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GDF8 and activin A are the key negative regulators of muscle mass in postmenopausal females: a randomized phase I trial

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Evolutionary pressures to protect against food scarcity likely resulted in highly-conserved pathways designed to minimize energy expenditure, one of which involves the minimization of muscle mass; these mechanisms may be counter-productive in a modern world suffering from obesity and sarcopenia. Growth differentiation factor 8 (GDF8)/myostatin, acting via ActRIIA/B receptors, is the best-characterized negative regulator of muscle mass, leading to therapeutic efforts to augment muscle growth by blocking GDF8 or ActRIIA/B. ActRIIA/B blockade approximately doubles the muscle increase of GDF8 blockade, and as ActRIIA/B responds to multiple other TGFβ-family members, this implies other ligands might also regulate muscle mass. Previously, we suggested that activin A (ActA) is the key second negative regulator acting via ActRIIA/B, as blockade of both GDF8 and ActA in mice/monkeys matches the muscle growth of ActRIIA/B blockade. Here, we extend these observations to humans in a two-part, randomized, placebo-controlled Phase 1 trial (www.clinicaltrials.gov, NCT02943239) conducted at two sites in New Zealand. Eligible subjects included healthy postmenopausal females aged 45–70 years and males aged 35–60 years not intending to father children, with a body mass index of 18–32 kg/m^2. Part I tested single-dose administration of anti-GDF8 alone, anti-ActA alone, several dose combinations of anti-GDF8 + anti-ActA, or placebo in healthy postmenopausal females; part II tested multiple-dose administration of anti-ActA alone or placebo in healthy postmenopausal females, combination anti-GDF8 + anti-ActA or placebo in healthy postmenopausal females, and anti-ActA alone or placebo in healthy males. The primary outcome measure was the incidence and severity of treatment-emergent adverse events through week 16 for the single-dose part of the study and through week 40 for the multiple-dose part of the study. Secondary endpoints included percent and absolute change in thigh muscle volume, percent and absolute change in total and regional body composition, pharmacokinetic profiles of the GDF8 and ActA mAbs in serum over time, changes in serum total GDF8 and total ActA levels over time, and the presence of anti-

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drug antibodies against the GDF8 mAb or the ActA mAb. Magnetic resonance imaging was used to quantitate changes in thigh muscle volume and dual x-ray absorptiometry was used to quantitate changes in regional body composition (total lean mass, appendicular lean body mass, android fat mass, and total fat mass). A total of 82 subjects were enrolled (48 in the single-dose part and 34 in the multiple-dose part of the study). Baseline demographic and clinical characteristics were generally balanced across the single- and multiple-dose parts of the study. Combining GDF8 and ActA blocking antibodies led to greater muscle growth than either antibody alone; increases in muscle were accompanied by reductions in fat. The observed clinical effects on muscle and fat paralleled mAb exposure in serum. The combination was generally well tolerated, and no subjects tested positive for anti-drug antibodies post-treatment. These results suggest that GDF8 and ActA are the dominant negative regulators of muscle mass in humans, and that combined blockade may be a promising therapeutic approach in muscle atrophy and obesity settings.

During most of evolutionary history, caloric scarcity was among the greatest threats to survival, resulting in enormous selection pressure for mechanisms to minimize energy expenditure. One of the evolutionary mechanisms for conserving energy involves the minimization of muscle mass, as muscle is a major energy sink, even at rest[1]. In the setting of widespread availability of excess calories and more sedentary lifestyles[2–5]—as exists in much of the modern world—mechanisms that reduce muscle mass to conserve energy are counter-productive, and unfortunately result in higher fat deposition and obesity, and can exacerbate the risk of sarcopenia[6].

It has long been known that growth differentiation factor 8 (GDF8, also known as myostatin), acting via the activin type II receptors ActRIIA/B in skeletal muscle, is a major negative regulator of muscle mass[7–9]. This has led to therapeutic efforts to promote muscle growth—in settings of muscle atrophy as well an in obesity-related conditions—involving blocking antibodies to GDF8 or ActRIIA/B[10]. However, studies in mice showed that receptor blockade had about twice the impact on muscle growth compared to GDF8 blockade[9], suggesting that there may be other negative regulators of muscle mass acting via these receptors; with greater efficacy came greater concerns about adverse events with receptor blockade, as this receptor system responds to multiple other transforming growth factor-β (TGFβ)-related ligands, involved in myriad biologic processes[11–13]. We previously suggested that activin A (ActA) might be the key second negative regulator acting via ActRIIA/B, based on studies in mouse and non-human primates showing that joint blockade of GDF8 and ActA with monoclonal antibodies led to synergistic increases in muscle mass, similar to that seen with broader ActRIIA/B blockade[14].

The current trial attempted to extend these preclinical findings to humans, by assessing the effects of administering blocking monoclonal antibodies (mAbs) to GDF8 (trevogrumab) and to ActA (garetosmab), alone or in combination, on body composition. Consistent with preclinical observations[14], we found that combination blockade of GDF8 and ActA led to dose-dependent, persistent, greater than additive increases in muscle mass while decreasing fat mass.

Results

Enrollment and baseline demographics

Herein we report results of a phase 1 clinical trial (www.clinicaltrials.gov, NCT02943239), involving a randomized, double-blind, placebo-controlled comparison assessing the safety, tolerability, pharmacokinetics, and pharmacodynamics of the GDF8 mAb, trevogrumab, and the ActA mAb, garetosmab, each given alone, as well as the GDF8 mAb given in combination with increasing dose levels of the ActA mAb; these antibodies have previously been described[14]. The primary population initially studied was healthy postmenopausal females. Females of non-reproductive potential were chosen out of an abundance of caution, given findings from a preclinical study in Sprague Dawley rats and a study of pre- and postnatal development in cynomolgus monkeys that raised questions about ActA blockade on reproductive function (see Supplementary Results for complete details of these preclinical studies). Males were also initially excluded since there were concerns about the potential for testicular toxicity at the outset of the trial that emanated from the aforementioned toxicology study in Sprague Dawley rats (see Supplementary Results for complete details of this preclinical study). The study included a single ascending dose part (part I; healthy postmenopausal females only) and a multiple dose part (part II; healthy postmenopausal females), which was intended to further establish safety and tolerability and corroborate findings observed after single doses. A panel of healthy males not intending to father children was also included in part II to receive multiple doses of the ActA mAb once toxicology studies in non-human primates revealed an absence of testicular toxicity (see Supplementary Results for complete details of these preclinical studies). Subjects in part I of the study received a single treatment at the beginning of the trial, which was anticipated to result in active mAb levels for the 8-week evaluation period (as confirmed by pharmacokinetic studies described below). Of the 82 total subjects, 48 were randomized to the single-dose part of the study (Figs. 1A, 2) and 34 to the multiple-dose part of the study (Figs. 1B, 2). Baseline demographic and clinical characteristics were generally balanced across the single- and multiple-dose parts of the study (Tables 1, 2).

Single-dose combination of anti-GDF8 and anti-ActA results in greater increases in muscle and lean mass measures, and greater decreases in fat measures, than either mAb alone

To understand the contribution of GDF8 and ActA on body composition in humans (healthy postmenopausal females), we administered single doses of the GDF8 and ActA mAbs alone or in combination, and used various measures to assess changes in muscle and fat at weeks 4 and 8: magnetic resonance imaging (MRI) was used to quantitate percent change in thigh muscle volume (Fig. 3A); and dual x-ray

