

ADMINISTRATION OF FIBRONECTIN BASED SCAFFOLD DOMAIN PROTEINS TO TREAT OVERWEIGHT, OBESITY, AND RELATED HEALTH CONDITIONS

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to, and the benefit of, U.S. Provisional Application No. 63/484286 (filed February 10, 2023) and U.S. Provisional Application No. 63/588035 (filed October 5, 2023). The entire contents of the aforementioned applications are incorporated herein by reference.

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BACKGROUND OF THE INVENTION

Myostatin, also known as growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor- β (TGF- β) superfamily of secreted growth factors. Myostatin expression is limited primarily to skeletal muscle and adipose tissue, where it has been shown to be a negative regulator of skeletal muscle development (Lee LS, *Immunol. Endocr. Metab. Agents Med. Chem.* 2010;10:183-194). Both genetic and pharmacological findings indicate that myostatin regulates energy metabolism and that its inhibition can significantly attenuate the progression of metabolic diseases, including overweight, obesity, and related health conditions. For example, myostatin null mice exhibit decreased body fat accumulation (McPherron & Lee, J. JCI 109:595, 2002) when compared with wild type mice of the same age. This reduction in body fat is a manifestation of reduced adipocyte number and size, implicating a significant role of myostatin in adipogenesis as well as in myogenesis. In addition, increases in skeletal muscle mass and strength are associated with metabolic adaptations which positively affect body composition, energy expenditure, glucose homeostasis and insulin requirements.

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Over the past two decades, recombinant DNA technology has led to the discovery of a significant number of protein therapeutics. For example, anti-myostatin Adnectins which effectively inhibit myostatin activity *in vitro* and *in vivo* have been described (US Patents 8,933,199; 8,993,265; 8,853,154; and 9,493,546). These anti-myostatin Adnectins are useful for the treatment of disorders, diseases and conditions for which inhibition of myostatin activity is

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beneficial, including, for example, muscle wasting diseases, metabolic disorders and conditions resulting in muscle atrophy.

Overweight and obesity are complex disease processes which have reached pandemic dimensions. The worldwide prevalence of obesity has nearly tripled since 1975, mainly due to the adoption of a progressively more sedentary lifestyle and the consumption of less healthy diets (Boutari, C. and Mantzoros, C., *Metabolism*. 2022 Aug; 133: 155217). Specifically, the global obesity prevalence has risen approximately 2 percentage points per decade (NCD Risk Factor Collaboration [NCD-RisC] Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet* [London, England] 2017;390:2627–2642). According to the WHO, there are about 2 billion adults who are overweight, while 650 million are obese. Assuming these rates continue on the same trajectory, it is expected that 2.7 billion adults will be overweight and over 1 billion will be obese by 2025 (Boutari, C. and Mantzoros, 2022).

Overweight and obesity, and associated co-morbidities, such as type 2 diabetes, hypertension, dyslipidemia, and coronary heart disease are key risks factors for overall mortality (Apovian *et. al.* 2015). However, obesity treatment is challenging and treatment of obesity or overweight, and their related comorbidities, in particular type 2 diabetes represent a substantial, unmet medical need. Accordingly, it is an object of the present invention to provide improved methods for treating patients with overweight, obesity and related comorbidities.

SUMMARY OF THE INVENTION

Provided herein are methods for improving glycemic control in a human patient, methods for treating, preventing, or reducing obesity and related comorbidities, and methods of treating or preventing type II diabetes, by administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin. In some embodiments, the polypeptide is administered (or is for administration) according to a particular clinical dosage regimen (*e.g.*, at particular doses and according to a specific schedule).

In one aspect, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin.

In another aspect, a method of treating, preventing, or reducing obesity and related
5 comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin.

In a further aspect, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin.

10 In one embodiment, the patient has a body mass index (BMI) ≥ 30 kg/m². In another embodiment, the patient has a BMI ≥ 25 and < 30 kg/m² with at least one weight-related comorbidity. In another embodiment, the patient has a BMI < 25 kg/m².

In one embodiment, the patient is overweight with or without a related health condition. In another embodiment, the patient is obese. In another embodiment, the patient has an
15 overweight or obesity related comorbidity. Exemplary overweight and obesity related comorbidities include, but are not limited to: type 2 diabetes, glucose intolerance, prediabetes, insulin resistance, hypertension, dyslipidemia, increased waist circumference, cardiovascular disease, non-alcoholic fatty liver disease, obstructive sleep apnea, physical impairment, osteoarthritis, osteoporosis, renal disease, sexual hormone(s) impairment, endocrine reproductive
20 disorders such as polycystic ovary syndrome or male hypogonadism, stroke, and gallstones. In one embodiment, the patient has type II diabetes.

In one embodiment, the polypeptide is administered to the patient as a formulation. Any suitable formulation comprising a polypeptide which comprises a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin can be utilized according to the methods described
25 herein. In one embodiment, the formulation comprises: (i) at least 10 mg/mL of the polypeptide; (ii) a disaccharide at a concentration of at least 5%; (iii) a histidine buffer at a concentration of between about 20 to about 60 mM; and (iv) a pharmaceutically acceptable aqueous carrier, wherein the formulation has a pH range of about 6.5 to about 7.8.

In one embodiment, the polypeptide concentration in the formulation is between about 10 mg/mL and 200 mg/mL, between about 10 mg/mL and 150 mg/mL, or between about 10 mg/mL and 85 mg/mL.

5 In one embodiment, the disaccharide is present at weight (w/w) ratio of at least 5:1 protein to sugar. In another embodiment, the formulation comprises about 5% to about 30% of the disaccharide. In another embodiment, the concentration of the disaccharide is about 150 mM to about 800 mM, or about 300 to about 700 mM. In another embodiment, the disaccharide is trehalose, and the formulation comprises about 5 to about 30% trehalose, about 15% to about 25% trehalose, or about 20% to about 25% trehalose. In another embodiment, the disaccharide
10 is trehalose dihydrate, and the concentration of trehalose dihydrate in the formulation is about 150 mM to about 800 mM, about 300 to about 700 mM, about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM or about 700 mM.

15 In one embodiment, histidine is present at a concentration of at least 20 mM.

In one embodiment, the viscosity of the formulation is from about 5 to 20 cps, from about 5 to 15 cps, or from about 7 to 12 cps.

In one embodiment, the pH is about 6.6 to 7.6, about 6.8 to 7.4, or about 7.0 to 7.3.

20 In one embodiment, the formulation comprises a surfactant at a concentration of between about 0.01% and 0.5%.

In one embodiment, the formulation comprises a chelator, wherein the concentration of the chelator is between about 0.01 mM and about 0.5 mM or between about 0.05 mM and 0.2 mM, and wherein the chelator is selected from the group consisting of DPTA, EDTA and EGTA.

25 In one embodiment, the formulation comprises about 10-140 mg/mL of the polypeptide; about 5-25% trehalose dihydrate, about 20-30 mM histidine, and a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

In one embodiment, the formulation comprises about 10-140 mg/mL of the polypeptide, about 5-25% trehalose dihydrate, about 20-30 mM histidine, about 0.02-0.06 mM DTPA, about 0.01-0.05% polysorbate 80, and a pharmaceutically acceptable aqueous carrier,

wherein the pH of the formulation is about 6.8 to 7.3.

In one embodiment, the formulation comprises about 10-140 mg/mL of the polypeptide, about 600 mM trehalose dihydrate, 25-30 mM histidine, and a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 7.0 to 7.3.

5 In one embodiment, the formulation comprises about 10-140 mg/mL of the polypeptide, about 600 mM trehalose dihydrate, 25-30 mM histidine, about 0.02-0.06 mM DTPA, about 0.01-0.05% polysorbate 80, and a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 7.0 to 7.3.

10 In one embodiment, the formulation comprises about 10-75 mg/mL of the polypeptide, about 5-25% trehalose dihydrate, about 20-30 mM histidine, and a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

In one embodiment, the formulation comprises about 10-75 mg/mL of the polypeptide; about 5-25% trehalose dihydrate, about 20-30 mM histidine, about 0.02-0.06 mM DTPA, about 0.01-0.05% polysorbate 80, and a pharmaceutically acceptable aqueous carrier,
15 wherein the pH of the formulation is about 6.8 to 7.3.

In one embodiment, the formulation comprises about 10-75 mg/mL of the polypeptide, about 600 mM trehalose dihydrate, about 30 mM histidine, about 0.05 mM DTPA, about 0.02% polysorbate 80, a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 7.1.

20 In one embodiment, the polypeptide is administered as a unit dosage form comprising about 1.0 mL or less of a formulation comprising: (i) about 10-75 mg/mL of the polypeptide, (ii) about 5-25% trehalose dihydrate, (iii) about 20-30 mM histidine, (iv) about 0.02-0.06 mM DTPA, (v) about 0.01-0.05% polysorbate 80; and (vi) a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

25 Any suitable polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin can be used according to the methods described herein. In one embodiment, the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively. In another embodiment, the ¹⁰F_n3

domain comprises the amino acid sequence set forth in SEQ ID NO: 8. In another embodiment, the polypeptide comprises the amino acid sequence set forth SEQ ID NO: 11. An exemplary polypeptide is BHV-2000 (also known as “taldefgrobep alfa”, “RO7239361” and “BMS-986089”). BHV-2000 is an anti-myostatin adnectin (engineered scaffold based on the 10th fibronectin type III domain) that exhibits high affinity for myostatin ($K_d = 0.17\text{--}0.45\text{ nM}$) and inhibits myostatin and GDF-11 second messenger signaling in cells ($IC_{50s} = 0.06\text{--}1\text{ nM}$ and $0.09\text{--}0.7\text{ nM}$, respectively). BHV-2000 comprises SEQ ID NO:78. Accordingly, in another embodiment, the polypeptide comprises the amino acid sequence set forth SEQ ID NO: 78.

