by AONs is hampered by poor cellular uptake after systemic administration and the tissue-specific skipping efficiency, which is better in skeletal muscle as compared to cardiac muscles (91). In animals, alternative strategies to improve the delivery efficiency of AONs have been developed (131). For instance, linking the antisense sequence to a small nuclear RNA (U7 snRNA) enhances the engagement in RNA processing (92). By using an AAV vector for delivery, exon-skipping was efficiently induced and

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dystrophin levels restored in this study (92). In addition, delivery efficiency of AONs to skeletal as well as cardiac muscle could be significantly enhanced by peptide-conjugated PMOs, providing another alternative method to rescue dystrophin levels in both tissues (132). Systemic delivery of PMOs could also be enhanced by natural carriers such as Saponin (133). Furthermore, the systemic delivery of a tricycle-DNA-AON was more efficient in improving muscle function compared to 2'OMe or PMOs (134). Importantly, dystrophin levels were not only restored in limb skeletal muscles, but also in diaphragm and the heart, showing the potential of this approach to enhance cardio-respiratory function (134; 135). The disadvantage of AONs to target individual exons can be overcome by using multi-exon-skipping targeting the mutation hot spot (exon 45-55), which would allow a common therapy of approximately 63% of DMD patients (136; 137). Finally, CRISPR/Cas9 as a tool for genome editing has emerged as a novel approach to treat muscular dystrophies (138). Experimentally, delivery of the CRISPR/Cas9 components Cas9 nuclease and specific single-guide RNAs to the muscle by AAV vectors lead to the rescue of dystrophin expression, either by inducing exon-skipping, generation of a full-length protein or inducing an in-frame deletion (139; 140). The use of Cpf1, an alternative nuclease to Cas9, exhibited similar results in regard to the restoration of dystrophin in mice (141). The ability to directly editing DNA and thereby induce permanent genomic changes is a major advantage of CRISPR/Cas9 technology (138). However, various hurdles, including delivery systems and potential off-target effects will have to be addressed before clinical application will be possible. Along the same lines, stem cell treatment of muscle dystrophies, either in a heterologous manner from healthy donors, or by engineering of autologous stem cells or inducible pluripotent stem cells (iPS), has been successfully used in pre-clinical experiments and clinical proof-of-concept studies, but systemic application in patients remains to be established (138; 142–144). Therefore, even though few therapies have arrived in the clinic so far, a wide variety of novel avenues is on the horizon to fix the current drought in drugs for muscle wasting pathologies.

Acknowledgments

The work in our laboratory is supported by the Swiss National Science Foundation, the European Research Council (ERC) Consolidator grant 616830-MUSCLE_NET, Swiss Cancer Research grant KFS-3733-08-2015, the Swiss Society for Research on Muscle Diseases (SSEM), SystemsX.ch, the "Novartis Stiftung für Medizinisch-Biologische Forschung" and the University of Basel.

Terms and Definitions

AAV	adeno-associated virus
ActRIIB	activin receptor type-IIB
AON	antisense oligonucleotides
BMD	Becker muscular dystrophy
CMD	congenital muscular dystrophy
DMD	Duchenne muscular dystrophy

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GH	growth hormone
GR	glucocorticoid receptor
LBM	lean body mass
LGMD	limb-girdle muscular dystrophy
MAFbx	muscle atrophy F-box protein
MuRF1	muscle RING finger-containing protein 1
MSTN	myostatin
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor κB
PGC-1α	peroxisome proliferator-activated receptor γ coactivator 1α
PTC	premature termination codon
SARM	selective androgen receptor modulator
TNFα	tumor necrosis factor α
TWEAK	TNF-like weak inducer of apoptosis

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Summary points

- Despite a high prevalence of secondary muscle wasting, i.e. in sarcopenia and cachexia, mechanistic insights into disease etiology and therapeutic options remain rare.
- Muscular dystrophies, many of which are fatal, suffer from a lack of treatment options.
- In recent years, new targets and approaches have been identified and are currently being tested in clinical trials.
- For secondary muscle wasting pathologies, multipronged approaches aimed at pharmaceutical, nutritional and exercise interventions will have to be considered.
- In muscular dystrophies, gene replacement and editing strategies might lead to future clinical breakthroughs.

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Sidebar

“Exercise mimetics”: a new principle for the pan-treatment of muscle wasting symptoms?