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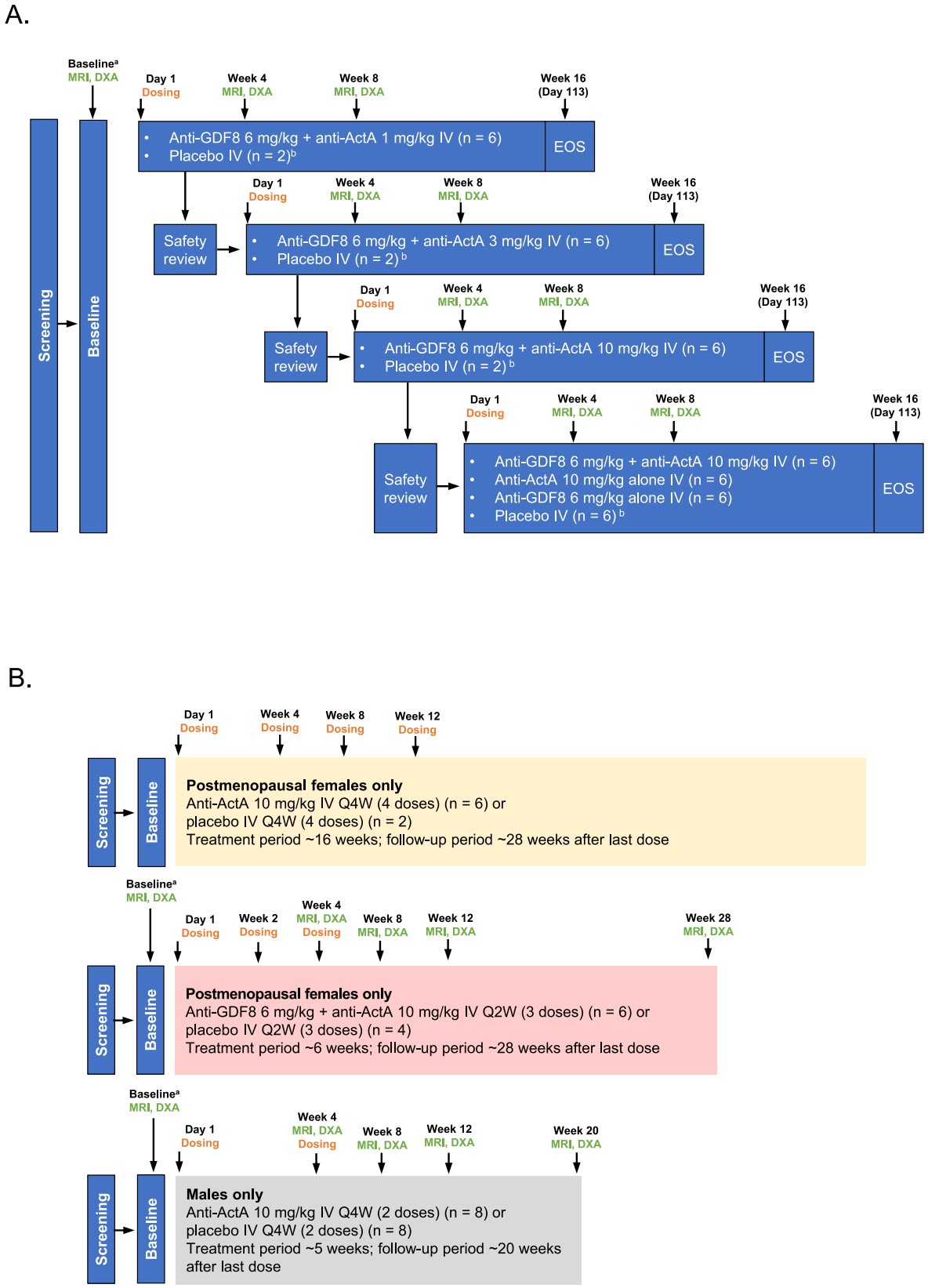

Fig. 1 | Study design. Study design schematics for the single-dose (part I; panel **A**) and multiple-dose (part II; panel **B**) parts of the study. [a]Two baseline MRIs and two baseline DXAs were obtained for each subject. [b]Placebo subjects were pooled for analysis. ActA activin A, EOS end of study, DXA dual X-ray absorptiometry, GDF8 growth differentiation factor 8, MRI magnetic resonance imaging, Q2W every 2 weeks, Q4W every 4 weeks.

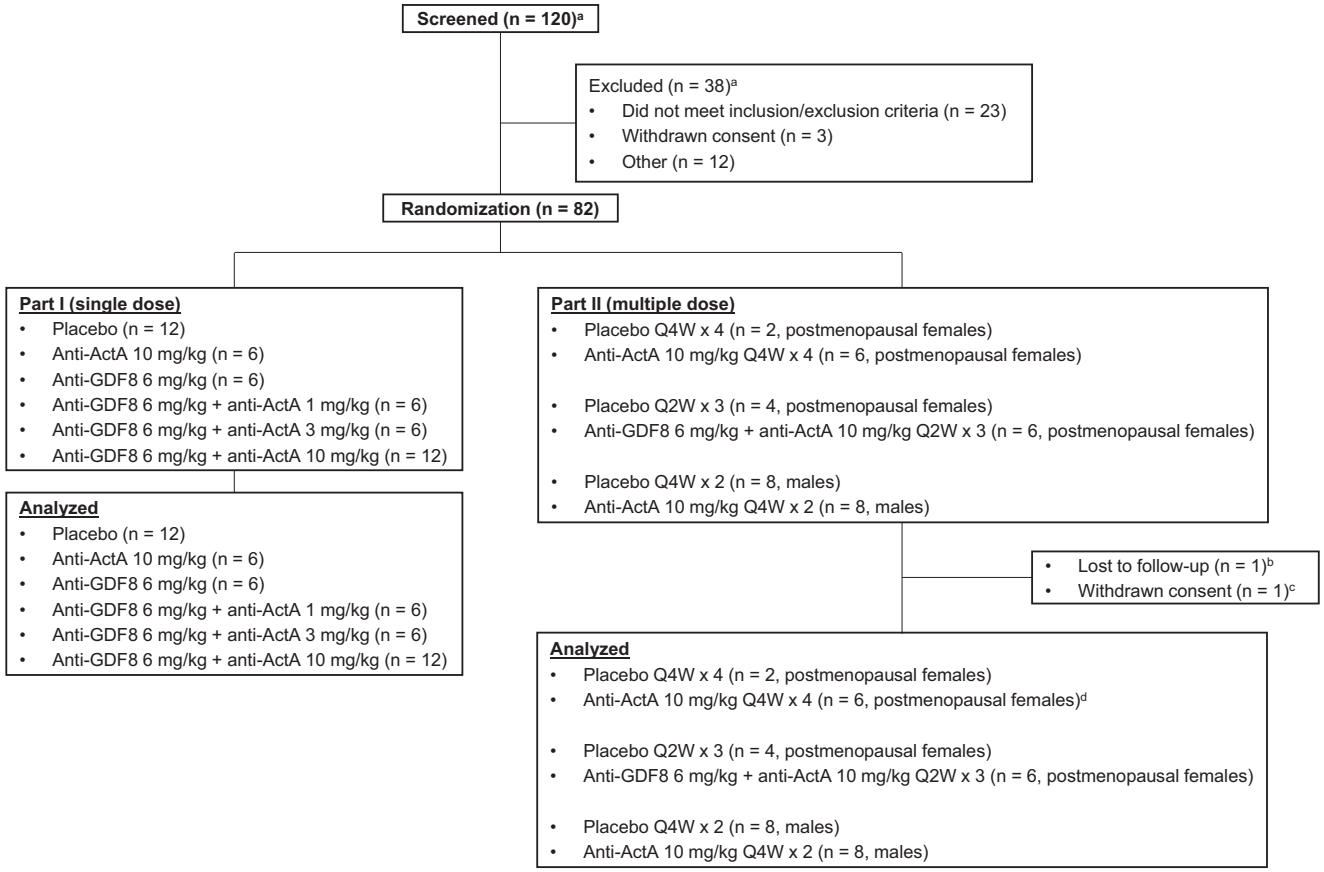

Fig. 2 | Flow diagram. Flow diagram for subjects randomized to the single-dose (part I) and multiple-dose (part II) parts of the study. All subjects in part I of the trial are postmenopausal females. [a]Number of subjects screened and excluded before randomization includes those in both the single-dose (part I) and multiple-dose (part II) parts of the study. [b]Placebo Q4W × 2 (male). [c]Anti-ActA 10 mg/kg Q4W × 2 (male); withdrew after completing all assigned doses of study drug due to new job demands and extra responsibilities. [d]One subject receiving Anti-ActA 10 mg/kg Q4W × 4 had study drug withdrawn due to a treatment-emergent adverse event. ActA activin A, GDF8 growth differentiation factor 8, Q2W every 2 weeks, Q4W every 4 weeks.

absorptiometry (DXA) was used to quantitate percent changes in total lean mass (Fig. 3B), appendicular lean body mass (a measure of lean tissue in the arms and legs[15]; Fig. 3C), android fat mass (Fig. 3D), and total fat mass (Fig. 3E). At week 8, treatment with the individual blocking mAbs each showed numeric increases in all the muscle and lean mass measures compared to placebo (Fig. 3A-C), as well as numeric decreases in the fat measures compared to placebo (Fig. 3D, E), but only the combination—and particularly using the highest dose—showed consistent nominally statistically significant differences in all the measures of muscle gain and fat loss. Comparison between week 4 and week 8 showed increasing benefit in most measures across this time period (Fig. 3F–J; Table 3; and Supplementary Table 1). Post hoc comparisons between the treatment arms, assessing the contribution of each ligand to change in thigh muscle volume at weeks 4 and 8 support that combination blockade leads to greater increases in muscle size and are presented in Supplementary Tables 2, 3.

