

Myostatin inhibitors as pharmacological treatment for muscle wasting and muscular dystrophy

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Abstract

Myostatin, a member of the transforming growth factor beta (TGF- β) superfamily that is highly expressed in skeletal muscle, was first described in 1997. It has been known that loss of myostatin function induces an increase in muscle mass in mice, cow, dogs and humans. Therefore, myostatin and its receptor have emerged as a therapeutic target for loss of skeletal muscle such as sarcopenia and cachexia, as well as muscular dystrophies. At the molecular level, myostatin binds to and activates the activin receptor IIB (ActRIIB)/Alk 4/5 complex. Therapeutic approaches therefore are being taken both pre-clinically and clinically to inhibit the myostatin signaling pathway. Several myostatin inhibitors, including myostatin antibodies, anti-myostatin peptibody, activin A antibody, soluble (decoy) forms of ActRIIB (ActR II B-Fc), anti-myostatin adnectin, ActR II B antibody have been tested in the last decade. The aim of this review is to present the current knowledge of several myostatin inhibitors as a therapeutic approach for patients with loss of skeletal muscle.

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Introduction

Myostatin, also known as growth differentiation factor-8 (GDF-8) is a member of the growth factor β (TGF- β) superfamily. It was first identified in 1997 [1]. Myostatin is synthesized as a precursor protein that undergoes proteolytic processing at a dibasic site to generate an N-terminal propeptide and a disulfide linked C-terminal dimer. Proteolytic cleavage of the propeptide by the bone morphogenetic protein (BMP)-1/tolloid family of metalloproteinases is necessary for activation of its protein function [2]. Myostatin is expressed almost exclusively in cells of the skeletal muscle lineage in humans, and evidence indicates that myostatin acts as an essential negative regulator of muscle bulk [3]. The role of myostatin in skeletal muscle was discovered using the method of gene disruption in animals [3-6]. In addition, a mutation at the myostatin locus that leads to the absence of myostatin expression has been reported leading to significant muscle over-growth in a child [7]. This “hypertrophy” phenotype has been replicated in animal models by pharmacological blockade of myostatin protein, and inactivation of myostatin in dystrophic animal models exerted positive effects on disease

progression [8]. It has therefore been suggested that myostatin may be a primary target of pharmacological interventions in muscular dystrophy [8-10]. Importantly, myostatin has been shown to be up-regulated in advanced age, and patients with chronic disease such as heart failure, chronic kidney disease, or chronic obstructive pulmonary disease have been described to have elevated levels [11-13]. The utility of myostatin inhibitors to treat muscle wasting in the setting of chronic diseases has generated keen interest worldwide.

The purpose of this review is to provide an overview of the clinical developments in the field of myostatin inhibitors. In addition, we summarize the results of clinical trials in patients with loss of skeletal muscle mass as a result of sarcopenia, cachexia, or muscular dystrophies.

The molecular mechanism of myostatin function

The myostatin signaling pathway and its role in regulating skeletal muscle has been recently reviewed [14,15]. In molecular biology, myostatin binds to its receptor complex activin type IIB (ActRIIB) /Alk 4 or 5 on skeletal muscle resulting in activation of two serine residues of small mothers against decapentaplegic

(Smad) 2/3, representing intracellular proteins that transduce extracellular signals from TGF- β ligands to the nucleus. One of the important downstream targets of Smad signaling is MyoD, a transcription factor that is involved in protein synthesis that ultimately gives rise to muscle wasting. Moreover, myostatin also down-regulates MyoD in a nuclear factor- κ B (NF- κ B) independent way, and inhibits pax3 expression, which is possibly an upstream target of MyoD [16,17]. Other TGF- β family members were shown to also activate mitogen-activated protein kinases (MAPKs), particularly p38 and extracellular signal-regulated kinase 1/2 (ERK1/2), and its signalling results in the down-regulation of myogenesis-related genes in a Smads-independent signaling fashion.