The peptide or formulation comprising the peptide can be administered to a patient by any suitable means. In one embodiment, the peptide is formulated for intravenous administration. In one embodiment, the peptide is formulated for subcutaneous administration.

The efficacy of the treatment methods provided herein can be assessed using any suitable means. In one embodiment, central adiposity is reduced.

In one embodiment, the method results in an improvement in glycemic control. In one embodiment an improvement in glycemic control is achieved by improving insulin sensitivity.

In one embodiment, the treatment results in an improvement in a Diabetes Treatment Satisfaction Questionnaire (DTSQ) score. In another embodiment, the treatment results in an improvement in an Impact of Weight on Quality of Life (IWQOL) score.

In one embodiment, the treatment results in a decrease in total body fat mass (FM) of at least 5 kg compared to baseline. For example, in one embodiment, the treatment results in a decrease in total body FM of at least 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11 kg or more. In one embodiment, the treatment results in a decrease in total body FM of at least 5 kg by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an at least 5% decrease in total body FM compared to baseline. For example, in one embodiment, the treatment results in an at least 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5 % or more

decrease in total body FM. In one embodiment, the treatment results in an at least 5% decrease in total body FM by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an at least 5% decrease in body weight compared to baseline. For example, in one embodiment, the treatment results in an at least 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5 % or more decrease in body weight. In one embodiment, the treatment results in an at least 5% decrease in body weight by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an at least 2% increase in body lean mass (LM) compared to baseline. For example, in one embodiment, the treatment results in at least 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 % or more increase in body LM compared to baseline. In one embodiment, the treatment results in an at least 2% increase in body LM by week 6, week 8, week 10, week 12, week 16, week 20, week 24, week 36, week 48, or week 60 compared to baseline. In another embodiment, the treatment results in an at least 25% increase in body LM by week 8, week 10, week 12, week 16, week 20, week 24, week 36, week 48, or week 60 compared to baseline.

FM, LM, and body fat can be assessed by any suitable means. In one embodiment, FM is assessed by dual-energy x-ray absorptiometry (DXA). In one embodiment, LM is assessed by DXA. In one embodiment, body fat is assessed by skinfold calipers, body circumference measurements, DXA, hydrostatic weighing, air displacement plethysmography (Bod Pod), bioelectrical impedance analysis (BIA), bioimpedance spectroscopy (BIS), or electrical impedance myography (EIM), a 3-D body scanner, a multi-compartment model, and/or magnetic resonance spectroscopy (MRI).

In one embodiment, the treatment results in a decrease in waist circumference (WC) and/or waist-to-hip ratio compared to baseline. For example, in one embodiment, the treatment results in a decrease in WC by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline. In another embodiment, the treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more compared to baseline. In another embodiment, the

treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more by week 12, week 15, week 18, week 24, week 36, week 48, or week 60 compared to baseline.

5 In one embodiment, the treatment results in a decrease in WC and a decrease in total body weight compared to baseline. For example, in one embodiment, the treatment results in a decrease in WC by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline and a decrease in total body weight by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline. In another embodiment, the treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more compared to baseline and a decrease in total body
10 weight by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline. In another embodiment, the treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more and a decrease in total body weight by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% by week 12, week 15, week 18, week 24, week 36, week 48, or week 60 compared to baseline.

15 In another embodiment, the treatment results in a decrease in waist-to-hip ratio compared to baseline. In one embodiment, the treatment results in a decrease in waist circumference (WC) and/or waist-to-hip ratio by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in a decrease in subcutaneous and abdominal visceral adipose tissue compared to baseline. For example, in one embodiment, the treatment
20 results in at least 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5 % or more decrease in subcutaneous and abdominal visceral adipose tissue compared to baseline. In one embodiment, the results in a decrease in subcutaneous and abdominal visceral adipose tissue by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an improvement in diabetes status. For
25 example, in one embodiment, the treatment results in an improvement in the patient's HbA1c. In another embodiment, the treatment results in an improvement as assessed by Homeostatic Model Assessment (HOMA). In another embodiment, the treatment results in an improvement as assessed by quantitative insulin-sensitivity check index (QUICKI). In another embodiment, the treatment results in an improvement as assessed by Matsuda Index. In one embodiment, the

treatment results in an improvement in diabetes status by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in a shift toward normal levels of one or more biomarkers selected from the group consisting of serum lipids, high-sensitivity C-reactive protein (hs-CRP), interleukin 6, leptin, and adiponectin. In one embodiment, the treatment results in a shift toward normal levels of one or more biomarkers selected from the group consisting of serum lipids, high-sensitivity C-reactive protein (hs-CRP), interleukin 6, leptin, and adiponectin by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in a reduction in insulin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in insulin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in insulin level by week 8, week 10, week 12, week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in a reduction in leptin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in leptin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in leptin level by week 8, week 10, week 12, week 24, week 36, week 48, or week 60 compared to baseline. In one embodiment, the treatment results in an improvement in hand grip strength, for example, as assessed by dynamometry. In one embodiment, the treatment results in an improvement in hand grip strength by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the peptide or formulation thereof is administered as a monotherapy according to the methods described herein. In another embodiment, the peptide or formulation

thereof is administered in combination with one or more additional therapeutic agents. In one embodiment, the peptide or formulation thereof and one or more therapeutic agents are co-administered at the same time (*e.g.*, simultaneously). In another embodiment, the peptide or formulation thereof is administered prior to administration of the one or more additional
5 therapeutic agents. In another embodiment, the peptide or formulation thereof is administered after administration of the one or more additional therapeutic agents.

The polypeptides (anti-myostatin Adnectin molecules) are administered in the target population at any suitable dose. In one embodiment, the polypeptides are administered as repeated subcutaneous injections. In one embodiment, the polypeptides are administered at a
10 dosing interval of once a week. In one embodiment, the polypeptides are administered at a dosing interval of once every two weeks. In one embodiment, the polypeptides are administered at a dosing interval of once a month.

In one embodiment, the polypeptides are administered at a dose of approximately 5-200mg. For example, the polypeptides can be administered at a dose of 5mg, 6mg, 7mg, 8mg,
15 9mg, 10mg, 11mg, 12mg, 13mg, 14mg, 15mg, 16mg, 17mg, 18mg, 19mg, 20mg, 21mg, 22mg, 23mg, 24mg, 25mg, 26mg, 27mg, 28mg, 29mg, 30mg, 31mg, 32mg, 33mg, 34mg, 35mg, 36mg, 37mg, 38mg, 39mg, 40mg, 41mg, 42mg, 43mg, 44mg, 45mg, 46mg, 47mg, 48mg, 49mg, 50mg, 51mg, 52mg, 53mg, 54mg, 55mg, 56mg, 57mg, 58mg, 59mg, 60mg, 61mg, 62mg, 63mg, 64mg, 65mg, 66mg, 67mg, 68mg, 69mg, 70mg, 71mg, 72mg, 73mg, 74mg, 75mg, 76mg, 77mg, 78mg,
20 79mg, 80mg, 81mg, 82mg, 83mg, 84mg, 85mg, 86mg, 87mg, 88mg, 89mg, 90mg, 91mg, 92mg, 93mg, 94mg, 95mg, 96mg, 97mg, 98mg, 99mg, 100mg, 101mg, 102mg, 103mg, 104mg, 105mg, 106mg, 107mg, 108mg, 109mg, 110mg, 111mg, 112mg, 113mg, 114mg, 115mg, 116mg, 117mg, 118mg, 119mg, 120mg, 121mg, 122mg, 123mg, 124mg, 125mg, 126mg, 127mg, 128mg, 129mg, 130mg, 131mg, 132mg, 133mg, 134mg, 135mg, 136mg, 137mg, 138mg,
25 139mg, 140mg, 141mg, 142mg, 143mg, 144mg, 145mg, 146mg, 147mg, 148mg, 149mg, 150mg, 151mg, 152mg, 153mg, 154mg, 155mg, 156mg, 157mg, 158mg, 159mg, 160mg, 161mg, 162mg, 163mg, 164mg, 165mg, 166mg, 167mg, 168mg, 169mg, 170mg, 171mg, 172mg, 173mg, 174mg, 175mg, 176mg, 177mg, 178mg, 179mg, 180mg, 181mg, 182mg,

183mg, 184mg, 185mg, 186mg, 187mg, 188mg, 189mg, 180mg, 191mg, 192mg, 193mg, 194mg, 195mg, 196mg, 197mg, 198mg, 199mg, or 200mg.

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections). In one embodiment, the ¹⁰Fn3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 11, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 78, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient BHV-2000, wherein BHV-2000 is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to

myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks.

- 5 In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections). In one embodiment, the ¹⁰F_n3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.

- 10 In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 11, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

- 15 In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 78, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

- 20 In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient BHV-2000, wherein BHV-2000 is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

- 25 In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in

SEQ ID NOs: 5, 6 and 7, respectively, and wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections). In one embodiment, the ¹⁰F_n3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.

5 In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 11, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

10 In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 78, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

15 In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient BHV-2000, wherein BHV-2000 is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

20 Further provided are kits that include a pharmaceutical formulation containing a polypeptide which comprises a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, such as BHV-2000, in a therapeutically effective amount adapted for use in the methods described herein.