Increases in muscle mass were also observed following a multiple-dose combination of anti-GDF8 and anti-ActA

Multiple-dose administration of GDF8 and ActA mAbs was assessed to gain further experience on the safety and tolerability of these mAbs and to corroborate the effects on muscle mass that were observed following single doses. Postmenopausal females were administered GDF8 and ActA mAbs in combination Q2W for three doses in total. In this portion of the study, with the included sample size, only thigh muscle volume was considered likely to show significant effects, as DXA is a more variable and less sensitive measure than MRI (see Methods section for additional statistical information).

Combination treatment significantly increased thigh muscle volume, as measured by MRI, from baseline to weeks 4, 8, and 12 compared with placebo (Supplementary Table 4), consistent with the effects observed after single doses. Following withdrawal of treatment, muscle size returned to baseline by week 28, showing the reversibility of the effect.

When evaluating additional measures of lean mass by the less-sensitive DXA modality, we observed numerical increases in lean mass over time that did not achieve nominal statistical significance (Supplementary Tables 4, 5). Numerical (not statistically significant) decreases in android and total fat mass were also observed (Supplementary Tables 4, 5).

A panel of males was also included primarily to assess the tolerability of ActA mAb alone (Q4W × 2 doses) and underwent MRI and DXA measures. No obvious changes in thigh muscle volume nor lean or fat mass were detected (Supplementary Tables 4, 5).

Clinical effects on muscle and fat parallel mAb exposure

Following a single administration of the GDF8 mAb, concentrations of total GDF8 in serum increased, reflecting formation of an inert complex between the mAb bound to GDF8 (Fig. 4); maintenance of high levels of this complex for ~8 weeks suggests that blockade is occurring during this time frame, consistent with the coincident effects on muscle and fat (Fig. 3F–J). At the same time, single administration of the ActA mAb at 1, 3, and 10 mg/kg showed both dose-dependent increases in serum activin mAb concentrations and dose-dependent

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Table 1 | Baseline characteristics for the single-dose part of the study (healthy postmenopausal females)

	Pooled placebo (*n* = 12)	Anti-ActA 10 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 1 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 3 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg (*n* = 12)
Age, years						
Mean (SD)	55.5 (5.8)	61.5 (4.0)	56.3 (3.5)	61.2 (7.0)	61.0 (4.8)	57.6 (5.0)
Age group, years, *n* (%)						
≥45 to <65	11 (91.7)	5 (83.3)	6 (100)	4 (66.7)	5 (83.3)	10 (83.3)
≥65	1 (8.3)	1 (16.7)	0	2 (33.3)	1 (16.7)	2 (16.7)
Ethnicity, *n* (%)						
Hispanic or Latino	0	1 (16.7)	0	0	0	0
Not Hispanic or Latino	12 (100)	5 (83.3)	6 (100)	6 (100)	6 (100)	12 (100)
Race, *n* (%)						
White	12 (100)	6 (100)	5 (83.3)	6 (100)	5 (83.3)	10 (83.3)
Native Hawaiian/Other Pacific Islander	0	0	0	0	0	1 (8.3)
Other	0	0	1 (16.7)	0	1 (16.7)	1 (8.3)
Height, cm						
Mean (SD)	162.3 (8.4)	163.5 (5.7)	165.2 (5.7)	166.3 (2.3)	165.0 (5.7)	163.8 (4.0)
Weight, kg						
Mean (SD)	68.6 (9.0)	71.0 (5.0)	73.7 (13.5)	69.6 (14.8)	70.6 (13.4)	69.3 (11.0)
BMI, kg/m^2						
Mean (SD)	26.2 (3.6)	26.6 (0.7)	26.8 (3.5)	25.1 (4.8)	25.8 (3.8)	25.8 (3.6)

Full analysis set presented.
ActA activin A, *BMI* body mass index, *GDF8* growth differentiation factor 8, *SD* standard deviation.

Table 2 | Baseline characteristics for the multiple-dose part of the study

		Healthy postmenopausal females		Healthy males
	Pooled placebo (*n* = 14)	Anti-ActA 10 mg/kg Q4W × 4 doses (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg Q2W × 3 doses (*n* = 6)	Anti-ActA 10 mg/kg Q4W × 2 doses (*n* = 8)
Age, years				
Mean (SD)	53.7 (5.4)	55.7 (3.1)	57.7 (5.3)	49.3 (5.6)
Age group, years, *n* (%)				
<45	1 (7.1)	0	0	1 (12.5)
≥45–<65	13 (92.9)	6 (100)	5 (83.3)	7 (87.5)
≥65 years	0	0	1 (16.7)	0
Ethnicity, *n* (%)				
Hispanic or Latino	0	0	0	0
Not Hispanic or Latino	13 (92.9)	6 (100)	6 (100)	7 (87.5)
Not reported	0	0	0	1 (12.5)
Unknown	1 (7.1)	0	0	0
Race, *n* (%)				
White	13 (92.9)	5 (83.3)	6 (100)	6 (75.0)
Asian	0	0	0	2 (25.0)
Native Hawaiian/Other Pacific Islander	0	1 (16.7)	0	0
Other	1 (7.1)	0	0	0
Height, cm				
Mean (SD)	173.3 (8.4)	162.9 (7.0)	163.7 (9.1)	178.5 (5.8)
Weight, kg				
Mean (SD)	80.7 (15.5)	64.0 (9.5)	74.5 (10.3)	83.5 (13.9)
BMI, kg/m^2				
Mean (SD)	26.7 (3.6)	24.0 (1.4)	27.8 (2.9)	26.1 (3.4)

Full analysis set presented.
ActA activin A, *BMI* body mass index, *GDF8* growth differentiation factor 8, *SD* standard deviation.

