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Myostatin inhibitors as therapies for muscle wasting associated with cancer and other disorders

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Purpose of review
This review summarizes recent progress in the development of myostatin inhibitors for the treatment of muscle wasting disorders. It also focuses on findings in myostatin biology that may have implications for the development of antimyostatin therapies.

Recent findings
There has been progress in evaluating antimyostatin therapies in animal models of muscle wasting disorders. Some programs have progressed into clinical development with initial results showing positive impact on muscle volume.
In normal mice myostatin deficiency results in enlarged muscles with increased total force but decreased specific force (total force/total mass). An increase in myofibrillar protein synthesis without concomitant satellite cell proliferation and fusion leads to muscle hypertrophy with unchanged myonuclear number. A specific force reduction is not observed when atrophied muscle, the predominant therapeutic target of myostatin inhibitor therapy, is made myostatindeficient.
Myostatin has been shown to be expressed by a number of tumor cell lines in mice and man.

Summary
Myostatin inhibition remains a promising therapeutic strategy for a range of muscle wasting disorders.

Keywords
ActRIIB, cancer cachexia, muscle function, muscle hypertrophy, muscle wasting, muscular dystrophy, myostatin, satellite cell

INTRODUCTION

Cachexia is a wasting syndrome exhibited by many cancer patients particularly at advanced stages of disease. It is characterized by the loss of skeletal muscle mass (with or without fat loss), despite adequate nutritional intake. Cancer cachexia is associated with diminished quality of life, functional performance and decreased survival. Cancer-related muscle loss is an independent predictor of poor outcome linked to increased immobility and mortality, and has also been associated with intolerance to chemotherapy. There has been increasing interest in therapeutic interventions that prevent, delay or treat cancer-related muscle wasting, in hopes of improving outcomes for the cancer patient. Some recent reviews and key articles in this area are available [1–16].

There are many commonalities at the molecular level in the pathways in skeletal muscle that result in atrophy, whether it is in the context of cancer cachexia or other noncancer muscle wasting situations. The mechanisms regulating skeletal muscle mass have recently been reviewed [17–21]. Myostatin has emerged as an intriguing therapeutic target [22]. Myostatin, a member of the TGFβ superfamily of growth factors, is a highly conserved negative regulator of skeletal muscle mass that is upregulated in many conditions of muscle wasting. Various induced or natural conditions leading to myostatin deficiency result in increased muscle mass and strength in normal animals and have been shown

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KEY POINTS

- Myostatin inhibitors can enhance strength and functional performance measures in mouse models of cancer cachexia.
- Reports of effects of myostatin inhibitors in animal models of rare or orphan neuromuscular diseases including muscular dystrophy generally demonstrate an increase in muscle mass but effects on strength, performance and/or survival are mixed.
- Several different types of myostatin pathway inhibitors including myostatin antibodies and ActRIIB antibody are in clinical development and preliminary results reported show that they can increase LBM and/or TMV in healthy volunteers, muscular dystrophy and cancer patients, although development of an ActRIIB-Fc (ACE-031) has been discontinued because of bleeding issues.
- Myostatin inhibitors work to induce muscle hypertrophy predominantly through effects on myofibrillar protein synthesis rather than stimulating satellite cell proliferation.
- Myostatin deficiency is able to increase total force in normal mice but specific force is compromised due, at least in part, to reduced MND size in the myofibers in these hypertrophied muscles. Treating disorders with, where muscle is atrophied and MND size is below normal, with myostatin inhibitors, has not been confounded by such issues. Myostatin has been reported to be expressed by a number of murine and human tumor cell lines.

to treat or prevent a range of muscle wasting conditions.

The myostatin signaling pathway and its role in regulating skeletal muscle has been recently reviewed [23,24]. At the molecular level, myostatin binds to and activates the activin receptor IIB (ActRIIB)/Alk 4/5 complex (Fig. 1). Although ActRIIB/Alk4/5 is broadly expressed, myostatin is produced and exhibits its effects primarily on skeletal muscle.