In recent years, the idea of designing and applying so-called “exercise mimetics”, pharmacological agents that regulate the effects of endurance and/or resistance exercise, has gained tremendous traction. Partial proof-of-concept has been provided for a range of compounds, mostly aimed at boosting an oxidative, high endurance muscle phenotype in experimental models. Currently, most of these agents ultimately affect the expression and/or activity of PGC-1α (reviewed in ref. 128). In several animal models of muscle wasting disorders, AICAR, resveratrol and other members of this type of compounds resulted in an amelioration of the disease phenotype. However, none of these “exercise mimetics” has been translated into clinical application to date. Moreover, the concept of “exercise mimetics” is highly controversial and compelling arguments have been put forward that a pharmacological agent is unable to elicit the pleiotropic and complex exercise-induced plastic changes in skeletal muscle and other organs (reviewed in refs. 128; 130).

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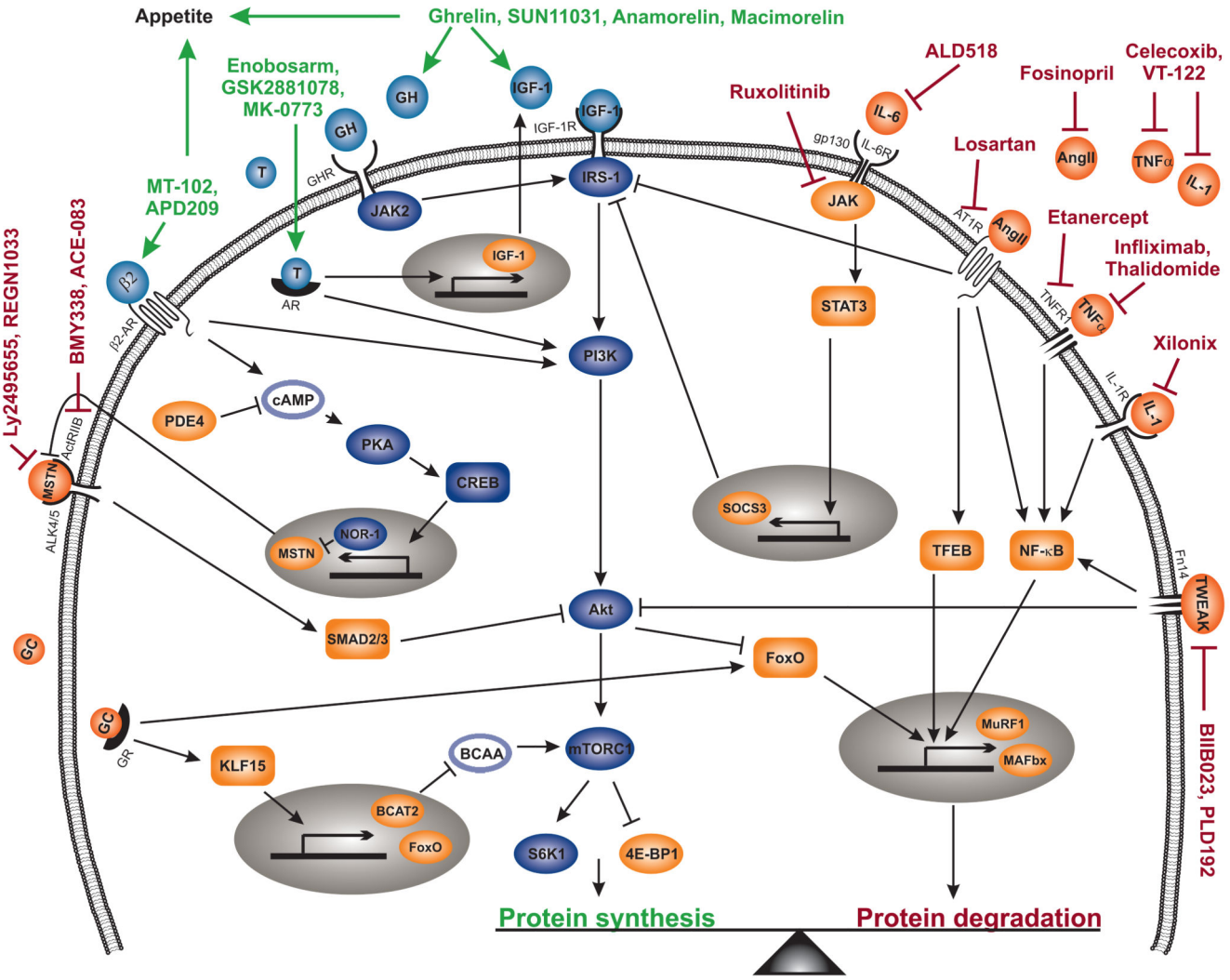

Figure 1. Signaling pathways involved in secondary muscle wasting and potential drugs to reduce loss of muscle mass.