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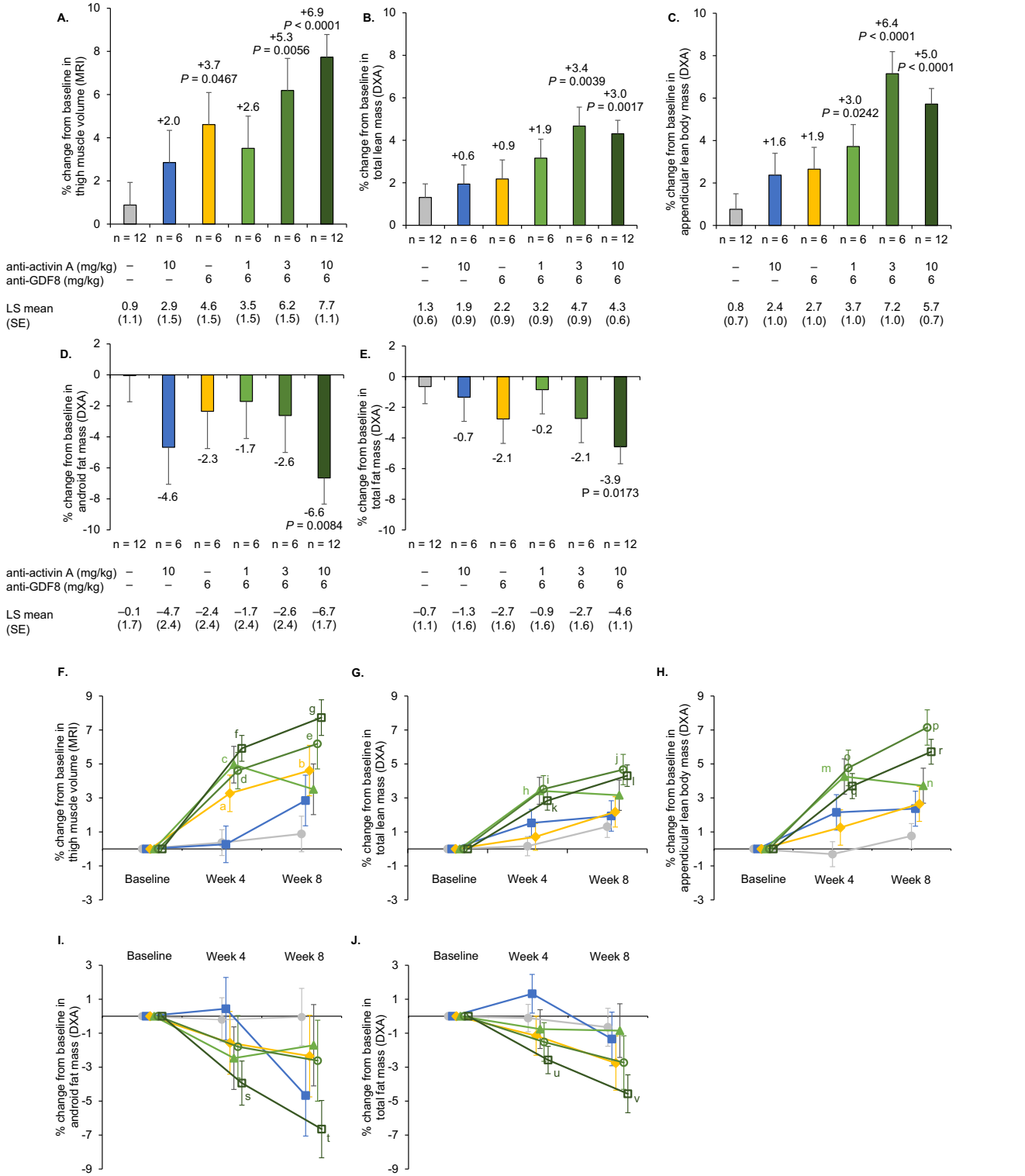

Fig. 3 | Single-dose blockade of GDF8 and ActA increased lean body mass and decreased fat mass. Panels **A**–**C** present the percent change from baseline to week 8 in indicators of lean body mass following a single dose of IV GDF8 mAb, ActA mAb, both in combination, or placebo: thigh muscle volume as assessed by MRI (panel **A**), total lean mass as assessed by DXA (panel **B**) and appendicular lean mass assessed by DXA (panel **C**). Panels **D**, **E** present the percent change from baseline to week 8 in indicators of fat mass following a single dose of IV GDF8 mAb, ActA mAb, both in combination, or placebo: android fat mass assessed by DXA (panel **D**), and total fat mass as assessed by DXA (panel **E**). Panels **F**–**H** present the percent change from baseline over time in indicators of lean body mass following a single dose of IV GDF8 mAb, ActA mAb, both in combination, or placebo: thigh muscle volume as assessed by MRI (panel **F**), total lean mass as assessed by DXA (panel **G**) and appendicular lean mass assessed by DXA (panel **H**). Panels **I**, **J** present the percent change from baseline over time in indicators of fat mass following a single dose of IV GDF8 mAb, ActA mAb, both in combination, or placebo: android fat mass assessed by DXA (panel **I**) and total fat mass as assessed by DXA (panel **J**). Data were least squares mean ± standard error. In panels **A**–**E**, column data labels indicate difference from placebo. Nominal *P* values were calculated using a two-sided *t*-test; *P* values less than 0.05 were considered statistically significant. [a]$P = 0.0335$; [b]$P = 0.0467$; [c]$P = 0.0012$; [d]$P = 0.0025$; [e]$P = 0.0056$; [f]$P < 0.0001$; [g]$P < 0.0001$; [h]$P = 0.0018$; [i]$P = 0.0013$; [j]$P = 0.0039$; [k]$P = 0.0015$; [l]$P = 0.0017$; [m]$P = 0.0009$; [n]$P = 0.0242$; [o]$P = 0.0003$; [p]$P < 0.0001$; [q]$P = 0.0004$; [r]$P < 0.0001$; [s]$P = 0.0488$; [t]$P = 0.0084$; [u]$P = 0.0358$; [v]$P = 0.0173$. Full analysis set presented. Source data are provided as a Source Data file. ActA activin A, DXA dual X-ray absorptiometry, GDF8 growth differentiation factor 8, mAb monoclonal antibody, MRI magnetic resonance imaging.

increases in total ActA, similarly reflecting complex formation between this mAb and ActA (Fig. 4). Effects on muscle volume and fat mass coincided with changes in total ActA concentrations, particularly in the 1 mg/kg ActA group (low-dose combination), where decreased effects at week 8 relative to week 4 (Fig. 3F–J) seemed to parallel decreased concentrations of total ActA in serum (i.e., the low dose of the ActA mAb did not appear to maintain blockade over the 8-week period, while the higher doses did).

Following multiple-dose administration of GDF8 and ActA mAbs in combination Q2W through week 4 (three doses in total), effects on muscle volume (Supplementary Table 4) coincided with changes in concentrations of total GDF8 and total ActA (Supplementary Fig. 1). Serum concentrations of both total GDF8 and total ActA increased shortly after the start of treatment (reflecting binding of the ligands with their respective mAbs), reached a plateau by approximately4 to 8 weeks, remained at a plateau until approximately week 12, and then declined to lower concentrations by week 28. This paralleled effects on muscle volume, which were highest at weeks 8 and 12 and tended to return to baseline by week 28. Effects on fat mass (Supplementary Tables 4, 5) showed a later onset relative to both effects on muscle volume and elevated concentrations of total ligands in serum (Supplementary Fig. 1).

GDF8 and ActA mAbs were generally well tolerated

The GDF8 and ActA antibodies were generally well-tolerated. In the single-dose part of the study, adverse events (AEs) occurring in ≥20% of subjects in any of the combination groups that did not occur in the placebo group were muscle spasms, mouth ulcerations/aphthous ulcers, and urinary tract infection (Table 4). Treatment-related muscle spasms and mouth ulcerations were reported more frequently in the anti-GDF8 + anti-ActA combination groups compared with placebo, with a difference in frequency greater than 40% in at least one dose group. All subjects receiving treatment had AEs that were mild-to-moderate and non-serious. No clinically meaningful differences were identified between the treatment groups from a review of vital signs, safety labs including routine blood chemistry and hematology, follicle-stimulating hormone, bone-specific alkaline phosphatase, or creatinine phosphokinase levels.

Headache, which occurred in all groups, was the most frequently reported AE following multiple-dose administration of the GDF8 + ActA mAb combination in postmenopausal females (Table 5). Muscle spasms were reported more frequently in the anti-ActA alone and anti-GDF8 + anti-ActA combination groups compared with the placebo. Mouth ulcerations/aphthous ulcer and upper respiratory tract infection were reported more frequently in the anti-ActA alone and anti-GDF8 + anti-ActA combination groups, respectively, compared with the placebo. Treatment-related muscle spasms were reported more frequently in the anti-GDF8 + anti-ActA combination groups compared with placebo, with a difference in frequency greater than 40%. One serious AE of diverticulitis occurred in the anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg Q2W × 3 group after week 28 in a subject with a prior history of diverticulosis and was considered not related to treatment by the investigator. One AE of herpes zoster that occurred in the anti-ActA alone group lead to withdrawal from the trial; this AE was considered related to treatment by the investigator. A summary of AEs is also presented for males treated with the ActA mAb alone Q4W for 2 doses in total (Table 5).

There were no observable trends in body weight (Supplementary Tables 6, 7) and no subjects tested positive for anti-drug antibodies post-treatment in either the single-dose or multiple-dose parts of the study.