Many approaches are being taken both preclinically and clinically to inhibit the myostatin signaling pathway (Fig. 1). The majority of these approaches acts extracellularly to block myostatin engaging with the ActRIIB/Alk4/5 receptor complex, either by binding directly to myostatin itself or by binding to components of this receptor complex. Due to the fact that multiple ligands signal through, and therefore bind, ActRIIB apart from myostatin (including activin A, gdf11, bmp9) [25–27] the approaches that target the ActRIIB receptor or use ActRIIB as a soluble decoy receptor may not specifically block myostatin action. Similarly, the naturally occurring myostatin binding proteins follistatin and Fstl3 are known to bind a number of growth factors in addition to myostatin [28,29]. The added risk/benefit of these multitargeted approaches is under investigation (see below).

PROGRESS IN VALIDATION OF MYOSTATIN AS A TARGET FOR MUSCLE WASTING DISORDERS

Preclinical results

Two recent studies, performed in mouse models of cancer cachexia, have examined the effects of myostatin inhibitors on physical performance and muscle function, building on previous data that showed positive effects on muscle mass [30,31]. Mice with Lewis Lung carcinoma treated with ActRIIB-Fc (Fig. 1), a soluble myostatin receptor that binds myostatin, activin and other ligands, showed increases in body weight and muscle weights with grip strength significantly increased and resting time significantly decreased by treatment [32▪]. A myostatin antibody in the same model was able to completely abrogate the tumor-induced reduction in total muscle force in various limb and diaphragm muscles [33▪]. The results of these recent studies are encouraging as the value of myostatin inhibitors to cancer patients exhibiting muscle wasting is ultimately to affect functional performance through increased muscle function.

Aside from models of cancer cachexia, most recently published preclinical activity with myostatin inhibitors has focused on developing therapies in the area of rare or orphan diseases, in which symptoms are devastating to patients and few if any significant treatment options are available. Testing of myostatin inhibitors in animal models of muscular dystrophy [34] has shown generally positive effects on muscle mass but inconsistent effects on muscle function and histopathology [reviewed in [35▪]]. ActRIIB-Fc or ActRIIB shRNA given to *mdx* mice, a well used but not ideal model of human muscular dystrophy [36,37▪▪], produced increases in muscle mass and total force but specific force was unchanged [38,39▪,40]. In contrast, a recent study reported an increase in specific force of the soleus muscle in *mdx* mice after long-term exposure to a myostatin propeptide [41]. Studies with myostatin inhibitors have not shown any improvement on eccentric contraction-induced force drop, a key measure of myofiber structural integrity [40,42,43]. Therefore, there is increasing evidence that myostatin inhibitors can improve muscle function in the *mdx* mouse through an increase in muscle mass and total force but do not