In one embodiment, a kit for improving glycemic control in a human patient is provided,
25 wherein the kit comprises:

- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3

amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and

- (b) instructions for using the polypeptide in the method of any one of the preceding claims.

5 In another embodiment, a kit for treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, wherein the kit comprises:

- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3
10 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and
- (b) instructions for using the polypeptide in the method of any one of the preceding claims.

15 In another embodiment, a kit for treating or preventing type II diabetes in a human patient is provided, wherein the kit comprises:

- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3
20 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and
- (b) instructions for using the polypeptide in the method of any one of the preceding claims.

25 In one embodiment, the ¹⁰F_n3 domain comprises the amino acid sequence set forth in SEQ ID NO:8. In one embodiment, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO:11. In one embodiment, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO:78. In one embodiment, the polypeptide is for administration as a unit dosage form comprising about 1.0 mL or less of a formulation comprising, (i) about 10-75 mg/mL of the polypeptide; (ii) about 5-25% trehalose dihydrate; (iii) about 20-30 mM histidine; (iv) about

0.02-0.06 mM DTPA; (v) about 0.01-0.05% polysorbate 80; and (vi) a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

Also provided are uses of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin for improving glycemic control in a human patient, wherein
5 the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

Further provided are uses of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin for treating, preventing, or reducing obesity and related
10 comorbidities in a human patient, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

Further provided are uses of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin for treating or preventing type II diabetes in a human patient,
15 wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, the polypeptide comprises a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and
20 wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively. In another embodiment, the ¹⁰Fn3 domain comprises the amino acid sequence set forth in SEQ ID NO:8. In another embodiment, the polypeptide comprises the amino acid sequence set forth SEQ ID NO:11. In another embodiment, the polypeptide comprises the amino
25 acid sequence set forth SEQ ID NO:78. In another embodiment, the polypeptide is for administration as a unit dosage form comprising about 1.0 mL or less of a formulation comprising (i) about 10-75 mg/mL of the polypeptide; (ii) about 5-25% trehalose dihydrate; (iii) about 20-30 mM histidine; (iv) about 0.02-0.06 mM DTPA; (v) about 0.01-0.05% polysorbate

80; and (vi) a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

BRIEF DESCRIPTION of the Drawings

5 **FIG. 1** is a schematic depicting the study design. Baseline was the start of treatment with vehicle or taldefgrobep.

FIG. 2 shows the change in baseline in total body weight (TBW) and body composition at Week 8: C57BL/6J Mice on taldefgrobep versus vehicle. Error bars represent standard error.

10 **FIG. 3** is a set of bar graphs that depict the change in fat mass from baseline in the taldefgrobep and vehicle groups. Error bars represent standard error.

FIG. 4 is a set of bar graphs that depict the change in lean mass from baseline in the taldefgrobep and vehicle groups.

FIGs. 5A-5B are bar graphs that depict insulin (**FIG. 5A**) and leptin (**FIG. 5B**) levels after 8 weeks of vehicle or taldefgrobep treatment.

15 **FIG. 6** is a line graph that shows the correlation between change in TBW and waist circumference (WC) across adult anti-obesity medication trials. The dotted line represents the identity line. The size of each data point is proportional to the square root of the sample size.

DETAILED DESCRIPTION OF THE INVENTION

20 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by the skilled artisan. Although any methods and compositions similar or equivalent to those described herein can be used in practice or testing of the present invention, the preferred methods and compositions are described herein.

 The singular form “a,” “an,” and “the” include plural reference unless the context clearly
25 dictates otherwise.

 The term “about”, particularly in reference to a given quantity or number, is meant to encompass deviations within plus or minus ten percent ($\pm 10\%$), (*e.g.*, $\pm 5\%$).

I. Polypeptides & Myostatin Binding Adnectin Molecules

Provided herein are methods for improving glycemic control in a human patient, methods for treating, preventing, or reducing obesity and related comorbidities, and methods of treating or preventing type II diabetes, by administering to the patient a polypeptide comprising a

5 fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin.

“Full-length myostatin” as used herein refers to the full-length polypeptide sequence described in McPherron et al. (1997), *supra*, as well as related full-length polypeptides including allelic variants and interspecies homologs. The term “myostatin” or “mature myostatin” refers to fragments of the biologically active mature myostatin, as well as related polypeptides including

10 allelic variants, splice variants, and fusion peptides and polypeptides. The mature C-terminal protein has been reported to have 100% sequence identity among many species including human, mouse, chicken, porcine, turkey, and rat (Lee *et al.*, *PNAS* 2001;98:9306). The sequence for human prepromyostatin is:

15 MQKLQLCVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACTWRQNTK
SSRIEAIKIQILSKLRLETAPNISKDVIRQLLPKAPPLRELIDQYDVQRDDSS
DGSLEDDDDYHATTETIITMPTESDFLMQVDGKPKCCFFKFSSKIQYNKVV
KAQLWIYLRPVETPTTVFVQILRLIKPMKDGTRYTGIRSLKLDMPGTGIW
QSIDVKTVLQNWLKQPESNLGIEIKALDENGHDLAVTFPGPGEDGLNPFLE
VKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRY
20 KANYCSGECEVFVLQKYPHTLVHQANPRGSAGPCCTPTKMSPINMLYF
NGKEQIIYGKIPAMVVDRCGCS (SEQ ID NO: 1).

The sequence for human pro-myostatin is:

25 NENSEQKENVEKEGLCNACTWRQNTKSSRIEAIKIQILSKLRLETAPNISKD
VIRQLLPKAPPLRELIDQYDVQRDDSSDGSLEDDDDYHATTETIITMPTESDF
LMQVDGKPKCCFFKFSSKIQYNKVVKAQLWIYLRPVETPTTVFVQILRLIK
PMKDGTRYTGIRSLKLDMPGTGIWQSIDVKTVLQNWLKQPESNLGIEIK
ALDENGHDLAVTFPGPGEDGLNPFLEVKVTDTPKRSRRDFGLDCDEHSTE
SRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEVFVLQKYPHTLV
30 HQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS
(SEQ ID NO: 2).

The sequence for mature myostatin (conserved in human, murine, rat, chicken, turkey, dog, horse, and pig) is:

DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFV
 FLQKYPTHVLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPA
 MVVDRCGCS (SEQ ID NO: 3).

As used herein, a "fibronectin based scaffold" or "FBS" protein or moiety refers to
 5 proteins or moieties that are based on a fibronectin type III ("Fn3") repeat. Fibronectin has 18
 Fn3 repeats, and while the sequence homology between the repeats is low, they all share a high
 similarity in tertiary structure. For reviews see Bork et al., *Proc. Natl. Acad. Sci. USA*,
 89(19):8990-8994 (1992); Bork et al., *J. Mol. Biol.*, 242(4):309-320 (1994); Campbell et al.,
Structure, 2(5):333-337 (1994); Harpez et al., *J. Mol. Biol.*, 238(4):528-539 (1994)). An Fn3
 10 domain is small, monomeric, soluble, and stable. It lacks disulfide bonds and, therefore, is stable
 under reducing conditions. Fn3 domains comprise, in order from N-terminus to C-terminus, a
 beta or beta-like strand, A; a loop, AB; a beta or beta-like strand, B; a loop, BC; a beta or beta-
 like strand, C; a loop, CD; a beta or beta-like strand, D; a loop, DE; a beta or beta-like strand, E;
 a loop, EF; a beta or beta-like strand, F; a loop, FG; and a beta or beta-like strand, G. The seven
 15 antiparallel β -strands are arranged as two beta sheets that form a stable core, while creating two
 "faces" composed of the loops that connect the beta or beta-like strands. Loops AB, CD, and EF
 are located at one face ("the south pole") and loops BC, DE, and FG are located on the opposing
 face ("the north pole").

Adnectins are a class of therapeutic FBS proteins with high-affinity and specific target-
 20 binding properties that are derived from the tenth human fibronectin type III domain (¹⁰Fn3):

VSDVPRDLEVVAATPTSLLISWDAPAVTVRYRITYGETGGNSPVQEFTV
PGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRT
 (SEQ ID NO: 4) (BC, DE, and FG loops are underlined).

Accordingly, as used herein, a "¹⁰Fn3 domain" or "¹⁰Fn3 moiety" or "¹⁰Fn3 molecule"
 25 refers to wild-type ¹⁰Fn3 and biologically active variants thereof, *e.g.*, biologically active variants
 that specifically bind to a target, such as a target protein.

A "region" of a ¹⁰Fn3 domain (or moiety or molecule) as used herein refers to either a
 loop (AB, BC, CD, DE, EF and FG), a β -strand (A, B, C, D, E, F and G), the N-terminus

(corresponding to amino acid residues 1-7 of SEQ ID NO: 1), or the C-terminus (corresponding to amino acid residues 93-94 of SEQ ID NO: 1).

A "scaffold region" refers to any non-loop region of a human ¹⁰Fn3 domain. The scaffold region includes the A, B, C, D, E, F and G β -strands as well as the N-terminal region (amino acids corresponding to residues 1-7 of SEQ ID NO: 1) and the C-terminal region (amino acids corresponding to residues 93-94 of SEQ ID NO: 1).

The term "anti-myostatin Adnectin" refers to a protein molecule that binds to and antagonizes myostatin and that comprises at least a one ¹⁰Fn3 domain derived from the human wild-type ¹⁰Fn3 domain (SEQ ID NO: 1). The anti-myostatin Adnectin can further comprise additional protein domains (*e.g.*, an Fc domain), and can also refer to multimer forms of the polypeptide, such as dimers, tetramers and hexamers.