Discussion

This is the first report of a clinical trial demonstrating that GDF8 and ActA work together to regulate muscle growth, and suggests these two ligands are the key dominant negative regulators of muscle mass in humans. Combination ligand blockade with the GDF8 (trevogrumab) and ActA (garetosmab) mAbs led to substantial dose-dependent increases in MRI-quantitated thigh muscle volume over placebo that was greater with combination than treatment with either mAb alone. Furthermore, we observed increases in total body muscle volume and lean body mass using additional imaging modalities (DXA), suggesting the effects of total ligand blockade are widespread and not limited to a single muscle group. Consistent effects of the combination treatment on thigh muscle MRI were observed following either single or multiple doses, suggesting maximal or near-maximal effects were observed after a single dose. In the single dose portion of the study where we had enough subjects receiving placebo and active antibodies to see effects by DXA, we also observed corresponding decreases in fat mass following combination blockade of GDF8 and ActA, consistent with the notion that increases in muscle will increase energy expenditure and correspondingly and indirectly decrease fat deposits.

Combination blockade of GDF8 and ActA was found to be generally well tolerated in this study, with muscle spasms and mouth ulcerations/aphthous ulcers appearing as the main treatment-related adverse events in the combination groups. There were otherwise no newly identified safety concerns. This is in contrast to the tolerability concerns raised for the ActA mAb garetosmab in patients with fibrodysplasia ossificans progressiva (FOP), an ultra-rare condition in which patients express a mutant activin type I receptor ACVR1 that leads to abnormal signaling by ActA and deposition of bone in skeletal muscle and connective tissues[16]. FOP has a high background morbidity and mortality, which makes the assessment of treatment-related adverse effects challenging. While the safety of garetosmab in FOP patients is currently being further investigated in a phase 3 trial, in the current study in otherwise healthy individuals, garetosmab alone as well as in combination with the GDF8 mAb was generally well tolerated, and we did not observe the pattern of AEs reported in patients with FOP, which may be disease specific.

In the current study, initial increases in thigh muscle volume with the combination blockade of GDF8 and ActA returned to baseline by week 28. This suggests that these ligands need to be constitutively blocked in order to maintain the effects on lean mass. GDF8 is a muscle rheostat[17], and GDF8 levels increase in proportion to muscle mass[18]. We speculate that GDF8 functions to keep muscle growth in check and that blockade of GDF8 signaling is a non-physiological state akin to leptin deficiency for adipose; the absence of GDF8 signaling likely suggests to the muscle that it has wasted away and needs to promote growth. We also know that ActA, like GDF8, signals through ActRIIA/B to activate the downstream transcription factors SMAD2/3, ultimately resulting in muscle atrophy[14], so it is also capable of acting as a second negative regulator of muscle in the absence of GDF8. Thus, once serum concentrations of the mAbs decline and blockade of these ligands ceases, muscle volume returns to baseline. Importantly, there was no exercise intervention in this study, so we were able to evaluate the effects of combination blockade in the least-confounded way possible.

Although no head-to-head trials are available, cross-study comparisons suggest that the efficacy of GDF8 and ActA mAbs in combination is similar to bimagrumab, a human mAb that binds and blocks activin type II receptors[19]. Thus, specific blockade using just our GDF8 and ActA mAbs produces similar benefits as does a receptor-blocking mAb that blocks multiple ligand pathways, while potentially avoiding adverse effects that might emerge as a result of complete ActRIIA and ActRIIB blockade.

As has been previously proposed, the ability to specifically block negative regulators of muscle mass could provide profound therapeutic benefit in multiple settings[10]—ranging from individuals (such as the elderly) suffering from muscle atrophy and weakness, to the obesity setting where increases in muscle can drive energy expenditure that can help with weight control but also provide metabolic

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Table 3 | Percent change from baseline to week 8 in lean body mass and fat mass following single-dose blockade of GDF8 and ActA (healthy postmenopausal females)

	Pooled placebo (*n* = 12)	Anti-ActA 10 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 1 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 3 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg (*n* = 12)
Thigh muscle volume (cm^3) by MRI						
Baseline						
Mean (SD)	411.50 (45.17)	425.72 (55.28)	401.08 (65.05)	393.56 (78.84)	405.58 (62.99)	416.33 (66.50)
Percent change from baseline						
Week 4						
LS mean (SE)	0.37 (0.76)	0.27 (1.08)	3.27 (1.08)	4.96 (1.08)	4.61 (1.07)	5.92 (0.76)
LS mean difference vs placebo (SE)		−0.11 (1.32)	2.90 (1.32)	4.59 (1.32)	4.24 (1.32)	5.55 (1.07)
95% CI		−2.77, 2.55	0.24, 5.55	1.92, 7.25	1.59, 6.90	3.38, 7.72
Nominal *P* value		0.9358	0.0335	0.0012	0.0025	<0.0001
Week 8						
LS mean (SE)	0.88 (1.05)	2.85 (1.49)	4.61 (1.49)	3.51 (1.49)	6.19 (1.48)	7.73 (1.05)
LS mean difference vs placebo (SE)		1.97 (1.82)	3.73 (1.82)	2.63 (1.82)	5.31 (1.82)	6.85 (1.48)
95% CI		−1.70, 5.65	0.06, 7.40	−1.05, 6.31	1.64, 8.98	3.86, 9.85
Nominal *P*-value		0.2846	0.0467	0.1569	0.0056	<0.0001
Total lean mass (kg) by DXA						
Baseline						
Mean (SD)	40.49 (4.38)	43.10 (2.76)	42.22 (6.51)	42.52 (5.02)	42.54 (5.90)	40.94 (5.07)
Percent change from baseline						
Week 4						
LS mean (SE)	0.16 (0.56)	1.53 (0.80)	0.71 (0.79)	3.42 (0.79)	3.52 (0.79)	2.84 (0.56)
LS mean difference vs placebo (SE)		1.37 (0.98)	0.55 (0.97)	3.26 (0.98)	3.36 (0.98)	2.69 (0.79)
95% CI		−0.61, 3.35	−1.41, 2.52	1.29, 5.23	1.39, 5.33	1.09, 4.28
Nominal *P* value		0.1702	0.5715	0.0018	0.0013	0.0015
Week 8						
LS mean (SE)	1.31 (0.64)	1.94 (0.90)	2.18 (0.89)	3.16 (0.89)	4.67 (0.89)	4.31 (0.63)
LS mean difference vs placebo (SE)		0.63 (1.11)	0.88 (1.10)	1.85 (1.10)	3.37 (1.10)	3.00 (0.89)
95% CI		−1.60, 2.87	−1.34, 3.10	−0.37, 4.08	1.14, 5.59	1.20, 4.81
Nominal *P* value		0.5716	0.4299	0.1004	0.0039	0.0017
Appendicular lean body mass (kg) by DXA						
Baseline						
Mean (SD)	17.44 (2.39)	18.02 (1.68)	18.10 (3.05)	17.92 (3.35)	18.67 (3.48)	17.32 (2.19)
Percent change from baseline						
Week 4						
LS mean (SE)	−0.31 (0.74)	2.15 (1.04)	1.26 (1.04)	4.26 (1.04)	4.77 (1.05)	3.70 (0.74)
LS mean difference vs placebo (SE)		2.46 (1.28)	1.57 (1.28)	4.57 (1.28)	5.08 (1.29)	4.01 (1.04)
95% CI		−0.12, 5.04	−1.02, 4.15	1.99, 7.15	2.48, 7.68	1.90, 6.11
Nominal *P*-value		0.0612	0.2276	0.0009	0.0003	0.0004
Week 8						
LS mean (SE)	0.76 (0.73)	2.37 (1.03)	2.65 (1.03)	3.72 (1.03)	7.15 (1.04)	5.72 (0.73)
LS mean difference vs placebo (SE)		1.61 (1.27)	1.90 (1.27)	2.96 (1.26)	6.39 (1.28)	4.97 (1.03)
95% CI		−0.95, 4.16	−0.66, 4.45	0.40, 5.51	3.82, 8.96	2.88, 7.05
Nominal *P*-value		0.2109	0.1418	0.0242	<0.0001	<0.0001
Total fat mass (kg) by DXA						
Baseline						
Mean (SD)	25.04 (6.52)	25.15 (4.74)	29.02 (7.80)	24.61 (10.55)	25.91 (8.58)	25.82 (8.31)
Percent change from baseline						
Week 4						
LS mean (SE)	−0.11 (0.816)	1.32 (1.14)	−1.15 (1.15)	−0.76 (1.14)	−1.52 (1.14)	−2.58 (0.81)