In addition to myostatin, ActRIIB binds to a diverse group of TGF- β family members, including activin A, BMP-2, BMP-7, and GDF-11. The soluble form of ActRIIB (sActRIIB), a fusion protein of the receptor extracellular domain with immunoglobulin Fc, acts as a decoy receptor, which is freely circulating and removes protein, myostatin and other negative regulators inhibiting the growth of skeletal muscle. Therefore, the majority of pharmacological approaches target extracellularly to block myostatin engaging the ActRIIB/Alk4/5 receptor complex, either by binding directly to myostatin itself or by binding to components of this receptor complex.

Clinical development of myostatin inhibitors for muscle wasting

Myostatin was first recognized as an endogenous inhibitor of muscle growth in 1997 [1]. Several lines of evidence indicated that antagonization of myostatin, activin A, and GDF11 signaling are a promising therapeutic approach for multiple types of muscle wasting. The first human trials of a myostatin inhibitors named MYO-029, a recombinant human antibody, began in 2004. Since then several myostatin inhibitors have been developed as myostatin antibodies, anti-myostatin peptibodies, activin A antibodies, soluble (decoy) forms of ActRIIB (ActR II B-Fc) / recombinant fusion proteins, anti-myostatin adnectin, and ActR II B antibodies (Figure1). Myostatin inhibitors have progressed into clinical development to prove therapeutic benefit as summarized in Table 1.

Myostatin antibodies

Wyeth Pharmaceuticals's stamulumab (previously named MYO-029) is a recombinant human immunoglobulin G (IgG)1(λ) antibody that binds to myostatin and neutralizes its activity by preventing its binding to the endogenous high-affinity receptor ActRIIB. It was developed for the treatment of muscular dystrophies in subjects with Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, or Limb-girdle muscular dystrophy. Phase II clinical trial of MYO-029 for patients with a variety of muscular dystrophies were completed in January 2007, and it was decided to

discontinue the development when the MYO-029 trials failed to show efficacy to increase muscle strength by manual muscle testing. Landogrozumab (LY2495655) is a humanized monoclonal antibody that neutralises the activity of the myostatin protein. It was developed by Eli Lilly & Co. Phase II clinical trials of LY2495655 in patients undergoing elective total hip replacement (completed February, 2014), cancer cachexia (completed January, 2016), sarcopenia (completed December, 2013) have ended, and are currently under review. Regeneron Pharmaceuticals Inc. and Sanofi have started a new global collaboration to develop a new myostatin antibody named Trevogrumab (REGN1033). The selected anti-myostatin antibody contains an IgG4 constant region. REGN-1033 has been studied in the treatment of sarcopenia, including disuse atrophy, chronic disease, changes in food and nutritional intake. Phase II clinical trials of REGN-1033 in patients with sarcopenia have been completed in February 2015. Efficacy evaluations are ongoing. Pfizer's Domagrozumab (PF-06252616), is an experimental anti-myostatin monoclonal antibody for intravenous infusion. PF-06252616 was granted orphan drug designation in July 2012 and fast track designation in November 2012 by the US Food and Drug Administration (FDA), and granted the investigational candidate orphan medical product designation by the European Medicines Agency (EMA) in February 2013. PF-06252616 is in clinical development for limb girdle muscular dystrophy, a highly heterogeneous group of very rare muscular disorders including Duchenne muscular dystrophy and Becker muscular dystrophy.

Anti-myostatin peptibodies

PINTA-745, previously named AMG-745, had been a novel anti-myostatin peptibody, that was originally developed by the US-based company Amgen. Structurally, it is a fusion protein with a human Fc at the N-terminus and a myostatin-neutralizing bioactive peptide at the C-terminus. Phase I / II clinical trials have been completed in January 2016) but it was unfortunately decided to discontinue the development of AMG-745 as it failed to meet efficacy endpoints, defined as the percent change from baseline to week 12 in lean body mass as measured by Dual Energy X-Ray Absorptiometry (DXA).