"Polypeptide" as used herein refers to any sequence of two or more amino acids, regardless of length, post-translation modification, or function. "Polypeptide," "peptide," and "protein" are used interchangeably herein. Polypeptides can include natural amino acids and non-natural amino acids such as those described in U.S. Pat. No. 6,559,126, incorporated herein by reference. Polypeptides can also be modified in any of a variety of standard chemical ways (*e.g.*, an amino acid can be modified with a protecting group; the carboxy-terminal amino acid can be made into a terminal amide group; the amino-terminal residue can be modified with groups to, *e.g.*, enhance lipophilicity; or the polypeptide can be chemically glycosylated or otherwise modified to increase stability or *in vivo* half-life). Polypeptide modifications can include the attachment of another structure such as a cyclic compound or other molecule to the polypeptide and can also include polypeptides that contain one or more amino acids in an altered configuration (*i.e.*, R or S; or, L or D). The peptides of the invention are proteins derived from the tenth type III domain of fibronectin that have been modified to bind to myostatin and are referred to herein as, "anti-myostatin Adnectin" or "myostatin Adnectin."

A "polypeptide chain", as used herein, refers to a polypeptide wherein each of the domains thereof is joined to other domain(s) by peptide bond(s), as opposed to non-covalent interactions or disulfide bonds.

An "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous
5 solutes. In preferred embodiments, the polypeptide will be purified (1) to greater than 95% by weight of polypeptide as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing condition using Coomassie blue or, preferably, silver stain. Isolated
10 polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

"Percent (%) amino acid sequence identity" herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a selected
15 sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or
20 Megalign (DNASTAR™) software. Those skilled in the art can readily determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or
25 comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of

amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

As used herein, "conservative substitution" denotes the replacement of an amino acid residue by another, without altering the overall conformation and function of the peptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, shape, hydrophobic, aromatic, and the like). Exemplary conservative substitutions include those fulfilling the criteria defined for an accepted point mutation in Dayhoff *et al.*, *Atlas of Protein Sequence and Structure*, 5:345-352 (1978 and Supp.) Examples of conservative substitutions include substitutions within the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. By "substituted" or "modified" the present invention includes those amino acids that have been altered or modified from naturally occurring amino acids. As such it should be understood that in the context of the present invention a conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

As used herein, the term "Adnectin binding site" refers to the site or portion of a protein (*e.g.*, myostatin) that interacts or binds to a particular Adnectin (*e.g.*, as an epitope is recognized by an antibody). Adnectin binding sites can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Adnectin binding sites formed by contiguous amino acids are typically retained on exposure to denaturing solvents, whereas Adnectin binding sites formed by tertiary folding are typically lost on treatment of denaturing solvents.

The terms "specifically binds," "specific binding," "selective binding," and "selectively binds," as used interchangeably herein refers to an Adnectin that exhibits affinity for a myostatin, but does not significantly bind (*e.g.*, less than about 10% binding) to a different polypeptide as measured by a technique available in the art such as, but not limited to, Scatchard analysis and/or competitive binding assays (*e.g.*, competition ELISA, BIACORE assay). The term is also applicable where *e.g.*, a binding domain of an Adnectin of the invention is specific for myostatin.

The term "preferentially binds" as used herein refers to the situation in which an Adnectin of the invention binds myostatin at least about 20% greater than it binds a different polypeptide as measured by a technique available in the art such as, but not limited to, Scatchard analysis and/or competitive binding assays (e.g., competition ELISA, BIACORE assay).

5 As used herein, the term "cross-reactivity" refers to an Adnectin which binds to more than one distinct protein having identical or very similar Adnectin binding sites.

 The term " K_D ," as used herein, is intended to refer to the dissociation equilibrium constant of a particular Adnectin-protein (e.g., myostatin) interaction or the affinity of an Adnectin for a protein (e.g., myostatin), as measured using a surface plasmon resonance assay or
10 a cell binding assay. A "desired K_D ," as used herein, refers to a K_D of an Adnectin that is sufficient for the purposes contemplated. For example, a desired K_D may refer to the K_D of an Adnectin required to elicit a functional effect in an *in vitro* assay, e.g., a cell-based luciferase assay.

 The term " k_{ass} ," as used herein, is intended to refer to the association rate constant for the
15 association of an Adnectin into the Adnectin/protein complex.

 The term " k_{diss} ," as used herein, is intended to refer to the dissociation rate constant for the dissociation of an Adnectin from the Adnectin/protein complex.

 The term " IC_{50} ," as used herein, refers to the concentration of an Adnectin that inhibits a response, either in an *in vitro* or an *in vivo* assay, to a level that is 50% of the maximal inhibitory
20 response, i.e., halfway between the maximal inhibitory response and the untreated response.

 The term "myostatin activity" as used herein refers to one or more of growth-regulatory or morphogenetic activities associated with the binding of active myostatin protein to ActRIIb and the subsequent recruitment of Alk4 or Alk5. For example, active myostatin is a negative regulator of skeletal muscle mass. Active myostatin can also modulate the production of muscle-
25 specific enzymes (e.g., creatine kinase), stimulate myoblast proliferation, and modulate preadipocyte differentiation to adipocytes. Myostatin activity can be determined using art-recognized methods, such as those described herein.

 The phrases "inhibit myostatin activity" or "antagonize myostatin activity" or "antagonize myostatin" are used interchangeably to refer to the ability of the anti-myostatin

Adnectins of the present invention to neutralize or antagonize an activity of myostatin *in vivo* or *in vitro*. The terms "inhibit" or "neutralize" as used herein with respect to an activity of an Adnectin of the invention means the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse e.g., progression or severity of that
5 which is being inhibited including, but not limited to, a biological activity or property, a disease or a condition. The inhibition or neutralization is preferably at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or higher.

For example, an anti-myostatin Adnectin in the pharmaceutical formulation may reduce circulating levels of biologically active myostatin normally found in a vertebrate subject, or a
10 reduction of circulating levels of biologically active myostatin in subjects with disorders that result in elevated circulating levels of myostatin. A reduction of myostatin activity may be determined using *in vitro* assays, e.g., binding assays, as described herein.

The term "PK" is an acronym for "pharmacokinetic" and encompasses properties of a compound including, by way of example, absorption, distribution, metabolism, and elimination
15 by a subject. A "PK modulation protein" or "PK moiety" as used herein refers to any protein, peptide, or moiety that affects the pharmacokinetic properties of a biologically active molecule when fused to or administered together with the biologically active molecule. Examples of a PK modulation protein or PK moiety include PEG, human serum albumin (HSA) binders (as disclosed in U.S. Publication Nos. 2005/0287153 and 2007/0003549, PCT Publication Nos. WO
20 2009/083804 and WO 2009/133208), human serum albumin, Fc or Fc fragments and variants thereof, and sugars (e.g., sialic acid).

The "half-life" of an amino acid sequence or compound can generally be defined as the time taken for the serum concentration of the polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the sequence or compound and/or clearance or sequestration of
25 the sequence or compound by natural mechanisms. The half-life can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally involve the steps of suitably administering to a subject a suitable dose of the amino acid sequence or compound of the invention; collecting blood samples or other samples from the subject at regular intervals;

determining the level or concentration of the amino acid sequence or compound of the invention in said blood sample; and calculating, from (a plot of) the data thus obtained, the time until the level or concentration of the amino acid sequence or compound of the invention has been reduced by 50% compared to the initial level upon dosing. Reference is, for example, made to the standard handbooks, such as Kenneth, A. et al., Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al., Pharmacokinetic Analysis: A Practical Approach (1996). Reference is also made to Gibaldi, M. et al., Pharmacokinetics, 2nd Rev. Edition, Marcel Dekker (1982).

Half-life can be expressed using parameters such as the $t_{1/2}$ -alpha, $t_{1/2}$ -beta, HL_Lambda_z, and the area under the curve (AUC). In the present specification, an "increase in half-life" refers to an increase in any one of these parameters, any two of these parameters, any three of these parameters or all four of these parameters. An "increase in half-life" in particular refers to an increase in the $t_{1/2}$ -beta, and/or HL_Lambda_z, either with or without an increase in the $t_{1/2}$ -alpha and/or the AUC or both.

The notations "mpk", "mg/kg", or "mg per kg" refer to milligrams per kilogram. All notations are used interchangeably throughout the present disclosure.

A. Exemplary Molecules

Polypeptides (*e.g.*, anti-myostatin Adnectin molecules) that may be used in the formulation provided herein comprise an Fn3 domain derived from the wild-type tenth module of the human fibronectin type III domain (¹⁰Fn3) (SEQ ID NO: 1).

In some embodiments, the anti-myostatin Adnectin in the pharmaceutical formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 and 7, respectively.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 and 7, respectively, wherein the BC loop comprises 1, 2 or 3 amino acid substitutions, such as conservative amino acid substitutions which allow the anti-myostatin Adnectin to maintain binding to myostatin.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 and 7, respectively, wherein at least one loop

of the BC, DE, and FG loops of the ¹⁰F_n3 domain has 1 amino acid substitution relative to the respective BC, DE, and FG loops of SEQ ID NOs: 5, 6 and 7.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 and 7, respectively, wherein one loop from
5 the BC, DE, or FG loop of the ¹⁰F_n3 domain has 1 amino acid substitution relative to the respective BC, DE, or FG loop of SEQ ID NOs: 5, 6 and 7.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively wherein (i) the serine at position 3 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the
10 group consisting of A, C, D, F, H, I, K, L, N, Q, R, T, V, W, or Y; (ii) the leucine at position 4 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from M or V; (iii) the proline at position 5 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, E, I, K, L, M, N, Q, R, S, T, V, or Y; (vi) the histidine at position 6 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the
15 group consisting of A, C, D, E, F, G, I, K, L, M, N, Q, R, S, T, V, W, or Y; (vii) the glutamine at position 7 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; (viii) the glycine at position 8 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid S; (ix) the lysine at position 9 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the
20 group consisting of A, C, D, E, F, G, H, I, L, M, N, Q, R, S, T, V, W, or Y; (x) the alanine at position 10 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, G, L, M, S, or T; or (xi) the asparagine at position 11 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, F, H, P, Q, R, S, or Y.