Table 3 (continued) | Percent change from baseline to week 8 in lean body mass and fat mass following single-dose blockade of GDF8 and ActA (healthy postmenopausal females)

	Pooled placebo (*n* = 12)	Anti-ActA 10 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 1 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 3 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg (*n* = 12)
LS mean difference vs placebo (SE)		1.42 (1.39)	−1.04 (1.41)	−0.65 (1.39)	−1.41 (1.39)	−2.47 (1.14)
95% CI		−1.39, 4.24	−3.89, 1.81	−3.47, 2.16	−4.23, 1.41	−4.77, −0.17
Nominal *P* value		0.3133	0.4646	0.6413	0.3180	0.0358
Week 8						
LS mean (SE)	−0.65 (1.12)	−1.34 (1.58)	−2.76 (1.60)	−0.85 (1.58)	−2.73 (1.58)	−4.57 (1.12)
LS mean difference vs placebo (SE)		−0.69 (1.93)	−2.11 (1.96)	−0.20 (1.93)	−2.08 (1.93)	−3.92 (1.58)
95% CI		−4.59, 3.21	−6.06, 1.84	−4.10, 3.70	−5.98, 1.82	−7.11, −0.73
Nominal *P* value		0.7217	0.2873	0.9187	0.2884	0.0173
Android fat mass (kg) by DXA						
Baseline						
Mean (SD)	2.04 (1.01)	1.85 (0.72)	2.35 (0.96)	1.81 (1.00)	2.05 (0.94)	2.10 (1.08)
Percent change from baseline						
Week 4						
LS mean (SE)	−0.21 (1.30)	0.44 (1.84)	−1.57 (1.85)	−2.47 (1.84)	−1.80 (1.83)	−3.94 (1.30)
LS mean difference vs placebo (SE)		0.65 (2.25)	−1.35 (2.26)	−2.25 (2.25)	−1.59 (2.25)	−3.73 (1.84)
95% CI		−3.89, 5.20	−5.91, 3.20	−6.80, 2.29	−6.13, 2.95	−7.43, −0.02
Nominal *P* value		0.7738	0.5518	0.3228	0.4830	0.0488
Week 8						
LS mean (SE)	−0.05 (1.69)	−4.67 (2.39)	−2.35 (2.40)	−1.71 (2.39)	−2.62 (2.38)	−6.65 (1.69)
LS mean difference vs placebo (SE)		−4.62 (2.93)	−2.31 (2.93)	−1.66 (2.93)	−2.57 (2.92)	−6.61 (2.38)
95% CI		−10.53, 1.28	−8.23, 3.62	−7.57, 4.25	−8.47, 3.32	−11.42, −1.79
Nominal *P* value		0.1217	0.4362	0.5731	0.3836	0.0084

Full analysis set presented. Nominal *P* values were calculated using a two-sided *t*-test; *P* values less than 0.05 were considered statistically significant.
ActA activin A, *CI* confidence interval, *DXA* dual X-ray absorptiometry, *GDF8* growth differentiation factor 8, *LS* least squares, *MRI* magnetic resonance imaging, *SD* standard deviation, *SE* standard error.

benefit. Moreover, it has recently been appreciated that emerging approaches to combat obesity by causing profound weight loss—such as with bariatric surgery or glucagon-like peptide-1 (GLP1)-receptor agonists—can often also result in profound muscle loss, with up to 40% of the associated weight loss accounted for by lean mass loss[20–22]. This loss of muscle, an undesirable effect on body composition, can lead to negative long-term consequences for patients with obesity. In an accompanying manuscript, we describe preclinical studies in obese mice and non-human primates showing that GDF8 and ActA mAbs in the setting of GLP1-induced weight loss can maintain and even increase muscle mass, with associated metabolic benefit including reduced adiposity, thus providing a potentially better approach to weight loss and maintenance[23], consistent with the findings observed when treating obese mice with bimagrumab and a GLP1-receptor agonist[24]. While these early results are encouraging and suggest the need for further investigation, the benefit-risk profile of these agents in settings of obesity will require larger and longer clinical studies. Clinical trials are now underway in patients with obesity using these mAbs in combination with GLP1-receptor agonists to explore this important possibility (www.clinicaltrials.gov, NCT06299098).

It is important to interpret these results in the context of study limitations. This is a phase 1 trial; therefore, a limited number of healthy volunteers were exposed to treatment for preliminary safety, pharmacodynamic, and pharmacokinetic effects. The key parts of the study and conclusions of the impact of the combination of ActA mAb and GDF8 mAb were based on a study in postmenopausal women only (males did not receive the combination). This was done for practical reasons at an early stage in development when there was uncertainty about potential adverse consequences of ActA blockade on reproductive potential (see Supplementary Results for complete details of the preclinical studies). Given that similar effects on muscle mass are observed in male cynomolgus monkeys, it appears likely that the phenomenon of the role of ActA and GDF8 in regulating muscle mass will be generalizable[14,23]. The effects on muscle mass that were observed by blocking both ActA and GDF8 following single doses of mAbs were corroborated by thigh muscle MRI following multiple dose administration. However, corroboration of effects on lean muscle mass and fat mass, as measured by DXA, following multiple doses were limited by the size of the study, given the variability of the measurements[25,26]. The variability of the DXA measurements, both at baseline and over time, were larger in the multiple-dose cohorts than in the single-dose cohorts. Reasons for the extra variability are unknown, but unsurprising given the small sample size in this safety cohort. Additional studies are being conducted to confirm these observations.

Methods

Trial oversight

This study was conducted in accordance with the principles of the Declaration of Helsinki and the Council for International Organizations of Medical Sciences International Ethical Guidelines, and was consistent with Good Clinical Practices of the International Conference on Harmonisation and applicable regulatory requirements. The study protocol was approved by an institutional review board prior to study initiation (Ministry of Health, Wellington, New Zealand). Monitoring and site supervision were performed with oversight by the sponsor.

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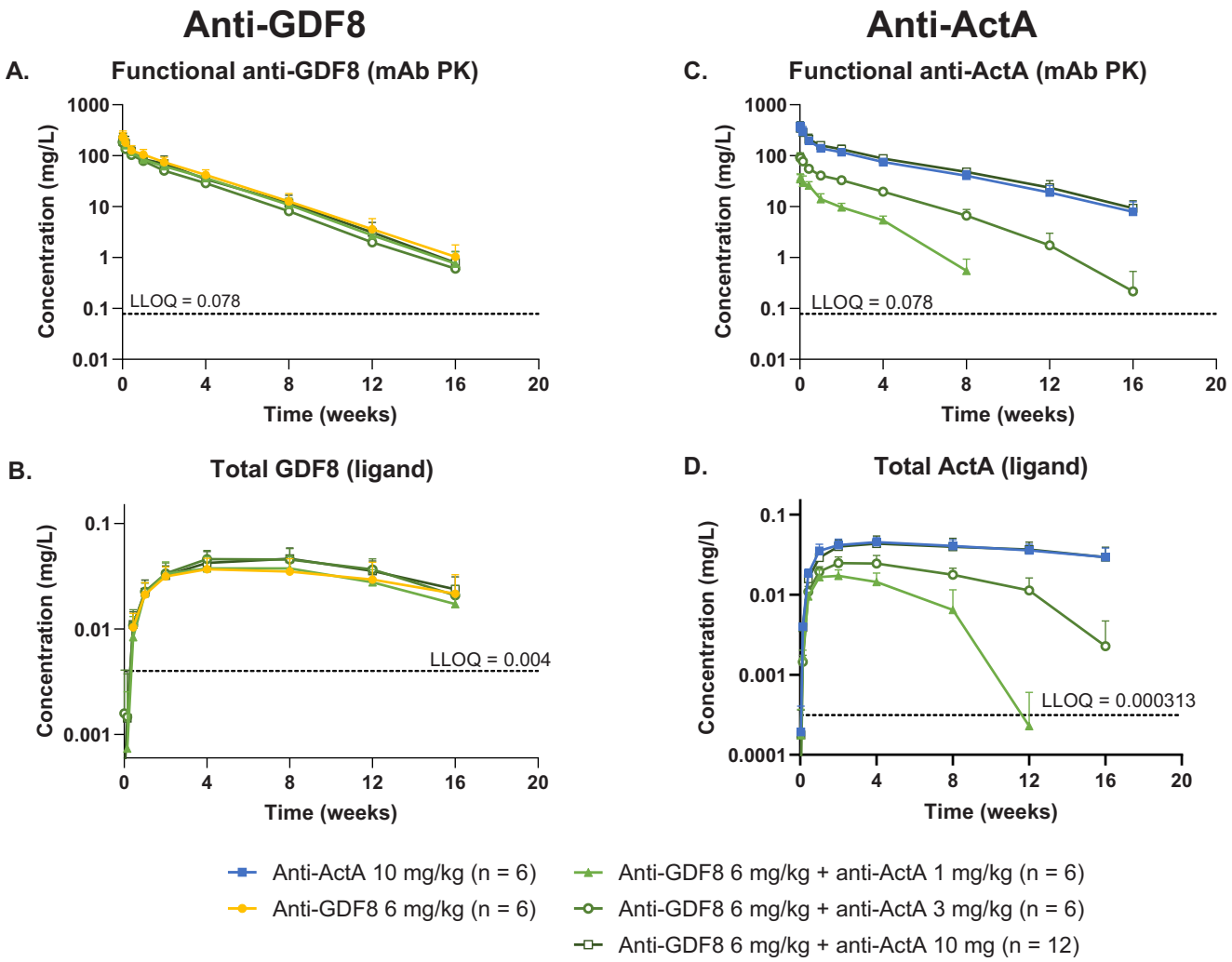