Activin A antibody

Regeneron Pharmaceuticals's REGN-2477, an antibody to Activin A was developed and was granted orphan drug designation on 18 November 2016 by EMA for the treatment of Fibrodysplasia ossificans progressive. Fibrodysplasia ossificans progressive is extremely rare human genetic disease in which muscle tissue and connective tissue such as tendons and ligaments are gradually replaced by bone, and the formation of extra-skeletal bone that causes progressive loss of mobility. REGN-2477 is currently being tested in a phase I study to

evaluate the safety, tolerability, and pharmacodynamics effects of REGN-2477 alone and in combination with REGN-1033 in healthy volunteers [18].

Figure 1 Myostatin and activin Treatments

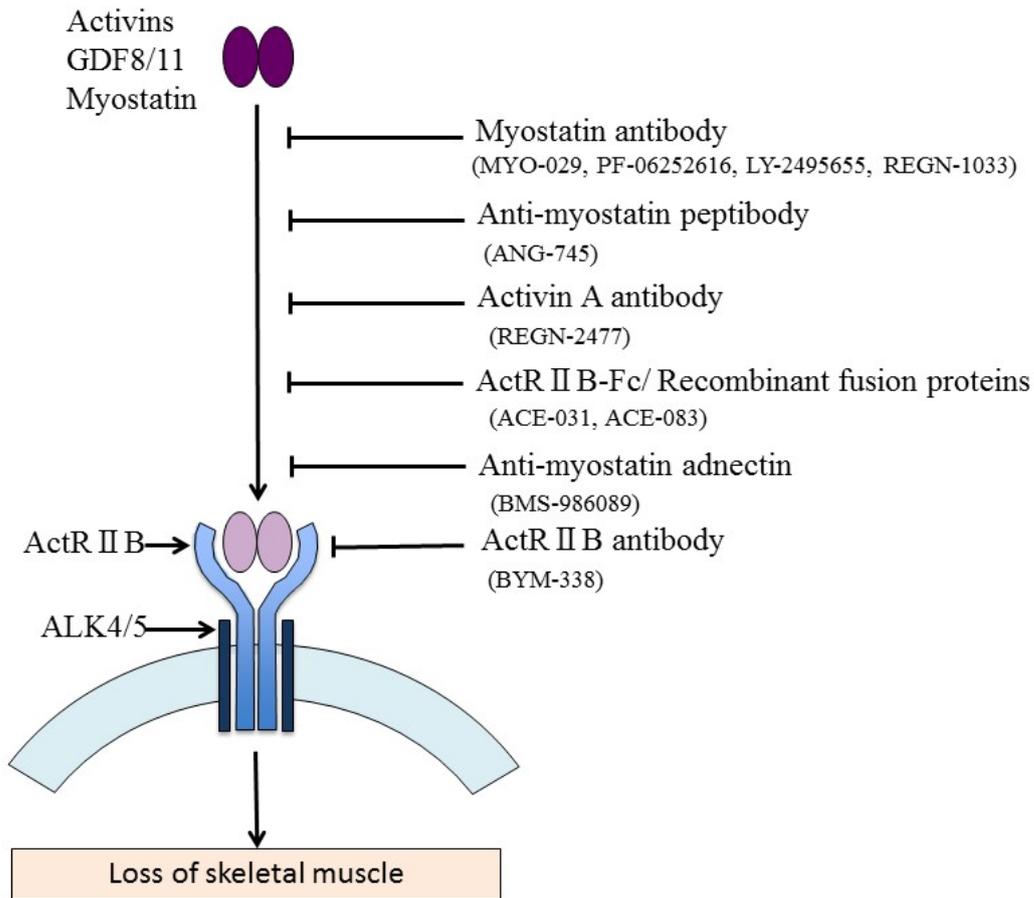


Figure legend

Myostatin and activin signal to target cell by binding to its receptor complex activin type IIB (ActRIIB) /Alk 4 or 5. Signalling through this pathway results in the inhibition of muscle differentiation and growth. Myostatin inhibitors act extracellularly by either binding myostatin and TGF- β family members including activin A, bone morphogenetic protein (BMP)-2, BMP-7, and GDF-11 directly (myostatin antibody, anti-myostatin peptibody, activin A antibody, anti-myostatin adnectin) or by binding its receptor complex (ActRIIB antibody) in order to block myostatin engaging its receptor complex and activating downstream signalling.