Several classes of drugs are tested in clinical trials aimed at either inhibiting catabolic signaling (drug name red) and thereby reducing protein degradation (orange), or stimulating anabolic pathways (drug name green) and thereby increasing protein synthesis (blue). Activation via IGF-1, but also other upstream signals such as GH, androgens or β2-agonist, stimulate the PI3K – Akt – mTOR pathway and, as a consequence, protein synthesis is increased. In addition, Akt suppresses protein degradation by phosphorylating FoxO and thereby reducing its transcriptional activity. Several catabolic pathways do not only contribute to muscle atrophy by increasing the expression of E3 ligases MAFbx and MuRF1 via the transcription factors FoxO, TFEB or NF-κB, which all stimulate protein degradation, but also by inhibiting PI3K – Akt signaling and thereby reduce protein synthesis. MSTN is a major negative regulator of muscle mass by inhibiting Akt signaling. The multi-factorial nature of secondary muscle wasting and the different signaling pathways involved in muscle

atrophy aggravates the development of effective drugs. Abbreviations not mentioned in the text: T = testosterone; GC = glucocorticoid

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Annu Rev Pharmacol Toxicol. Author manuscript; available in PMC 2019 August 20.

Table 1

Table summarizing clinical trials performed to ameliorate secondary muscle wasting or muscular dystrophies

Drug name	Type of drug	Company	Completed (NCT-ID)	Phase	Patients	Results	Ongoing (NCT-ID)	Phase	Patients
Secondary muscle wasting									
Anti-inflammatory									
Etanercept	Soluble TNFα receptor	Mayo Clinic Cancer Center	NCT00046904	Phase III	Cancer patients	↔ BW, appetite			
Etanercept (Enbrel)	Soluble TNFα receptor	Amgen	NCT00802815	Phase I	Body myositis	No results			
Infliximab	Anti-TNFα	Various	NCT00060502	Phase II	Cancer patients	↔ BW, appetite			
			NCT00040885	Phase III					
Lenalidomide (Revlimid)	Anti-TNFα		NCT01127386	Phase I/II	Cancer patients	No results found			
Thalidomide	Anti-TNFα	Celgene Corporation	NCT00379353	Phase II	Cancer patients	↔ BW, appetite			
Xilonix (MABp1)	Anti-IL-1α	XBiotech, Inc.	NCT01021072	Phase I	Cancer patients	NCG	NCT03207724	Phase I	Cancer patients
						↑ LBM	NCT02138422	Phase III	
ALD518	Anti-IL-6	Alder Biopharmaceuticals, Inc.	NCT00866970	Phase II	Cancer patients	↔ LBM ↓ fatigue			
Ruxolitinib	JAK 1/2 inhibitor	Novartis					NCT02072057	Phase II	Cancer patients
BIIB023	Anti-TWEAK	Biogen	NCT01407406	Phase I	Healthy volunteers	Pharmacokinetics No results found on muscle atrophy			
			NCT01943513	Phase I					

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Drug name	Type of drug	Company	Completed (NCT-ID)	Phase	Patients	Results	Ongoing (NCT-ID)	Phase	Patients
PDL192	Anti-TWEAK receptor	Abbott Biotherapeutics Corp.	NCT00738764	Phase II	Cancer patients	Tolerability			
OHR/AVR 118	Peptide nucleic acid immune-modulator	OHR Pharma	NCT01206335	Phase II	Cancer patients	NCG ↔ BW, GS			
Celecoxib (Celebrex)	Cox2 inhibitor	Pfizer		Phase II	Cancer patients	Study with NCG: ↑ LBM, GS ↓ serum TNFα Study with CG: ↔ LBM			
VT-122	β-blocker + COX inhibitor	Vicus Therapeutics	NCT00527319	Phase II	Cancer patients	No statistics found	NCT01265576	Phase II	Cancer patients
Anti-MSTN/anti-ActRII									
ACE-083	Soluble ActRIIB	Acceleron Pharma, Inc.	NCT02257489	Phase I	Healthy volunteers	↑ MV	NCT02927080	Phase II	Facioscapulo humeral Muscular Dystrophy
BMY338 (Bimagrumab)	Anti-ActRII	Novartis Pharmaceuticals	NCT01433263	Phase II	Cancer patients, COPD, body myositis	↑ LBM, MV ↔ FP	NCT02152761	Phase II	Elderly
			NCT01669174	Phase II			NCT02333331	Phase II	
			NCT01925209	Phase II/III			NCT02468674	Phase II	
			NCT02573467	Phase III					
LY2495655	Anti-MSTN	Eli Lilly and Company	NCT01604408	Phase II	Elderly, cancer patients	↑ LBM, MV mixed results on FP			
			NCT01369511	Phase II					
			NCT01505530	Phase II					
REGN1033 (SAR391786)	Anti-MSTN	Regeneron Pharmaceuticals	NCT01963598	Phase II	Elderly	↑ LBM ↔ FP			