Fig. 4 | Pharmacokinetics and total ligand concentrations in serum of healthy postmenopausal females following single-dose administration of the GDF8 mAb (trevogrumab) and the ActA mAb (garetosmab). Pharmacokinetics are shown in panels **A, C**, and concentration-time profiles of the total ligands are shown in panels **B**, **D**. For the anti-GDF8 6 mg/kg + anti-ActA 1 mg/kg treatment group, no data were presented for week 12 and week 16 in panel **C** nor for week 16 in panel **D** because these values were below the LLOQ. Data were mean ± standard deviation; mean serum concentrations reported as zero are not plotted. The pharmacokinetic analysis set is presented. Source data are provided as a Source Data file. ActA activin A, GDF8, growth differentiation factor 8, LLOQ lower limit of quantification, mAb monoclonal antibody; PK pharmacokinetics.

Study design

This was a randomized, double-blind, placebo-controlled phase 1 clinical trial assessing the safety, tolerability, pharmacokinetics, and pharmacodynamics of the GDF8 and ActA mAbs, trevogrumab and garetosmab, alone and in combination. The study was registered at ClinicalTrials.gov No. NCT02943239 on October 16, 2016. The first part of the trial (part I) was a single ascending dose assessment in healthy postmenopausal females (Fig. 1A); the second part of the trial (part II) was an exploratory multiple-dose assessment in healthy postmenopausal females and healthy adult males (Fig. 1B). The trial was conducted at two sites in New Zealand from December 8, 2016 to April 30, 2019. The protocol is provided in the Supplementary Materials.

Participants

Eligible subjects included healthy postmenopausal females aged 45–70 years and males aged 35–60 years not intending to father children, with a body mass index of 18–32 kg/m^2, and who were willing to maintain their current diet and physical activity level. Sex was self-reported by the subject. Because there were potential reproductive abnormalities observed in preclinical toxicity studies in Sprague Dawley rats and cynomolgus monkeys (see Supplementary Results for complete details of these preclinical studies), out of an abundance of caution, we restricted female subjects to postmenopausal women of nonchildbearing potential. Postmenopausal status was confirmed by either a duration of 12 months since the last menses or a follicle-stimulating hormone level of ≥30 mIU/mL. Males were initially excluded, given findings from the aforementioned toxicology study in Sprague Dawley rats that suggested a potential risk of testicular toxicity (see Supplementary Results for complete details of this preclinical study). Later in the study, upon availability of additional preclinical toxicology studies in non-human primates that was reassuring (see Supplementary Results for complete details of these preclinical studies), a panel of healthy males not intending to father children was included. Full inclusion and exclusion criteria are detailed in the protocol provided in the Supplementary Materials. All subjects or legally authorized representatives provided written informed consent prior to study enrollment.

Treatment intervention and assessments

The trial was divided into two parts comprising single-dose and multiple-dose administration (Supplementary Table 8). The rationale for dose selection for both parts of the study is described in

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Table 4 | Summary of TEAEs in the single-dose part of the study (healthy postmenopausal females)

	Pooled placebo (*n* = 12)	Anti-ActA 10 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg (n = 6)	Anti-GDF8 6 mg/kg + anti-ActA 1 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 3 mg/kg (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg (*n* = 12)
TEAEs, n	34	15	16	28	31	73
Subjects with, *n* (%)						
≥1 TEAE	11 (91.7)	5 (83.3)	6 (100)	6 (100)	6 (100)	12 (100)
Serious TEAE	0	0	0	0	0	0
Infusion reaction TEAE	0	0	0	0	0	1 (8.3)
TEAE leading to permanent discontinuation of study drug	0	0	0	0	0	0
TEAE leading to death	0	0	0	0	0	0
TEAE of special interest[a]	0	0	0	0	0	0
TEAEs occurring in ≥20% of subjects in any treatment group, *n* (%)						
Headache	6 (50.0)	2 (33.3)	4 (66.7)	4 (66.7)	4 (66.7)	8 (66.7)
Muscle spasms	0	0	0	4 (66.7)	3 (50.0)	7 (58.3)
Back pain	2 (16.7)	1 (16.7)	0	1 (16.7)	0	4 (33.3)
Myalgia	2 (16.7)	0	1 (16.7)	0	2 (33.3)	2 (16.7)
Nausea	2 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)	4 (33.3)
Mouth ulceration	0	0	0	0	3 (50.0)	3 (25.0)
Upper respiratory tract infection	2 (16.7)	2 (33.3)	2 (33.3)	1 (16.7)	0	4 (33.3)
Urinary tract infection	0	0	0	0	0	3 (25.0)

Safety analysis set presented.
[a]TEAEs of special interest were only applicable to male subjects, none of which were included in the single-dose part (part I) of the study of postmenopausal women.
ActA activin A, *GDF8* growth differentiation factor 8, *TEAE* treatment-emergent adverse event.

Table 5 | Summary of TEAEs in the multiple-dose part of the study

	Healthy postmenopausal females			Healthy males	
	Pooled placebo[a] (*n* = 6)	Anti-ActA 10 mg/kg Q4W × 4 doses (*n* = 6)	Anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg Q2W × 3 doses (*n* = 6)	Placebo (*n* = 8)	Anti-ActA 10 mg/kg Q4W × 2 doses (*n* = 8)
TEAEs, *n*	28	26	48	13	18
Subjects with, *n* (%)					
≥1 TEAE	6 (100)	6 (100)	6 (100)	6 (75.0)	5 (62.5)
Serious TEAE	0	0	1 (16.7)	0	0
Infusion reaction TEAE	0	0	0	0	0
TEAE leading to permanent discontinuation of study drug	0	1 (16.7)	0	0	0
TEAE leading to death	0	0	0	0	0
TEAE of special interest[b]	0	0	0	0	0
TEAEs occurring in ≥20% of subjects in any treatment group, *n* (%)					
Upper respiratory tract infection	2 (33.3)	1 (16.7)	4 (66.7)	2 (25.0)	0
Aphthous ulcer		3 (50.0)		0	1 (12.5)
Diarrhea	0	1(16.7)	2 (33.3)	0	0
Muscle spasms	1 (16.7)	3 (50.0)	4 (66.7)	0	0
Back pain	2 (33.3)	0	1 (16.7)	1 (12.5)	0
Headache	5 (83.3)	2 (33.3)	2 (33.3)	0	4 (50.0)
Cough	2 (33.3)	0	0	0	1 (12.5)
Nasal congestion	2 (33.3)	0	0	0	0
Madarosis	0	0	2 (33.3)	0	0
Fatigue	0	1 (16.7)	2 (33.3)	1 (12.5)	0