Name	Mechanism of Action	Manufacturer	Condition	Phase of development	CT Identifier	Current status
IMO-029/ Stamulumab	Myostatin antibody	Wyeth	Healthy volunteers	phase1	NCT00563810	Discontinue development
PF-06252616/ Domagrozumab	Myostatin antibody	Pfizer	Healthy volunteers LGMD2i muscular dystrophy	phase1/2 phase1 phase2 phase2	NCT00104078 NCT01616277 NCT01616277 NCT02310763 NCT02907619	On-going development
LY-2495655/ Landogrozumab	Myostatin antibody	Eli Lilly	Advanced cancer volunteers Elective total hip replacement Older patients who have fallen recently Pancreatic cancer	phase1 phase1 phase2 phase2	NCT01524224 NCT01341470 NCT01369511 NCT01604408 NCT01505530	Under review development
REGN-1033/ Trevogrumab	Myostatin antibody	Regeneron Pharmaceuticals/ Sanofi	Healthy volunteers	phase1	NCT02741739	On-going development
AMG-745/ PINTA-745	Myostatin peptibody	Amgen/ Atara Biotherapeutics	Healthy volunteers	phase1	NCT02943239	Discontinue development
REGN-2477	Activin A antibody	Regeneron Pharmaceuticals	Healthy volunteers	phase1	NCT02870400	On-going development
ACE-031/ Ramatercept	ActRIIb-Fc	Acceleron/ Shire	Healthy volunteers	phase1	NCT00952887	Discontinue development
BMS-986089	anti-myostatin adnectin	Bristol-Myers Squibb	Duchenne muscular dystrophy	phase1 phase2 phase2	NCT00755638 NCT01239758 NCT01099761	Discontinue development
ACE-083	Recombinant fusion protein	Acceleron	Healthy volunteers	phase1/2	NCT02145234 NCT02515669	On-going development
BYM-338/ Bimagrumab	ActRIIB antibody	Novartis/ Morphosys	Ambulatory boys with DMD Healthy volunteers Facioscapulothoracic muscular dystrophy	phase1 phase2	NCT02257489 NCT02927080	On-going development
			Sarcopenia COPD with cachexia fracture surgery (Lung or Pancreas) sIBM	phase2 phase2 phase2 phase2 phase2	NCT02333331 NCT02468674 NCT01669174 NCT02152761 NCT01433263 NCT03005288 NCT02250443 NCT02573467	On-going development

ActR II B-Fc/ Recombinant fusion proteins

Ramatercept (ACE-031) binds ActRIIB ligands including myostatin, GDF11, and activins, acts as a ligand trap to block the interaction of these ligands with endogenous ActRIIB receptors. ACE-031 was awarded orphan status and accelerated review by the FDA for muscular dystrophy in 2010, however the development ACE-031 had to be discontinued due to safety concerns such as minor nosebleeds, gum bleeding, and/or small dilated blood vessels within the skin (completed June, 2011). Currently, Acceleron Pharma is developing ACE-083, an investigational drug that also acts as a ligand trap for members in the transforming growth factor-beta (TGF- β) superfamily. Unlike ActRIIB-Fc, ACE-083 does not bind the ligands BMP9/10, which are multifunctional cytokines belonging to the TGF- β superfamily, and are the only ligands of the TGF- β superfamily that can bind to both type I and type II receptors with rather equally high affinity [19]. ACE-083 has been designed to clinical development for patients with muscular dystrophy such as facioscapulohumeral muscular dystrophy. Facioscapulohumeral muscular dystrophy is one of the most common type of inherited muscular dystrophies. It is an autosomal dominant disorder and as many as 90% of affected patients are characterized by a unique pattern of affected musculature, typically arising with a reduction of facial and shoulder girdle muscle mass followed by weakness of the lower extremities muscles [20]. ACE-083 is currently being tested in a phase II study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics in patients with facioscapulohumeral muscular dystrophy and Charcot-Marie-Tooth disease [21].