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Drug name	Type of drug	Company	Completed (NCT-ID)	Phase	Patients	Results	Ongoing (NCT-ID)	Phase	Patients
Anti-catabolic									
Fosinopril	ACE inhibitor	Imperial College London, Medical Research Council	NCT01014338	Phase IV	COPD	↔ MV, MAFbx and MuRF1 mRNA			
Losartan	Ang-receptor blocker	Johns Hopkins University	NCT01989793	Phase II	Elderly	No results yet			
Anabolic									
Enobosarm (GTx-024)	SARM	GTx	NCT00467844	Phase II	Cancer patients	↑ LBM ↑ stair climbing ↔ GS, 6MWT	NCT02463032	Phase II	Cancer patients
			NCT01355484	Phase III					
			NCT01355497	Phase III					
GSK2881078	SARM	GSK Clinical Trials, GlaxoSmithKline	NCT02045940	Phase I	Healthy volunteers, elderly women	Pharmacokinetics and tolerability	NCT03359473	Phase II	COPD
			NCT02567773	Phase I					
Ly2452473	SARM	Dana-Farber Cancer Institute					NCT02499497	Phase II	Cancer patients
MK-0773	SARM	Merck Sharp & Dohme Corp.	NCT00529659	Phase II	Elderly women	↑ LBM ↔ FP			
Anamorelin HCl	Ghrelin receptor agonist	Helsinn Therapeutics (U.S.), Inc	NCT01387282	Phase III	Cancer patients	↑ BW, LBM ↔ GS	NCT03035409	Phase II	Cancer patients
			NCT01395914	Phase III					
Ghrelin	Natural ghrelin	Cantonal Hospital St. Gallen KSSG	NCT00933361	Phase I/II	Cancer patients	Phamacokinetics			
Macimorelin (AEZS-130)	Ghrelin receptor agonist	AEterna Zentaris					NCT01614990	Phase II	Cancer patients

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Drug name	Type of drug	Company	Completed (NCT-ID)	Phase	Patients	Results	Ongoing (NCT-ID)	Phase	Patients
SUN11031	Synthetic analog of human ghrelin	Daiichi Sankyo, Inc.	NCT00698828	Phase II	COPD	↑ BW, LBM ↔ FP			
APD209	Combination of megestrol and formoterol (β2- agonist)	Acacia Pharma Ltd	NCT00895726	Phase II	Cancer patients	only 7/14 finished; ↑ MV ↔ GS			
MT-102 (Espindolol)	Non-selective β- blocker with central seretonin 1A receptor and partial β2 receptor agonist effects	PsiOxus Therapeutics Ltd	NCT01238107	Phase II	Cancer patients	↑ LBM, GS ↔ FP			
NGM120	Anti-GFRAL	NGM Biopharmaceuticals, Inc					NCT03392116	Phase I	Healthy volunteers
Muscular dystrophies									
Gene therapy									
AAV-GALGT2	rAAVrh74.MCK.GrAAVrh74.MCK.GALGT2 (β-1,4 Nacetylgalactosaminyltransferase 2(GALGT2 or B4GALNT2)) (via a major lower limbartery)	The Research Institute at Nationwide Children's Hospital					NCT03333590	Phase I/II	DMD
AAV-microdystrophin	rAAVrh74.MCK.M icro-Dystrophin (i.m.)	The Research Institute at Nationwide Children's Hospital	NCT02376816	Phase I	DMD	No results available yet			
AAV-microdystrophin	SGT-001 (AAV9 vector containing muscle-specific promoter and microdystrophin construct); (i.v.)	Solid Biosciences, LLC					NCT03368742	Phase I/II	DMD
AAV-microdystrophin	rAAVrh74.MHCK 7.micro-dystrophin (i.v.)	The Research Institute at Nationwide Children's Hospital					NCT03375164	Phase I/II	DMD
AAV-minidystrophin	rAAV2.5-CMV-minidystrophin (d3990) (i.m.)	Asklepios Biopharmaceutical,Inc. and the Research Institute at Nationwide Children's Hospital	NCT00428935	Phase I	DMD	Detection of vector DNA;Mini-dystrophin protein detected in 2/4 patients			
AAV-minidystrophin	PF-06939926 (rAAV9 carrying a truncated mini-dystrophin); (i.v.)	Pfizer					NCT03362502	Phase I	DMD