25 In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively, wherein (i) the serine at position 3 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, F, I, V, W, or Y; (ii) the histidine at position 6 of the BC loop (SEQ ID NO: 6) is substituted with an amino acid selected from the group consisting of C, D, E, F, G, I,

K, L, M, N, Q, R, S, T, V, W, or Y; (iii) the lysine at position 9 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, G, H, I, L, M, N, Q, R, S, V, W, or Y; (iv) the alanine at position 10 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of G, L, M, or S; or (v) the asparagine at
5 position 11 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, H, Q, S, or Y.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively, wherein (i) the serine at position 3 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid F or W; (ii) the
10 histidine at position 6 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, F, G, I, K, L, M, N, R, S, T, V, W, or Y; (iii) the glutamine at position 7 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, E, F, H, I, K, L, M, P, R, S, T, V, or Y; (iii) the lysine at position 9 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group
15 consisting of A, C, H, L, M, N, R, V, W, or Y; (iv) the alanine at position 10 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid G or L; or (v) the asparagine at position 11 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid H or Q.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively, wherein the valine at
20 position 5 of the DE loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, I, K, L, M, N, Q, S, or T. In some embodiments, the valine at position 5 of the DE loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, E, I, L, M, Q, or T. In some embodiments, the valine at position 5 of the DE loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of
25 C, E, I, L, or M.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively, wherein (i) the valine at position 2 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, I, L, M, Q, T, W, or Y; (iii) the threonine at position 3 of the FG

loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, G, H, I, K, L, M, N, Q, R, S, V, W, or Y; (iv) the aspartic acid at position 4 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; (v) the threonine at position 5 of the FG loop (SEQ ID NO: 7) is substituted to with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; (vi) the glycine at position 6 of the FG loop (SEQ ID NO: 7) is substituted to with an amino acid selected from the group consisting of A, C, D, E, F, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; (vii) the tyrosine at position 7 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, H, I, L, M, N, P, S, T, V, or W; (viii) the leucine at position 8 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, H, I, K, M, N, Q, R, S, T, V, W, or Y; (ix) the lysine at position 9 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; (x) the tyrosine at position 10 of the FG loop (SEQ ID NO: 7) is substituted with the amino acid F or W; or (xi) the lysine at position 11 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively, wherein (i) the valine at position 2 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, I, L, or M; (ii) the threonine at position 3 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, H, I, L, M, Q, R, S, V, W, or Y; (iii) the aspartic acid at position 4 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, H, I, L, M, N, P, Q, S, T, V, W, or Y; (iv) the threonine at position 5 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, V, W, or Y; (v) the glycine at position 6 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, D, E, F, H, I, L, M, N, Q, S, T, V, W, or Y; (vi) the tyrosine at position 7 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid

selected from the group consisting of C, F, I, L, M, P, T, V, or W; (vii) the leucine at position 8 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, H, I, K, M, N, Q, R, T, V, W, or Y; (viii) the lysine at position 9 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, I, L, M, N, P, Q, R, S, T, V, W, or Y; (ix) the tyrosine at position 10 of the FG loop (SEQ ID NO: 7) is substituted with the amino acid W; or (x) the lysine at position 11 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, G, H, L, M, N, P, Q, R, S, T, or V.

In some embodiments, the anti-myostatin Adnectin in the formulation comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6 or 7, respectively, wherein (i) the valine at position 2 of the FG loop (SEQ ID NO: 7) is substituted with the amino acid I; (ii) the threonine at position 3 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, I, L, M, V, W, or Y; (iii) the aspartic acid at position 4 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, H, I, L, M, N, Q, S, T, or V; (iv) the threonine at position 5 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, F, G, I, L, M, N, Q, S, V, W, or Y; (v) the glycine at position 6 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, S, T, or W; (vi) the tyrosine at position 7 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of F, I, V, or W; (vii) the leucine at position 8 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of F, H, I, M, V, W, or Y; (viii) the lysine at position 9 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, G, I, L, M, T, V, or W; or (x) the lysine at position 11 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, G, L, M, P, Q, or R.

In certain embodiments, the anti-myostatin Adnectin comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence set forth in SEQ ID NO: 8:

EVVAATPTSLISWSLPHQGKANYRITYGETGGNSPVQEFTVPGRGVTA
TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT (SEQ ID NO: 8)

In some embodiments, the polypeptide in the formulation contains a ¹⁰F_n3 domain which binds myostatin comprising an amino acid sequence at least 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of 8, SEQ ID NO: 9 or SEQ ID NO: 10. For example, in some embodiments, the non-ligand binding sequences of ¹⁰F_n3, i.e., the "¹⁰F_n3 scaffold", may also be altered provided that the ¹⁰F_n3 retains ligand binding function and/or structural stability. A variety of mutant ¹⁰F_n3 scaffolds have been reported. In some embodiments, one or more of Asp 7, Glu 9, and Asp 23 is replaced by another amino acid, such as, for example, a non-negatively charged amino acid residue (e.g., Asn, Lys, etc.). These mutations have been reported to have the effect of promoting greater stability of the mutant ¹⁰F_n3 at neutral pH as compared to the wild-type form (see, e.g., PCT Publication No. WO 02/04523). A variety of additional alterations in the ¹⁰F_n3 scaffold that are either beneficial or neutral have been disclosed. See, for example, Batori et al., Protein Eng., 15(12):1015-1020 (December 2002); Koide et al., Biochemistry, 40(34):10326-10333 (Aug. 28, 2001).

In certain embodiments, the ¹⁰F_n3 domain of the polypeptide in the formulation comprises SEQ ID NO: 8, SEQ ID NO: 9 or SEQ ID NO: 10. In one embodiment, the ¹⁰F_n3 domain of the polypeptide in the formulation comprises SEQ ID NO: 10.

20 B. Extension Sequences

In certain embodiments, the polypeptides (e.g., anti-myostatin Adnectin molecules) in the formulation are modified to comprise an N-terminal extension sequence and/or a C-terminal extension sequence. For example, an MG sequence may be placed at the N-terminus of the ¹⁰F_n3 defined by SEQ ID NO: 4. The M will usually be cleaved off, leaving a G at the N-terminus. In some embodiments, the anti-myostatin Adnectin may comprise the amino acid sequence of SEQ ID NO: 8, and an N-terminal extension sequence as shown in Table 1. In addition, an M, G or MG may also be placed N-terminal to any of the N-terminal extensions shown in Table 1. In some embodiments, the anti-myostatin Adnectin in the formulation may be truncated at the threonine corresponding to T94 of SEQ ID NO: 4. Alternatively, C-terminal extensions may be

added after the C-terminal residue of SEQ ID NO: 8. Exemplary C-terminal extension sequences are shown in Table 1.

Table 1: Summary of N-terminal and C-terminal Extension Sequences			
SEQ ID NO	Description	Name	Sequence
11	Exemplary leader	AdNT1	MGVSDVPRDL
12	Exemplary leader	AdNT2	GVSDVPRDL
13	Exemplary leader	AdNT3	VSDVPRDL
14	Exemplary leader	AdNT4	SDVPRDL
15	Exemplary leader	AdNT5	DVPRDL
16	Exemplary leader	AdNT6	VPRDL
17	Exemplary leader	AdNT7	PRDL
	Exemplary leader	AdNT8	RDL
	Exemplary leader	AdNT9	DL
20	Exemplary tail	AdCT1	EIDKPSQ
	Exemplary tail	AdCT2	EI
22	Exemplary tail	AdCT3	EIEPKSS
23	Exemplary tail	AdCT4	EIDKPC
24	Exemplary tail	AdCT5	EIDKP
25	Exemplary tail	AdCT6	EIDK
26	Exemplary tail	AdCT7	EIDKPS
27	Exemplary tail	AdCT8	EIEKPSQ
28	Exemplary tail	AdCT9	EIDKPSQLE
29	Exemplary tail	AdCT10	EIEDEDEDEDED
30	Exemplary tail	AdCT11	EGSGS
31	Exemplary tail	AdCT12	EIDKPCQ
32	Exemplary tail	AdCT13	GSGC
33	Exemplary tail	AdCT14	EGSGC
34	Exemplary tail	AdCT15	EIDKPCQLE
35	Exemplary tail	AdCT16	EIDKPSQH H H H H H
36	Exemplary tail	AdCT17	GSGCH H H H H H
37	Exemplary tail	AdCT18	EGSGCH H H H H H
38	Tag	T1	H H H H H H

- 5 In certain embodiments, the C-terminal extension sequences (also called "tails"), comprise E and D residues, and may be between 8 and 50, 10 and 30, 10 and 20, 5 and 10, and 2

and 4 amino acids in length. In some embodiments, tail sequences include ED-based linkers in which the sequence comprises tandem repeats of ED. In exemplary embodiments, the tail sequence comprises 2-10, 2-7, 2-5, 3-10, 3-7, 3-5, 3, 4 or 5 ED repeats. In certain embodiments, the ED-based tail sequences may also include additional amino acid residues, such as, for example: EI, EID, ES, EC, EGS, and EGC. Such sequences are based, in part, on known Adnectin tail sequences, such as EIDKPSQ (SEQ ID NO: 20), in which residues D and K have been removed. In exemplary embodiments, the ED-based tail comprises an E, I or EI residues before the ED repeats.