Anti-myostatin adnectin

Bristol-Meyers-Squibb has developed another myostatin antibody-like drug called BMS-986089, an anti-myostatin adnectin (an engineered alternative scaffold biologic based on the 10th fibronectin type III domain) that exhibits high affinity for myostatin. Phase I / II clinical trials of BMS-986089 are ongoing, and phase II / III clinical trials are being designed to evaluate the efficacy, safety and tolerability in ambulatory boys with

Duchenne muscular dystrophy. The primary outcome measure of this clinical trial is changes in the stair climb velocity from baseline to week 48. The estimated completion date for the multi-center, randomized, double-blind, placebo-controlled period study is July, 2020.

ActR II B antibody

Bimagrumab (BYM-338) is a human monoclonal antibody developed to bind competitively to ActRII with greater affinity than its natural ligands myostatin and activin A. BYM-338 was initially created by MorphoSys, a product of its HuCAL antibody library, was then licensed to Novartis. In addition, Novartis announced that the FDA has granted breakthrough therapy designation to BYM-338 for sporadic inclusion body myositis on August 20, 2013. Sporadic inclusion body myositis is the commonest idiopathic inflammatory myopathy, in which characterized by slowly progressive muscle weakness and atrophy, with typical pathological changes of inflammation, degeneration and mitochondrial abnormality in affected muscle fibers. BYM338 dramatically increased skeletal muscle beyond sole inhibition of myostatin in mice, and highlights its therapeutic potential [22]. Phase II / III clinical trials of BYM-338 in patients with Sporadic Inclusion Body Myositis have been completed in January, 2016 and currently being reviewed. Clinical development of BYM-338 for patients after hip fracture surgery, sarcopenia patients, and obese patients with type 2 diabetes are continuing as planned.

Clinical results of myostatin inhibitors

A variety of myostatin inhibitors have currently progressed into clinical development in several indications, mainly sarcopenia, early recovery after surgery, and cachexia. Myostatin inhibitors for the treatment of muscular dystrophy are also being tested in early clinical trials. The myostatin/ActRIIB pathway has recently attracted a lot of attention as a main target for the development of drugs for muscle wasting, however, the available information about compounds in development is limited (Table 2).

Sarcopenia and early recovery after surgery

There has been increasing interest in therapeutic interventions that prevent, delay the onset or aim to treat muscle wasting in the hope of improving outcomes for patients with sarcopenia and for patients undergoing major surgery. The clinical trials of LY-2495655 have been completed [23] evaluating the efficacy and safety for sarcopenia in older patients after falls or with muscle weakness, but also in patients undergoing elective total hip arthroplasty. Becker et al. reported that LY-2495655 treatment in patients aged 75 years or older who had fallen in the past year increase in appendicular lean body mass, a reflection of their skeletal muscle mass in least-squares mean, by -0.123 kg (95% CI -0.287 to 0.040) in the placebo group and by $+0.303$ kg (95% CI 0.135 to 0.470) in the LY-2495655 group within 24 weeks. The absolute difference was $+0.43$ kg (95% CI 0.192 to 0.660 , $p < 0.0001$) [24]. Unfortunately, compared with placebo, no significant effect was detected for upper and lower extremities muscle strength and performance-based measures including stair climbing power test, 6-minute walking distance, and chair stand test at week 24. Adverse events were more frequent in the LY-2495655 group ($p = 0.007$), and this difference was driven by injection site reactions including injection site pain, bruising, erythema, rash, and pruritus (30% of LY-2495655 group vs. 9% of placebo group, $p < 0.001$). Woodhouse et al. also demonstrated that LY-2495655 treatment in patients after hip fracture surgery led to a dose-dependent increase in appendicular lean body mass and a decrease in fat mass, however, the appendicular lean body mass did not reach the superiority threshold at week 12 [25]. Novartis is currently recruiting participants to evaluate the efficacy, safety, and pharmacokinetics of BYM-338 in a phase II trial for sarcopenia, obese patients with type 2 diabetes mellitus, and patients after hip fracture surgery [26]. The primary outcome measures of these clinical trials were changes in total lean and total fat mass or appendicular skeletal muscle index from baseline into week 24 and 48 measured by dual energy X-ray absorptiometry (DXA), gait speed, 6-minute walk test, and short physical performance battery test. The estimated completion date for the randomized, double-blind, placebo-controlled study of intravenous BYM-338 is between October, 2018 and January, 2019.