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Drug name	Type of drug	Company	Completed (NCT-ID)	Phase	Patients	Results	Ongoing (NCT-ID)	Phase	Patients
AAV-dysferlin	rAAVrh74.MHCK 7.DYSF.DV (i.m.)	The Research Institute at Nationwide Children's Hospital					NCT02710500	Phase I	Dysferlinopathy
AAV-SGCA	rAAV1.tMCK.human-alpha-sarcoglycan (i.m. and via a major lower limb artery)	The Research Institute at Nationwide Children's Hospital	NCT00494195	Phase I	LGMD2D	No adverse effects SGCA mRNA and protein detectable in 2/3	NCT01976091	Phase I/II	LGMD2D
AAV-SGCG	AAV1-gamma-sarcoglycan vector injection (i.m.)	Genethon	NCT01344798	Phase I	LGMD2C	No adverse effects SGCG mRNA level detectable in 4/9 and protein in 1/9			
PTC read-through									
Ataluren (Translarna,PTC124)	PTC read-through	PTC Therapeutics	NCT00264888	Phase II	DMD, BMD	Well tolerated ↓ CK levels ↔ 6MWT overall ↑ 6MWT in subgroup	NCT0281955	Phase II	DMD, BMD
			NCT00592553	Phase II			NCT01557400	Phase III	
			NCT01826487	Phase III			NCT02090959	Phase III	
			NCT01247207	Phase III					
			NCT02369731	PASS					
			NCT03179631	Phase III					
Gentamicin	PTC read-through	The Research Institute at Nationwide Children's Hospital	NCT00005574	Phase I	DMD, BMD	↓ CK levels Oto-nephrotoxicity			
			NCT00451074	Phase I					
NPC-14 (Arbekacin)	PTC read-through	Kobe University					NCT01918384	Phase II	DMD
Exon-skipping									

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Drug name	Type of drug	Company	Completed (NCT-ID)	Phase	Patients	Results	Ongoing (NCT-ID)	Phase	Patients
DS-5141b	Skipping exon 45 (s.c.); ENA	Daiichi Sankyo Co., Ltd.				NCT02667483	Phase I/II	DMD	
SRP-4045	Skipping exon 45 (i.v.); PMO	Sarepta Therapeutics					NCT02530905	Phase I	DMD
							NCT02500381	Phase III	
Eteplirsen (EXODYS 51, AVI-4658)	Skipping exon 51 (i.v. and i.m NCT00159250); PMO	Sarepta Therapeutics	NCT00159250	Phase I/II		Well tolerated ↑ 6MWT ↓ incidence of loss of ambulation	NCT03218995	Phase II	DMD
			NCT00844597	Phase I/II			NCT02286947	Phase II	
			NCT01396239	Phase II			NCT02420379	Phase II	
			NCT01540409	Phase II			NCT02255552	Phase III	
SRP-5051	Skipping exon 51 (i.v.); PPMO	Sarepta Therapeutics					NCT03375255	Phase I	DMD
SRP-4053	Skipping exon 53 (i.v.); PMO	Sarepta Therapeutics					NCT02310906	Phase I/II	DMD
							NCT02500381	Phase III	
NS-065/ NCNP-01	Skipping exon 53 (i.v.); PMO	NS Pharma, Inc.					NCT02740972	Phase II	DMD
							NCT03167255	Phase II	

Abbreviations: BW = body weight; LBM = lean body mass; MV = muscle volume; GS = grip strength; 6MWT = 6-minute walk test; FP = functional parameters; NCG = no control group, CG = control group; i.m. = intramuscular; i.v. = intravenous; SGCA = α-sarcoglycan; SGCG = γ-sarcoglycan; PMO = phosphorodiamidate morpholino oligonucleotides; PPMO = next-generation peptide PMO; ENA = 2'-O,4'-C-ethylene-bridged nucleic acid; PASS = post-approval safety study.

Clinical trials performing intervention studies to counteract secondary muscle wasting (i.e. cachexia, sarcopenia) by targeting muscle tissue were searched on January 2018 using www.clinicaltrial.gov; Phase I and II trials were only selected if finished within the last 5 or 10 years, respectively or if followed-up with additional trials; no combined interventions were included (e.g. drug and exercise).

Clinical trials performing intervention studies to ameliorate muscular dystrophies were searched on February 2018 using www.clinicaltrial.gov; only gene therapy, PTC read-through and exon-skipping were included in the search.