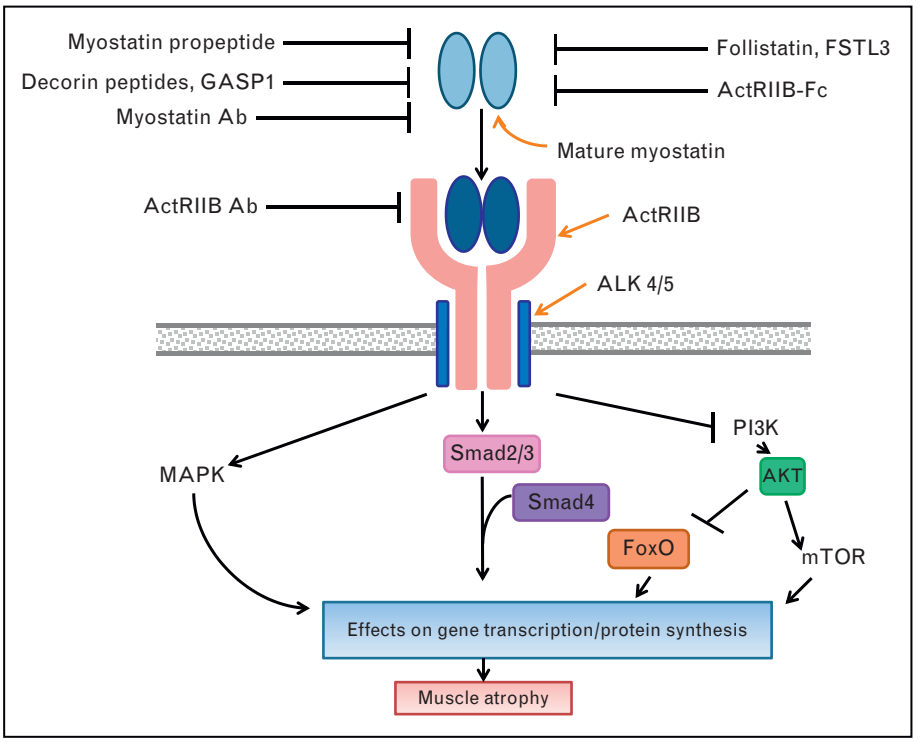

FIGURE 1. Summary of therapeutic invention points in the myostatin signaling pathway. Myostatin binds to its receptor complex ActRIIB/Alk 4 or 5 on skeletal muscle resulting in activation of the Smad 2/3, mitogen-activated protein kinase and inhibition of the PI3K intracellular signaling pathways that together result in gene transcriptional changes and effects on protein synthesis that ultimately give rise to muscle atrophy. Myostatin pathway inhibitors act extracellularly by either binding myostatin directly (Fstl3, Follistatin, myostatin antibody, GASP1, myostatin propeptide, decorin peptides, ActRIIB-Fc) or by binding its receptor complex (ActRIIB antibody) in order to block myostatin engaging its receptor complex and activating downstream signaling. Some of the inhibitors are naturally occurring (myostatin propeptide, Gasp1, follistatin, Fstl3) whereas others are engineered (myostatin antibody, ActRIIB antibody, ActRIIB-Fc). —I represent inhibitory activities. → represent activating activities. Ab = antibody.

consistently improve the underlying weakness of dystrophic muscle. There has been hope that myostatin inhibitors might attenuate the muscle fibrosis that is a hallmark of muscular dystrophy, given myostatin's role in inducing dystrophic muscle fibroblast proliferation [44▪] and the observation of decreased connective tissue in myostatin null mice [45▪]. Although earlier observations in *mdx* mice [34] and more recent observations in the golden retriever muscular dystrophy model [GRMD [46]], showed improvement in fibrosis with myostatin antibody or myostatin propeptide treatment, respectively, no improvement on muscle histopathology was seen after ActRIIB-Fc treatment of *mdx* mice [40,42]. It has been suggested that the degree of muscle disease at the time of treatment may influence outcome [43]. Human muscular dystrophy disorders display paradoxical muscle wasting and selective hypertrophy of skeletal muscles, leading to imbalance, contractures and postural instabilities [37▪▪]. When the muscle hypertrophic myostatin heterozygote whippet [47] was crossed with the GRMD dog, selective muscle hypertrophy seen in the GRMD dog was exaggerated resulting in more pronounced postural instability and worsened clinical scores, cautioning that further hypertrophy of already selective hypertrophic muscle in muscular dystrophy may not be beneficial [37▪▪]. Dysferlin null mice, a model of dysferlin-deficiency muscular dystrophy [48], expressing the myostatin inhibitor follistatin, demonstrated a transient increase in muscle mass followed by decreased muscle mass and function and increased muscle fibrosis [Lee *et al.* MDA meeting, San Diego, 2013].

There is excitement regarding disease-modifying therapies currently in clinical development for muscular dystrophy based on exon skipping methods, which overcome the underlying genetic defect of the dystrophin gene and improve specific muscle force without effects on muscle mass [reviewed in [49,50]]. Myostatin inhibitors are currently being investigated preclinically as possible adjunct therapy with these molecules [39▪,42,51–53].

The recently described increase in axon number together with delay in age-related neural degeneration in myostatin null mice have added support to

the investigation of myostatin inhibitors for the treatment of severe neuromuscular disorders [54▪,55]. However, SOD1 null mice, a model of amyotrophic lateral sclerosis, did not exhibit any improvements in survival (despite improvements in muscle mass) when exposed to myostatin inhibitors [56]. In another report, crossing of SMN null mice, a model of Spinal Muscular Atrophy, with myostatin null mice did not lead to increases in muscle mass or effects on survival [57], consistent with results using myostatin inhibitors from Sumner *et al.* [58] but inconsistent with the positive effects reported by Rose *et al.* [59]. In contrast to the above reports, treatment of the myotubularin-deficient mouse, a model of X-linked myotubular myopathy, with ActRIIB-Fc did lead to transient increases in muscle mass and strength and a 17% increase in survival [60▪▪].