Cachexia in chronic disease

Cachexia is a syndrome occurring at terminal stages of diseases such as cancer, chronic heart failure, chronic kidney disease, or chronic pulmonary obstructive disease [27,28]. This syndrome is characterized by loss of body weight as a consequence of pathological changes in different metabolic pathways. Novartis completed a phase II trial of BYM338 versus placebo for advanced chronic obstructive pulmonary disease (COPD) with cachexia. The objective measurements embraced thigh muscle mass measured by magnetic resonance imaging

(MRI) and 6 minutes walk distance. Over 8 weeks, 30 mg/kg of BYM-338 was efficacious at increasing thigh muscle mass in adult COPD patients with cachexia compared to placebo (at week 24: 5.04 ± 4.87 % vs. -1.31 ± 4.28 %, $p < 0.001$), but did not provide significant improvement in the 6-minute walk distance compared to placebo [29]. Similarly, BYM-338 has completed phase II clinical testing for the treatment of muscle wasting in patients with cancer of the lung or the pancreas. The objective measurements were thigh muscle volume, total lean mass and bone mineral density, body weight, and physical activity. Unfortunately, no effect was detected for body composition improvement or physical activity [30]. A phase I study of LY2495655 in patients with advanced cancer not receiving chemotherapy, with an abstract reporting that LY2495655 was well tolerated and provided durable improvement in muscle volume, hand grip strength and functional tests at American society clinical oncology annual meeting [31]. However, there is no clear trend in dose-dependent efficacy, possibly due to extremely small sample sizes and patient heterogeneity. A phase II clinical trial of LY-2495655 in combination with chemotherapy was also completed in patients with locally advanced or metastatic pancreatic cancer cachexia, and is ongoing to evaluate the efficacy in improving survival as well as lean body mass and physical performance. Furthermore, phase I clinical trials of AMG-745 treatment for prostate cancer patients with androgen deprivation therapy have been completed, AMG-745 significantly increased percentage change of lean body mass in the AMG-745 (3 mg/kg) group vs. the placebo groups on day 29 (1.5% vs. -0.7% , with between group difference of $2.2\% \pm 0.8\%$ SE, $p = 0.008$), and decrease fat mass (-1.7% vs. 0.8% , with a between group difference of $-2.5\% \pm 1.0\%$ SE, $p = 0.021$) was observed. However, no overall effects for either short physical performance battery and 1-repetition maximum of knee extension were noted [32].

Muscular dystrophy

Muscular dystrophies include more than 30 different inherited diseases, such as Duchene muscular dystrophy, Becker muscular dystrophy, and facioscaplohumeral muscular dystrophy, which are caused by mutations that affect distinct genes, yet all result in muscle degeneration, impaired locomotion and, in most cases, premature death [33,34]. Importantly, inactivation of myostatin in muscular dystrophic mice exerted beneficial effects on disease progression, suggesting that myostatin is a primary target of pharmacological development in muscular dystrophies. A phase II trial of ACE-031 in Duchenne muscular dystrophy demonstrated trends to increase lean body mass and reduce fat mass. However, non-muscle-related adverse events such as epistaxis, gum bleedings, and telangiectasias, contributed to the decision to discontinue the study [35,36]. On the other hand, Acceleron Pharma reported that as part of a phase I study in healthy volunteers, ACE-083 produced