C. Anti-Myostatin Adnectin Immunoglobulin Fc Fusions

In one aspect, formulations containing a polypeptide (*e.g.*, anti-myostatin Adnectin) fused to an immunoglobulin Fc domain, or a fragment or variant thereof, are provided. As used herein, a "functional Fc region" is an Fc domain or fragment thereof which retains the ability to bind FcRn. In some embodiments, a functional Fc region binds to FcRn, but does not possess effector function. The ability of the Fc region or fragment thereof to bind to FcRn can be determined by standard binding assays known in the art. In other embodiments, the Fc region or fragment thereof binds to FcRn and possesses at least one "effector function" of a native Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.*, an anti-myostatin Adnectin) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, *e.g.*, from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent

polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95% sequence identity therewith.

- 5 In an exemplary embodiment, the Fc domain is derived from an IgG1 subclass, however, other subclasses (e.g., IgG2, IgG3, and IgG4) may also be used. Shown below is the sequence of a human IgG1 immunoglobulin Fc domain:

10 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 39)

The core hinge sequence is underlined, and the CH2 and CH3 regions are in regular text. It should be understood that the C-terminal lysine is optional.

- 15 The fusion may be formed by attaching an anti-myostatin Adnectin to either end of the Fc molecule, i.e., Fc-anti-myostatin Adnectin or anti-myostatin Adnectin-Fc arrangements. In certain embodiments, the Fc and anti-myostatin Adnectin are fused via a linker. Exemplary linker sequences include GAGGGGSG (SEQ ID NO: 40), EPKSSD (SEQ ID NO: 41), D, ESPKAQASSVPTAQPQAEGLA (SEQ ID NO: 42), ELQLEESAAEAQDGELD (SEQ ID NO:
 20 43), GQPDEPGGS (SEQ ID NO: 44), GSGSGSGSGSGSGS (SEQ ID NO: 45), ELQLEESAAEAQEGELE (SEQ ID NO: 46), GSGSG (SEQ ID NO: 47), GSGC (SEQ ID NO: 48), AGGGGSG (SEQ ID NO: 49), GSGS (SEQ ID NO: 50), QPDEPGGS (SEQ ID NO: 51), GSGSGS (SEQ ID NO: 52), TVAAPS (SEQ ID NO: 53), KAGGGGSG (SEQ ID NO: 54), KGSGSGSGSGSGS (SEQ ID NO: 55), KQPDEPGGS (SEQ ID NO: 56),
 25 KELQLEESAAEAQDGELD (SEQ ID NO: 57), KTVAAPS (SEQ ID NO: 58), KAGGGGSGG (SEQ ID NO: 59), KGSGSGSGSGSGSG (SEQ ID NO: 60), KQPDEPGGSG (SEQ ID NO: 61), KELQLEESAAEAQDGELDG (SEQ ID NO: 62), KTVAAPSG (SEQ ID NO: 63) AGGGGSGG (SEQ ID NO: 64), AGGGGSG (SEQ ID NO: 65), GSGSGSGSGSGSG (SEQ ID NO: 66), QPDEPGGSG (SEQ ID NO: 67), and TVAAPSG (SEQ ID NO: 68).

In some embodiments, the Fc region used in the anti-myostatin Adnectin fusion comprises the hinge region of an Fc molecule. As used herein, the "hinge" region comprises the core hinge residues spanning positions 1-16 of SEQ ID NO: 39) (DKTHTCPPCPAPELLG; SEQ ID NO: 69) of the IgG1 Fc region.

5 In certain embodiments, the anti-myostatin Adnectin-Fc fusion in the formulation adopts a multimeric structure (e.g., dimer) owing, in part, to the cysteine residues at positions 6 and 9 of SEQ ID NO: 39 within the hinge region. In other embodiments, the hinge region as used herein, may further include residues derived from the CH1 and CH2 regions that flank the core hinge sequence, as shown in SEQ ID NO: 39. In yet other embodiments, the hinge sequence is
10 GSTHTCPPCPAPELLG (SEQ ID NO: 70).

In some embodiments, the hinge sequence, may include substitutions that confer desirable pharmacokinetic, biophysical, and/or biological properties. Some exemplary hinge sequences include EPKSSDKTHTCPPCPAPELLGGPS (SEQ ID NO: 71; core hinge region underlined), EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO 72; core hinge region
15 underlined), EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 73; core hinge region underlined), DKTHTCPPCPAPELLGGPS (SEQ ID NO: 74; core hinge region underlined), and DKTHTCPPCPAPELLGGSS (SEQ ID NO: 75; core hinge region underlined). In one embodiment, the residue P at position 18 of SEQ ID NO: 39 has been replaced with S to ablate Fc effector function; this replacement is exemplified in hinges having any one of SEQ ID NOs:
20 72, 73 or 75. In another embodiment, the residues DK at positions 1-2 of SEQ ID NO: 39 have been replaced with GS to remove a potential clip site; this replacement is exemplified in SEQ ID NO: 73. In another embodiment, the C at position 103 of SEQ ID NO: 76, which corresponds to the heavy chain constant region of human IgG1 (i.e., domains CH1-CH3), has been replaced with S to prevent improper cysteine bond formation in the absence of a light chain; this
25 replacement is exemplified in SEQ ID NOs: 71-73.

30 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC

LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 76)

In certain embodiments, an anti-myostatin Adnectin-Fc fusion may have the following configurations: 1) anti-myostatin Adnectin-hinge-Fc or 2) hinge-Fc-anti-myostatin Adnectin.

5 Therefore, any anti-myostatin Adnectin of the present invention can be fused to an Fc region comprising a hinge sequence according to these configurations. In some embodiments, a linker may be used to join the anti-myostatin Adnectin to the hinge-Fc moiety, for example, an exemplary fusion protein may have the configuration anti-myostatin Adnectin-linker-hinge-Fc or hinge-Fc-linker-anti-myostatin Adnectin. Additionally, depending on the system in which the
 10 fusion polypeptide is produced, a leader sequence may be placed at the N-terminus of the fusion polypeptide. For example, if the fusion is produced in a mammalian system, a leader sequence such as METDTLLLWVLLLWVPGSTG (SEQ ID NO: 77) may be added to the N-terminus of the fusion molecule. If the fusion is produced in *E. coli*, the fusion sequence will be preceded by a methionine.

15 In one embodiment, the polypeptide an Fc-anti-myostatin Adnectin construct known as BHV-2000. In one embodiment, the Fc-anti-myostatin Adnectin construct comprises the amino acid sequence:

20 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSP*ELQLFEESAAEAQEGELEGVSDVPR*
DLEVVAATPTSLISWSLPHQGKANYRITYGETGGNSPVQEF*TVPGRGVTATI*
SGLKPGVDYTITVYAVTVTDTGYLKYPISINRTEI (SEQ ID NO: 78).

25 The hinge region is underlined, the linker is in italics, the leader sequence is in bold, and the anti-myostatin Adnectin sequence is underlined and in italics.

In one embodiment, the formulation contains Fc-anti-myostatin Adnectin construct comprising the amino acid sequence:

30 GVSDVPRDLEVVAATPTSLISWSLPHQGKANYRITYGETGGNSPVQEF
TVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYLKYPISINRTEIEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE

YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 79)

The Fc domain comprises the human IgG1 CH2 and CH3 regions as follows:

5 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSP (SEQ ID NO: 80)

10 and the hinge sequence DKTHTCPPCPAPELLG (SEQ ID NO: 69).

II. Stable Formulations

The polypeptides described herein can be administered as a formulation.

A "stable" formulation or drug product is one in which the anti-myostatin Adnectin
15 therein essentially retains its physical and chemical stability and integrity upon storage. Stability
of the anti-myostatin Adnectin molecule formulations can be measured at selected temperatures
after selected time periods. For example, an increase in aggregate formation following
lyophilization and storage is an indicator for instability of a lyophilized anti-myostatin Adnectin
molecule formulation. In addition to aggregate formation, retention of original clarity, color and
20 odor throughout shelf life are indicators utilized to monitor stability of anti-myostatin Adnectin
molecule solutions. HMW species are multimers (i.e. tetramers, hexamers, etc.), which have a
higher molecular weight than anti-myostatin Adnectin molecule monomers or dimers. Typically,
a "stable" drug product may be one wherein the increase in aggregation, as measured by an
increase in the percentage of high molecular weight species (% HMW), is less than about 5% and
25 preferably less than about 3%, when the formulation is stored at 2-8°C for one year. Preferably,
the manufactured drug product comprises less than about 25% HMW species, preferably less than
about 15% HMW species, more preferably less than about 10% HMW species, most preferred
less than about 5% HMW species.

"Shelf-life" of a pharmaceutical product, e.g., a protein comprising an anti-myostatin
30 adnectin, is the length of time the product is stored before decomposition occurs. For example,

shelf-life may be defined as the time for decomposition of 0.1%, 0.5%, 1%, 5%, or 10% of the product.

The terms "lyophilized" and "freeze-dried" are used interchangeably herein and refer to a material that is dehydrated by first freezing and then reducing the surrounding pressure to allow
5 the frozen water in the material to sublime.