Safety analysis set presented.
[a]Pooled placebo of healthy postmenopausal females.
[b]TEAEs of special interest were only applicable to male subjects, none of which were included in the single-dose part (part I) of the study of postmenopausal women, and included epididymitis, orchitis (inflammation of the testicles), hydrocele (fluid buildup around 1 or both testicles), scrotum pain, and scrotum swelling.
ActA activin A, *GDF8* growth differentiation factor 8, *TEAE* treatment-emergent adverse event.

the Supplementary Methods. Part I tested single administration on day 1 as follows: three groups of postmenopausal females were randomized 6:2 to receive intravenous (IV) doses of co-administered anti-GDF8 6 mg/kg + anti-ActA 1, 3, or 10 mg/kg in sequence or placebo, in three 8-participant cohorts. After completion of the single-dose cohorts, an expansion panel randomized postmenopausal females at a ratio of 1:1:1:1 to receive IV doses of anti-ActA 10 mg/kg alone, anti-GDF8 6 mg/kg alone, co-administration of anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg, or placebo. Part II tested multiple administration as follows: eight healthy postmenopausal females were randomized 6:2 to receive IV doses of anti-ActA 10 mg/kg alone Q4W for four doses, or matching placebo; another group of 10 healthy postmenopausal females were randomized 6:4 to receive IV doses of combination anti-GDF8 6 mg/kg + anti-ActA 10 mg/kg Q2W for three doses, or matching placebo; and 16 healthy males were randomized 1:1 to receive IV doses of anti-ActA 10 mg/kg Q4W for two doses, or matching placebo. Subjects were randomized according to a central randomization scheme provided by an interactive voice/web response system to the designated study pharmacist or qualified designee.

Muscle was assessed by measuring thigh muscle volume by MRI, a highly sensitive indicator of muscle size[26]. Total and appendicular lean body mass, total fat mass, and android fat mass were also assessed by DXA, which measures changes in body composition over time and has been commonly used in other clinical research studies, enabling a comparison with efficacy measures in other published studies[19,27]. Additional analyses included measurement of biomarkers such as follicle-stimulating hormone, bone-specific alkaline phosphatase, and creatinine phosphokinase. Presented safety assessments included vital signs, clinical laboratory tests, and adverse events, with a complete description of all safety assessments detailed in the protocol (provided in the Supplementary Materials).

Endpoints

The primary outcome measure was the incidence and severity of treatment-emergent AEs through week 16 for the single-dose part of the study and through week 40 for the multiple-dose part of the study. Secondary endpoints included percent and absolute change in thigh muscle volume as measured by MRI (except for the group of healthy postmenopausal females randomized receive anti-ActA 10 mg/kg alone Q4W for four doses), percent and absolute change in total and regional body composition (lean mass and fat mass) as measured by DXA (except for the group of healthy postmenopausal females randomized receive anti-ActA 10 mg/kg alone Q4W for four doses), pharmacokinetic profiles of the GDF8 and ActA mAbs (assessed via measurement GDF8 mAb and ActA mAb concentrations) in serum over time, changes in serum total GDF8 and total ActA levels over time, and the presence of anti-drug antibodies against the GDF8 mAb or the ActA mAb. One group of healthy postmenopausal females that received the ActA mAb alone did not have any MRI or DXA assessments and was therefore not included in those analyses.

Statistical analyses

Safety analyses were performed in the safety analysis set, which included all randomized subjects who received any study drug based on the treatment received (as treated). Efficacy analyses were performed in the full analysis set, which included all randomized subjects based on the treatment allocated (as randomized). The pharmacokinetic analysis set included all treated subjects who received any study drug and who had at least one non-missing post-dose pharmacokinetic result following the administration of the study drug.

As described in the statistical analysis plan, for the single-dose part of the study, assuming an anti-GDF8 + anti-ActA combination treatment effect of 6% over placebo in thigh muscle volume by MRI and an anti-GDF8 alone treatment effect of 3% over placebo, sample sizes of 12 for the anti-GDF8 + anti-ActA combination, six for anti-GDF8 alone, and 12 for placebo (pooled) were estimated to provide 99% power in demonstrating a treatment difference between the anti-GDF8 + anti-ActA combination final dose and placebo at a two-sided 0.05 level based on an assumed common standard deviation of 3%. A power statement was not provided for the DXA endpoints of the study nor for the multiple-dose part of the study, which was primarily conducted for safety and tolerability purposes and was considered descriptive; the sample size was consistent with a phase 1 study. While no power statements were conducted for the lean and fat mass endpoints, DXA lean body mass measures are more variable and less specific to muscle size than measurements of muscle volume by MRI[26].

The percent change and change in efficacy endpoints from baseline were analyzed using an analysis of covariance model with treatment group as a fixed effect and the baseline value as a continuous covariate. Treatment differences between the anti-GDF8 + anti-ActA combination and placebo were assessed by least squares mean, 95% confidence intervals, and nominal P values from the model. No adjustment was made for multiple testing. Missing efficacy data was not imputed. Placebo subjects from the single-dose and multiple-dose parts of the study were pooled separately for analysis. Nominal P values <0.05 were considered statistically significant. Post hoc comparisons of percent change in thigh muscle volume between the treatment arms utilized the same statistical methodology as the pre-specified efficacy endpoints described above. All analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The statistical analysis plan is provided in the Supplementary Materials.

Antibody production

The GDF8 mAb (trevogrumab, also known as REGN1033) was generated using VelocImmune HumAb mouse[28,29] and developed as published[14,30]. The resulting antibody is a fully human immunoglobulin (Ig) G4 mAb specific for GDF8, with no cross-reactivity to GDF11, a closely related member of the TGF-β family[30]. The ActA mAb (garetosmab, also known as REGN2477) was generated using VelocImmune HumAb mouse[28,29] and developed as published[14]. The resulting antibody contains an IgG4 constant region and is a fully human mAb specific to ActA[14].

Pharmacokinetics and target ligands

To evaluate pharmacokinetics, serum samples were analyzed for functional GDF8 mAb and functional ActA mAb using validated enzyme-linked immunosorbent assays (ELISAs) with a lower limit of quantitation (LLOQ) of 78 ng/mL in undiluted human serum. Functional antibody is defined as antibody with at least one of the two fragment antigen-binding (Fab) domains available to bind target.

To evaluate ligands, serum samples were analyzed for total GDF8 and total ActA using non-validated ELISAs. Total ligand is defined as the sum of free and bound ligand (i.e., ligand not bound to antibody plus ligand bound to antibody). The total GDF8 and total ActA assays had LLOQs in undiluted human serum of 4 and 0.313 ng/mL, respectively. Both assays used mAbs directed against the respective ligands as capture and detection reagents. The assays included acid pretreatment of serum samples to dissociate soluble complexes formed between the ligands and mAb drugs.

Anti-drug antibodies were assessed in serum samples using electrochemiluminescence bridging immunoassays.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan) that support

the methods and findings reported in this manuscript. Data requests, including individual anonymized participant data, will be considered for sharing (1) once the product and indication has been approved by major health authorities (e.g., FDA, EMA, PMDA, etc.) or development of the product has been discontinued globally for all indications on or after April 2020 and there are no plans for future development, (2) if there is legal authority to share the data, and (3) there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/ (the typical response time is 6–12 months). Source data are provided with this paper.

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Author contributions

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Competing interests

D.G.T., S.A., P.P., A.B., K.M., B.J.M., P.M., D.J.G., E.G., J.T., J.D.D., B.H., R.P., G.D.Y., and G.A.H. are employees and stockholders of Regeneron Pharmaceuticals, Inc. J.M. and M.W.S. are employees and stockholders of Regeneron Pharmaceuticals, Inc. who report having a patent pending, which has been licensed and receiving royalties with Regeneron Pharmaceuticals, Inc. S.D. was an employee of and holds stocks in Regeneron Pharmaceuticals, Inc. C.W. is an employee of and shareholder in New Zealand Clinical Research. The authors declare no other competing interests.

Additional information

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