Other animal models of muscle wasting have been used to determine if inhibition of myostatin has therapeutic potential in treating a range of muscle wasting conditions. Positive results have been reported in models of chronic kidney disease, disuse atrophy and age and hypogonadism-induced muscle loss [61,62▪,63]. Overexpression of the myostatin interacting protein GASP1 [64] has been shown to induce muscular hypertrophy in mice but has not yet been tested in models of muscle wasting. Identification of myostatin-blocking decorin peptides are at an even earlier stage of preclinical development [65]. There is increasing preclinical evidence to suggest that inhibition of myostatin may also have metabolic benefits. Myostatin deficiency or myostatin inhibition in mice has been shown to result in decreased fat mass and increased insulin sensitivity raising the therapeutic potential of myostatin inhibition in obesity and insulin resistance associated with obesity [reviewed in [23]].

Clinical results

Some myostatin inhibitors have progressed into clinical development as summarized in Table 1.

LY2495655 is a myostatin antibody that is currently in clinical development for muscle wasting associated with cancer and other disorders (see Table 1). Results of a study in healthy volunteers demonstrated the drug to be well tolerated and led to an increase in thigh muscle volume (TMV) [66▪▪]. Interim results of a Phase 1 safety study of LY2495655 in advanced cancer patients without chemotherapy reported that a maximum tolerated dose was not reached and increased muscle volume with concomitant increases in hand grip strength and other functional measures were observed; however, a clear dose–response was not observed, ascribed to small sample size and patient heterogeneity [66▪▪]. A Phase 2 trial of LY2495655 in patients with locally advanced/inoperable or metastatic pancreatic cancer receiving standard of care chemotherapy is ongoing with overall survival as the primary endpoint of the trial (Table 1).

BYM-338 is an antibody directed to ActRIIB that is currently in Phase 2 for the treatment of cachexia in patients with stage IV nonsmall cell lung cancer or Stage III/IV adenocarcinoma of the pancreas. The primary endpoint of the trial is TMV at 8 weeks as measured by MRI. BYM-338 is also in Phase 2 trials for other muscle wasting disorders (Table 1). Single infusions of BYM-334 in healthy volunteers were reported to be well tolerated and resulted in an increase in TMV (D. Rook; International conference on sarcopenia research, Orlando, December 2012).

The correlation of increases in muscle volume to clinically meaningful functional outcomes for patients treated with myostatin inhibitors still awaits validation. Interestingly, myostatin protein levels were found to be upregulated in muscle biopsies taken from early stage gastric cancer patients even before significant weight loss (>10%) had occurred, leading to the suggestion of early intervention to prevent cancer cachexia [67▪].

Clinical development of myostatin inhibitors for the treatment of muscular dystrophy has made recent progress [49,68]. Phase 1/2 results of MYO-029, a myostatin antibody, failed to show effects on muscle strength or function in adult Becker, limb-girdle and facioscapulohumeral muscular dystrophy patients [69]. PF-06252616 is a myostatin antibody currently in Phase 1 testing in healthy volunteers; it was recently given orphan drug designation by the European Medical Agency (EMA) for treatment of Duchenne muscular dystrophy (DMD). ACE-031, a human ActRIIB-Fc, in single and multiple ascending studies in healthy volunteers showed significant increases in lean body mass (LBM) and TMV [70▪▪] [Borgstein *et al.* World Muscle Society, Japan, 2010]. ACE-031 was awarded orphan status and accelerated review by the Food and Drug Administration for muscular dystrophy in 2010. Results from a Phase 2 study with ACE-031 in DMD boys showed an increase in LBM and attenuation of declines in TMV and six minute walk distance (6MWD) [71▪▪]. However, the observation of reversible nosebleeds and skin telangiectasias in the healthy volunteer MAD study as well as in the Phase 2 muscular dystrophy study [71▪▪] has led to the termination of these trials. The underlying mechanism behind these adverse events is not understood although the genetic associations of mutations in the ALK1 type I receptor with hereditary haemorrhagic

Table 1. Summary of clinical development of myostatin inhibitors for treatment of muscle wasting associated with cancer and other disorders