substantial dose-dependent increases in muscle volume [37]. Based on these results, the company initiated a two-part phase II trial in patients with facioscapulohumeral muscular dystrophy patients, and Charcot-Marie-Tooth disease, patients who suffer from muscle weakness causing foot drop and reduced mobility. A clinical trial of MYO-029 has been conducted on behalf of Wyeth Pharma (now Pfizer) and completed in phase I / II for the treatment of Becker muscular dystrophy, Facioscapulohumeral muscular dystrophy, and Limb-girdle muscular dystrophy. MYO-029 was assessed through manual muscle testing, quantitative muscle testing measured by MRI and DXA scan, timed function tests, subject-reported outcomes, and muscle biopsies. MYO-029 showed only minimal improvements in muscle strength and the pathology of patients with muscular dystrophies [38], and these disappointing results lead to the discontinuation of further development of this compound for muscular dystrophies in 2008 [39]. BYM-338 has completed phase II / III testing in the treatment of muscle size in patients with sporadic inclusion body myositis. The objective measurements were 6-minute walking distance and lean body mass. BYM-338 showed a dose-dependent increase in lean body mass compared to placebo (3mg/kg, 10mg/kg vs. placebo) and substantial reductions in body fat ($p < 0.01$). In addition, participants on placebo and 1mg/kg of BYM-338 had a mean 6-min walk distance decrease from baseline to week 52 of -8.96 ± 10.77 m and -10.27 ± 10.72 m, though 3mg/kg and 10mg/kg BYM-338 treatment resulted in an increase of 9.63 ± 10.77 m and 8.63 ± 10.93 m, respectively. On the other hand, no consistent differences in quadriceps quantitative muscle strength were observed in the BYM-338 compared to the placebo group. However, differences between BYM-338 treatment vs. placebo did not reach statistical significance and the primary endpoint of improving 6-minute walk distance or muscle strength was likewise not reached [40,41].

The function of myostatin inhibitors in the regulation adipose tissue

Adipose tissues are mainly composed of adipocytes and play important roles in storage for excess energy and metabolically dynamic organ [42]. Several factors have been demonstrated to contribute to cachexia induced adipose tissue wasting, including increased lipid mobilization due to enhanced adipocyte triglyceride lipolysis, reduced lipogenesis and fatty acid esterification due to a decrease in both fatty acid synthase and lipoprotein lipase activity, and impairment of fat cell turnover, resulting in a disruption in the organization and development of adipose tissue [43-46]. Myostatin can be detected not only in muscle tissue but also adipose tissue. Myostatin null mice had a decreased amount of adipose tissue [47,48]. Several studies showed the negative effect of myostatin on pre-adipocyte differentiation and proliferation in myostatin knockout

mice [49,50]. It is still unclear whether the effect of myostatin on decreased adipose tissue is the result of direct regulation or an indirect consequence of increased skeletal muscle mass. On the other hand, the combined effects of increased lean body mass and reduced fat mass suggest potential benefit in obesity. The direct and indirect effects of myostatin on adipose tissue are still unclear. Additional experiments are required to determine the role of myostatin inhibitors on adipose tissue as well as on muscle wasting and physical performance in cachectic or obese patients.

Conclusions and perspectives for future research

As reviewed herein, myostatin inhibition may have the potential to be a potent therapy for muscle wasting, however, there currently are no clear drug candidates as most of the compounds in development have had very limited effectiveness in larger clinical trials. Despite this, a number of smaller clinical trials has demonstrated that inhibition of myostatin/ActRII signaling may help to improve muscle mass in patients with muscle wasting. Most of these pharmacological therapies have currently progressed into an early stage of clinical testing only, and results are awaited to confirm improved muscle mass, muscle strength, physical performance and outcomes that are clinically meaningful in sarcopenia, in patients after major surgery, in patients with cachexia, or in patients with muscular dystrophy. In addition, evidence of positive effects on muscle wasting through exercise training and nutritional supplementation are emerging [51-53]. Therefore, there is a need to determine the effects of hybrid therapies combining myostatin inhibitors with other novel approaches, including exercise and nutritional therapy to treat muscle wasting.

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Conflict of interest

Masakazu Saitoh, Junichi Ishida, Nicole Ebner, Stefan D. Anker, Jochen Springer and Stephan von Haehling declare that they have no conflict of interest with relevance to this article.

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