Name	Type of inhibitor	Sponsor	Condition	Patient population	Phase of development	Current status	Outcome	CT identifier/Ref.
LY2495655	Myostatin antibody	Lilly	–	Healthy volunteers	Phase 1 SAD	Completed	Well tolerated, increased TMV	[66▪▪]
			–	Healthy Japanese volunteers	Phase 1; SAD, MAD	Completed		NCT01341470
			Muscular atrophy	Hip arthroplasty	Phase 2	Actively recruiting		NCT01369511
			Muscle weakness	Older weak fallers	Phase 2	Active, not recruiting		NCT01604408
			Advanced cancer	Cancer patients	Phase 1	Active, not recruiting	Well tolerated, increased muscle volume (interim report)	[66▪▪]; NCT01524224
			Advanced cancer	Pancreatic cancer	Phase 2	Recruiting		NCT01505530
MYO-029	Myostatin antibody	Wyeth	–	Healthy volunteers	Phase 1	Completed		NCT00563810
			Adult muscular dystrophy	BMD; Facioscapulohumeral; muscular dystrophy; Limb-Girdle muscular dystrophy	Phase 1/2	Terminated	Increase in LBM; no effects on strength or function; skin hypersensitivity at highest doses	[70▪▪]; NCT00104078
ACE-031/ Ramatercept	ActRIIb-Fc	Acceleron/Shire	Muscle loss	Healthy postmenopausal women	Phase Ia (SAD)	Completed	Generally well tolerated, increased LBM and TMV	[70▪▪]; NCT00755638
			Muscle atrophy	Healthy postmenopausal women	Phase Ib (MAD)	Terminated	Common AE: nosebleed. Increased LBM, TMV	Borgstein *et al.* 2010, WMS, Japan; NCT00952887
			DMD	DMD boys	Phase 2; MAD	Terminated	Reversible telangiectasia and nosebleed; increased LBM, attenuated TMV and 6MWD	[71▪▪]; NCT01239758; NCT01099761
PF-06252616	Myostatin antibody	Pfizer	–	Healthy volunteers	Phase I; SAD, MAD	Recruiting		NCT01616277
BYM338	ActRIIB antibody	Novartis	Muscle wasting	Healthy volunteers	Phase 1 SAD		Well tolerated, increase in TMV	D. Rook, Intl conference on Sarcopenia research, Orlando, Dec, 2012
			sIBM	sIBM	Phase 2; Single dose	Completed	Well tolerated, increase in TMV, LBM, quadriceps strength and 6MWD	Amato *et al.* MDA meeting San Diego, 2013; NCT01423110
			COPD	COPD patients with cachexia	Phase 2	Recruiting		NCT01669174
			Skeletal muscle	Sarcopenic adults	Phase 2	Recruiting		NCT01601600
			Cachexia	Cancer cachexia (lung or pancreas)	Phase 2	Recruiting		NCT01433263

REGN1033/ SAR391786	Myostatin antibody	Regeneron/ Sanofi	Rehabilitation postorthopedic surgery	Healthy volunteers	Phase 1 SAD, MAD	Active, not recruiting		NCT01507402 NCT01720576
FS344	Follistatin-AAV gene therapy	Nationwide Children's Hospital/ Milo Biotech	BMD and SIBM	BMD and SIBM	Phase 1	Enrolling by invitation		NCT01519349
AMG-745	Myostatin peptibody	Amgen	Age-associated muscle loss	Age-associated muscle loss	Phase 2	Withdrawn prior to enrolment		NCT00975104

BMD, Becker muscular dystrophy; COPD, chronic obstructive pulmonary disease; DMD, Duchenne muscular dystrophy; CT Identifier, clinical trial identifier at ClinicalTrials.gov; LBM, lean body mass ; MAD, multiple ascending dose; SAD, single ascending dose; sIBM, sporadic inclusion body myositis; TMV, thigh muscle volume; 6MWD, six minute walk distance.

telangiectasias type 2 [72], and the elucidation of the Alk1/ActRIIB complex as the main signaling pathway for BMP9 [27,73] warrant further investigation into this particular ligand.

Skeletal muscle weakness is associated with a large array of conditions that involve muscle wasting ranging from age-related atrophy, termed sarcopenia, to the wasting associated with immobility, termed disuse atrophy (reviewed in [18,22]). Several clinical trials are in progress that seek to prove the concept that myostatin inhibitors may be therapeutically beneficial and provide meaningful benefit to these wasting states. Trials are ongoing in chronic obstructive pulmonary disease (COPD) patients, in rehabilitation postorthopedic surgery/ hip replacement subjects and in the sarcopenic adult and older weak fallers (Table 1). In a Phase 2 trial in sporadic inclusion body myositis (sIBM), a rare autoimmune disorder [74], BYM-338 was able to increase LBM, TMV and improve quadriceps strength and 6MWD (Amato *et al.* Annual MDA conference, San Diego, 2013; Table 1).

EMERGING MYOSTATIN PATHWAY BIOLOGY WITH IMPLICATIONS FOR THERAPEUTIC TARGETING: ROLE OF SATELLITE CELLS IN HYPERTROPHY OF SKELETAL MUSCLE INDUCED BY MYOSTATIN DEFICIENCY

In general muscles enlarged beyond normal size, or 'supersized' as a result of myostatin deficiency have increased total force but reduced specific force [75–78]. In myostatin null mice, the increase in total force does not match the increase in muscle mass [79]. Analysis of the contractility of single fibers from MSTN null mice demonstrated that the specific force deficits were at the level of the muscle myofiber [80,81▪▪]. Historically, it has been thought that a major function of myostatin was to maintain muscle satellite cell quiescence and that the relief of this inhibitory influence led to satellite proliferation and fusion to existing myofibers resulting in hypertrophy, akin to the mechanisms of muscle enlargement after exercise [81▪▪,82]. However, recent evidence demonstrates that myostatin exerts its effects directly on the myofiber with little effect on satellite cell activity [83▪]. The number of myonuclei in muscle is unchanged between normal and myostatin null mice resulting in larger ratios of cytoplasm volume : nuclei or myonuclear domains (MNDs) in null mice [81▪▪,84]. Qaisar *et al.* [81▪▪] suggested that there is a threshold size of MND under which the fiber is able to maintain the myofibrillar contractile apparatus and hence specific force. However, when a specific MND threshold is

reached the fiber is unable to maintain the specific force. Interestingly, food restriction of the MSTN$^{-/-}$ mouse, by reducing muscle fiber size, restored the MND to normal with a corresponding normalization of force generation capacity of the muscle [85]. Lee *et al.* [86▪▪] demonstrated that muscle hypertrophy still occurred in animals with satellite cell deficiencies treated with myostatin inhibitors and in mice with a myofiber specific ablation of the myostatin receptor ActRIIB, confirming that the myofiber itself is the target of myostatin action. Myostatin deficiency leads to an increase in myofibrillar protein synthesis although a decrease in protein degradation may also be at play [80,87▪,88▪]. These findings suggest that myostatin inhibition in mice leading to 'supersized' muscles occurs with minimal satellite cell activation and can lead to a MND size in which there may not be concomitant increases in function. It remains to be determined if this same mechanism is active in man but with the increased attention and speculation around misuse of myostatin inhibitors this might prove to be a blessing in disguise [89,90]. As exemplified above, treating disorders with myostatin inhibitors in which muscle is atrophied and MND size is below normal has not been confounded by negative effects on specific force measures. These results also suggest that myostatin inhibitors would be effective in states of satellite cell dysfunction or depletion such as muscular dystrophy [91].

Emerging linkage of myostatin with tumor biology

Perhaps not surprisingly skeletal muscle tumors, specifically rhabdomyosarcomas (RMS), the most common soft tissue tumor in children, are known to overexpress myostatin [92]. Blocking myostatin activity with a dominant negative form of ActRIIB resulted in decreased proliferation and promoted differentiation of a human RMS cell line suggesting that myostatin inhibition may be a valuable target for interventions for RMS [93]. More interestingly, Lokireddy *et al.* [94▪▪] for the first time reported that myostatin protein is expressed and secreted from the mouse adenocarcinoma cell line C26, and from several human cancer cell lines. A characterization of myostatin expression in primary tumors is warranted in light of these initial findings.

CONCLUSION

There are a number of intervention points being exploited to inhibit myostatin signaling in order to enhance muscle mass under the conditions of muscle atrophy. Many of these therapies have now progressed into early stage clinical trials. Results of several Phase 2 trials underway are awaited to determine if increased muscle volumes translate into muscle strength, performance and outcomes that are clinically meaningful to patients.

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Conflicts of interest

The authors are currently full time employees of Eli Lilly and Company.

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