A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized formulation in an aqueous carrier such that the anti-myostatin Adnectin molecule is dissolved in the reconstituted formulation. The reconstituted formulation is suitable for intravenous administration (IV) or subcutaneous (SC) administration to a patient in need thereof.

10 [0044] An "isotonic" formulation is one which has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsmol/KgH₂O. The term "hypertonic" is used to describe a formulation with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

15 The term "buffering agent" refers to one or more components that when added to an aqueous solution is able to protect the solution against variations in pH when adding acid or alkali, or upon dilution with a solvent. Pharmaceutically acceptable buffers include, but are not limited to, histidine, TRIS® (tris (hydroxymethyl) aminomethane), citrate, succinate, glycolate and the like, as described herein.

20 The term "pKa" refers to the negative logarithm (p) of the ionization (acid dissociation) constant (K_a) of an acid which is equal to the pH value at which equal concentrations of the acid and conjugate base forms of a buffer are present (in which half of the acid molecules are ionized).

When the p of a buffering agent equals the pH of the solution to be buffered, the buffering system is most effective.

An "acid" is a substance that yields hydrogen ions in aqueous solution. A "pharmaceutically acceptable acid" includes inorganic and organic acids which are nontoxic at the concentration and manner in which they are formulated.

A "base" is a substance that yields hydroxyl ions in aqueous solution. "Pharmaceutically acceptable bases" include inorganic and organic bases which are non-toxic at the concentration and manner in which they are formulated.

A "preservative" is an agent that reduces bacterial action and may be optionally added to the formulations herein. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol.

A "surfactant" is a surface active molecule containing both a hydrophobic portion (e.g., alkyl chain) and a hydrophilic portion (e.g., carboxyl and carboxylate groups). Surfactants suitable for use in the formulations of the present invention include, but are not limited to, polysorbates (e.g. polysorbates 20 or 80); poloxamers (e.g. poloxamer 188); sorbitan esters and derivatives; Triton; sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetadine; lauryl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauramidopropyl-cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or

isostearamidopropylbetaine (e.g., lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUATTM series (Mona Industries, Inc., Paterson, N.J.), polyethylene glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g., Pluronic, PF68 etc.).

5 A "drug substance" refers to the starting material utilized in formulation of the final drug product. Typical anti-myostatin adnectin drug substance compositions comprise a protein concentration from 10 mg/mL and 200 mg/mL, pH from 6.6 to 7.6 and % HMW species of < 5%.

A "formulated bulk solution" refers to the final formulation prior to filling of the container such as the formulated solution prior to filling the vials for lyophilization, or the formulated
10 solution prior to filling the syringe for IV and/or SC injection.

A "drug product" refers to the final formulation packaged in a container which may be reconstituted before use, such as with a lyophilized drug product; diluted further before use, such as with a liquid drug product; or utilized as is, such as with a SC solution drug product.

A. Exemplary Formulations

15 In some embodiments, the concentration of the polypeptide (e.g., anti-myostatin adnectin) in the formulation is between about 10 mg/mL and 200 mg/mL. In other embodiments, the concentration of the polypeptide in the formulation is between about 10 mg/mL and 140 mg/mL. In other embodiments, the concentration of the polypeptide in the formulation is least about 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL,
20 40 mg/mL, 45 mg/mL, 50, mg/mL, 60 mg/mL, 65 mg/mL, 70 mg/mL, 75 mg/mL, 80 mg/mL, 85 mg/mL, 90 mg/mL, 95 mg/mL or higher. In certain embodiments, the concentration of the polypeptide in the formulation is at least about 110 mg/mL, 115 mg/mL, 120 mg/mL, 125 mg/mL, 130 mg/mL, 135 mg/mL, 140, mg/mL or 145, mg/m L. In certain embodiments, the concentration of the polypeptide in the formulation is 10.7 mg/mL, 21.4 mg/mL, 50.0 mg/mL or
25 71.4 mg/mL.

The stabilizing sugar in the formulation is a disaccharide in a weight (w/w) ratio of at least 5:1 protein to sugar. In some embodiments, the protein:sugar weight ratio is between about 5:1 to 10:1. In some embodiments, the protein:sugar ratio is about 6:1, 7:1, 8:1, 9:1 or 10:1. In some embodiments, the protein:sugar ratio is about 6.75:1.

In some embodiments, the formulation comprises about 5% to about 30% of the disaccharide. In some embodiments, the formulation comprises about 10% to about 28% of the disaccharide. In some embodiments, the formulation comprises about 15% to about 25% of the disaccharide. In some embodiments, the formulation comprises about 20% to about 25% of the disaccharide. In some embodiments, the formulation comprises about 18%, 19%, 20%, 21%, 22%, 23%, 24% or about 25% of the disaccharide.

In some embodiments, the concentration of the sugar in the formulation is about 150 mM to about 800 mM. In some embodiments, the concentration of the sugar in the formulation is about 300 to about 700 mM. In other embodiments, the concentration of the sugar in the formulation is about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM or about 700 mM.

In some embodiments, the disaccharide is trehalose. In some embodiments, the formulation comprises about 5 to about 30% trehalose. In some embodiments, the formulation comprises about 10% to about 28% trehalose. In some embodiments, the formulation comprises about 15% to about 25% trehalose. In some embodiments, the formulation comprises about 20% to about 25% trehalose. In some embodiments, the formulation comprises about 18%, 19%, 20%, 21%, 22%, 23%, 24% or about 25% trehalose. In one embodiment, the formulation comprises 22% trehalose. In another embodiment, the formulation comprises 23% trehalose.

In some embodiments, the disaccharide is trehalose dihydrate. In some embodiments, concentration of trehalose dihydrate in the formulation is about 150 mM to about 800 mM. In some embodiments, the concentration of the trehalose dihydrate in the formulation is about 300 to about 700 mM. In other embodiments, the concentration of the trehalose dihydrate in the formulation is about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM or about 700 mM. In one embodiment, the concentration of trehalose dihydrate in the formulation is 600 nM.

The stabilizing sugar in the formulation is employed in an amount no greater than that which may result in a viscosity undesirable or unsuitable for administration via SC syringe. In

some embodiments, the viscosity of the formulation is from about 5 to 20 cps. In some embodiments, the viscosity of the formulation is from about 7 to 12 cps. In some embodiments, the viscosity is about 7-10 cps. In some embodiments, the viscosity of the formulation is less than 8 cps.

5 The buffering agent in the formulation is present in an amount of at least 20 mM, and is preferably between about 20 mM and about 40 mM. In some embodiments, the buffering agent is histidine at a concentration of about 20 mM, about 25 mM, about 30 mM or about 35 mM. In one embodiment, the formulation comprises about 30 mM histidine.

10 The pH of the formulation is maintained at a range from about 6.5 to about 7.8. In certain embodiments, the pH is maintained at a range from about pH from 6.6 to 7.6. In certain embodiments, the pH of the formulation is about 6.8 to 7.4. In certain embodiments, the pH of the formulation is about 7.0 to 7.3. In some embodiments, the pH of the formulation is 6.9, 7.0, 7.1, 7.2 or 7.3. In some embodiments, the pH of the formulation is about 7.1.

15 The aqueous carrier used in the formulations herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (e.g., phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

20 The formulations may further comprise a surfactant to further reduce the formation of visible particulates. Preferred surfactants include poloxamer and polysorbate at a concentration of between about 0.01% and 0.5%. In some embodiments, the concentration of the surfactant is between about 0.02% and about 0.1%. In one embodiment, the surfactant is poloxamer 188. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80. In one embodiment, the surfactant is polysorbate 80.

25 The formulations may further comprise a chelator at a concentration between about 0.01 mM and about 0.5 mM, preferably, between about 0.05 mM and 0.2 mM. Preferred chelators include, but are not limited to DPTA, EDTA and EGTA. In one embodiment, the chelator in the formulation is DPTA at a concentration of about 0.05 mM.

A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation.

In some embodiments, the formulation provided herein comprises:

- 5 (i) about 10-140 mg/mL of the polypeptide;
(ii) about 5-25% trehalose dihydrate; and
(iii) about 20-30 mM histidine,

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation provided herein consists essentially of:

- 10 (i) about 10-140 mg/mL of the polypeptide;
(ii) about 5-25% trehalose dihydrate; and
(iii) about 20-30 mM histidine,

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation provided herein comprises:

- 15 (i) about 10-140 mg/mL of the polypeptide;
(ii) about 5-25% trehalose dihydrate;
(iii) about 20-30 mM histidine;
(iv) about 0.02-0.06 mM DTPA; and
(v) about 0.01-0.05% polysorbate 80

20 wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation provided herein consists essentially of:

- (i) about 10-140 mg/mL of the polypeptide;
(ii) about 5-25% trehalose dihydrate;
(iii) about 20-30 mM histidine;
25 (iv) about 0.02-0.06 mM DTPA; and
(v) about 0.01-0.05% polysorbate 80

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation comprises:

- (i) about 10-75 mg/mL of the polypeptide;

(ii) about 600 mM trehalose dihydrate; and

(iii) 25-30 mM histidine,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation consists essentially of:

5 (i) about 10-75 mg/mL of the polypeptide;

(ii) about 600 mM trehalose dihydrate; and

(iii) 25-30 mM histidine,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation comprises:

10 (i) about 10-75 mg/mL of the polypeptide;

(ii) about 600 mM trehalose dihydrate;

(iii) 25-30 mM histidine;

(iv) about 0.02-0.06 mM DTPA; and

(v) about 0.01-0.05% polysorbate 80,

15 wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation consists essentially of:

(i) about 10-75 mg/mL of the polypeptide;

(ii) about 600 mM trehalose dihydrate;

(iii) 25-30 mM histidine;

20 (iv) about 0.02-0.06 mM DTPA; and

(v) about 0.01-0.05% polysorbate 80,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation comprises

(i) about 10-75 mg/mL of the polypeptide;

25 (ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In some embodiment, the formulation consists essentially of:

- (i) about 10-75 mg/mL of the polypeptide;
- (ii) about 600 mM trehalose dihydrate;
- (iii) about 30 mM histidine;
- 5 (iv) about 0.05 mM DTPA; and
- (v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

- (i) about 10.7 mg/mL of the polypeptide;
- 10 (ii) about 600 mM trehalose dihydrate;
- (iii) about 30 mM histidine;
- (iv) about 0.05 mM DTPA; and
- (v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

15 In one embodiment, the formulation comprises or consists essentially of:

- (i) about 21.4 mg/mL of the polypeptide;
- (ii) about 600 mM trehalose dihydrate;
- (iii) about 30 mM histidine;
- (iv) about 0.05 mM DTPA; and
- 20 (v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

- (i) about 50 mg/mL of the polypeptide;
- (ii) about 600 mM trehalose dihydrate;
- 25 (iii) about 30 mM histidine;
- (iv) about 0.05 mM DTPA; and
- (v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

- (i) about 71.4 mg/mL of the polypeptide;
- (ii) about 600 mM trehalose dihydrate;
- (iii) about 30 mM histidine;
- (iv) about 0.05 mM DTPA; and
- 5 (v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

- The recommended storage condition for the liquid formulation is from 2-8°C, with a recommended shelf life of at least 12 months. In order to ensure efficacy and safety during the time course of the shelf life of pharmaceutical compositions, the composition is stability tested.
- 10 Typically, the stability tests include but are not limited to tests regarding identity, purity and potency of the composition. The stability is tested both at the intended storage temperature and at elevated temperature or temperatures. Purity tests may include but are not limited to SDS-PAGE, CE-SDS, isoelectrofocusing, immunoelectrophoresis, Western blot, reversed-phase chromatography, Size-exclusion chromatography (SEC), ion exchange and affinity
- 15 chromatography. Other tests may include, but are not limited to: visual appearance such as color and transparency, particulates, pH, protein concentration measurement, moisture and reconstitution time.

- The degradation profile regarding, in particular, purity and potency, during the stability time course is intimately coupled to the composition and/or the formulation of the
- 20 pharmaceutical product. In particular, proper choice of formulation may significantly change the degradation profile. Typical degradation profiles for protein molecules products derived from FBS includes the formation of covalent and non-covalent high molecular weight aggregates, fragments, deamidation and oxidation products. Particularly, de-amidation and oxidation products as well as other acidic species usually develop during the time course of the stability
- 25 testing. In some cases, the acidic species limits the acceptable shelf life of the pharmaceutical composition. The formation of acidic species due to, for example, deamidation, can be tested by, *e.g.*, imaged capillary isoelectrofocusing (icIEF). In other cases, the formation of high molecular weight aggregates limits the acceptable shelf life of the pharmaceutical composition. The

formation of aggregates may be tested by for example SEC (size exclusion chromatography), DLS, MFI, SDS-PAGE or CE-SDS.

For example, an anti-myostatin Adnectin formulation with pharmaceutically acceptable stability can be one wherein, when stored at a temperature of about $5 \pm 3^\circ\text{C}$ or $25 \pm 2^\circ\text{C}$ for a period of least about 3 months, preferably about 6 months, and more preferably about 12 months or longer, such as 18 months or longer, such as for at least 24 or even 36 months, the percentage of aggregates is less than about 10%, preferably less than about 5%, more preferably less than about 2%, when determined using SEC analysis. Additionally or alternatively, a stable anti-myostatin Adnectin formulation of the invention can be one wherein, when stored at a temperature of about $5 \pm 3^\circ\text{C}$ or $25 \pm 2^\circ\text{C}$ for a period of least about 3 months, preferably about 6 months, and more preferably about 12 months or longer, the changes of main isoform are less than 15%, preferably less than 10%, more preferably less than 8%, most preferably less than 5%, when determined using icIEF analysis.

B. Preparation of the Formulation

The manufacturing process developed for SC formulations typically involves compounding with sugar, chelating agent and surfactant, followed by aseptic sterile filtration and filling into vials or syringes, optionally preceded by diafiltration (buffer exchange) and concentration of drug substance using an ultrafiltration unit. The protein purification is the first stage after production in the fermentation bioreactor. The protein is purified using multiple column and filtration steps and concentrated using tangential flow filtration into the formulation buffer. The concentrated drug substance is diluted with the formulation buffer at the target concentrations and this solution is sterile filtered and filled into sterile vials/syringes for patient use. One skilled in the art would be aware of the need to overfill the container so as to compensate for vial, needle, syringe hold-up during preparation and injection. For example, a 5-10% overage of drug product is incorporated into each vial of liquid formulation to account for withdrawal losses and guarantee that required dose (label claim) of drug product can be withdrawn from the vial.

Preparation of unit dosage forms for the formulation comprising the polypeptide comprising the ¹⁰F_n3 domain which binds myostatin (also referred interchangeably herein as

“anti-myostatin adnectin”) syringes involves protein production in a recombinant cell line, purification via multiple column steps, concentration and buffer exchange into formulation buffer using tangential flow filtration. The concentrated protein for the tangential flow filtration is further processed by dilution with formulation buffer to the target protein concentrations and the diluted product, after filtration, is filled into 1 mL syringes (*e.g.*, insulin syringe, tuberculin syringe, BioPak syringe, NeoPak syringe). In one embodiment, the syringes are then equipped with an UltraSafe Passive needle guard.

The unit dosage form of the formulation typically contains about 0.3 to 1.5 mL of the formulation. In certain embodiments, the unit dosage form contains a volume of 0.3, 0.5, 0.7, 0.8, 1.0, 1.2, 1.4 or 1.4 mL. In certain embodiments, the unit dosage form is provided at a volume of 0.7 mL. In some embodiments, the unit dosage form contains 5-100 mg of the polypeptide comprising the ¹⁰Fn3 domain which binds myostatin. In some embodiments, the unit dosage form comprises 7.5 mg, 15 mg, 35 mg or 50 mg of the anti-myostatin adnectin.

In some embodiments, the formulations are manufactured as disclosed herein and are stored in bulk at -60°C, for example, in 12L FFtp bags at polypeptide concentration of 85-150 mg/mL. In some embodiments, the formulations are stored at -60°C at a polypeptide concentration of 85 mg/mL. The bulk formulations are then thawed and diluted to the appropriate polypeptide concentration for preparation of the unit dosage forms. In some embodiments, the polypeptide concentration of the formulation in the unit dosage form is about 10 mg/mL to about 140 mg/mL. In some embodiments, the polypeptide concentration of the formulation in the unit dosage form is about 10 mg/mL to about 75 mg/mL. In certain embodiments, the polypeptide concentration of the formulation in the unit dosage form is 10.7 mg/mL, 20.4 mg/mL, 50 mg/mL or 71.4 mg/mL.

C. Administration

A pharmaceutical formulation comprising a polypeptide as described herein can be administered to a subject at risk for or exhibiting pathologies as described herein. The formulations provided herein are particularly useful for peripheral systemic delivery by intravenous, intraperitoneal or subcutaneous injection. In preferred embodiments, the formulations are delivered by subcutaneous injection.

A therapeutically effective dose refers to a dose that produces the therapeutic effects for which it is administered. An effective amount of a pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the binding agent molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient.

The exact dosage is determined based on factors related to the subject requiring treatment and can be ascertained using standard techniques. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that can be considered include the severity of the disease state, the general health of the subject, the age, weight, and sex of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy.

The polypeptides (anti-myostatin Adnectin molecules) are administered in the target population at any suitable dose. In one embodiment, the polypeptides are administered as repeated subcutaneous injections. In one embodiment, the polypeptides are administered at a dosing interval of once a week. In one embodiment, the polypeptides are administered at a dosing interval of once every two weeks. In one embodiment, the polypeptides are administered at a dosing interval of once a month.

In one embodiment, the polypeptides are administered at a dose of approximately 5-200mg. For example, the polypeptides can be administered at a dose of 5mg, 6mg, 7mg, 8mg, 9mg, 10mg, 11mg, 12mg, 13mg, 14mg, 15mg, 16mg, 17mg, 18mg, 19mg, 20mg, 21mg, 22mg, 23mg, 24mg, 25mg, 26mg, 27mg, 28mg, 29mg, 30mg, 31mg, 32mg, 33mg, 34mg, 35mg, 36mg, 37mg, 38mg, 39mg, 40mg, 41mg, 42mg, 43mg, 44mg, 45mg, 46mg, 47mg, 48mg, 49mg, 50mg, 51mg, 52mg, 53mg, 54mg, 55mg, 56mg, 57mg, 58mg, 59mg, 60mg, 61mg, 62mg, 63mg, 64mg, 65mg, 66mg, 67mg, 68mg, 69mg, 70mg, 71mg, 72mg, 73mg, 74mg, 75mg, 76mg, 77mg, 78mg, 79mg, 80mg, 81mg, 82mg, 83mg, 84mg, 85mg, 86mg, 87mg, 88mg, 89mg, 90mg, 91mg, 92mg, 93mg, 94mg, 95mg, 96mg, 97mg, 98mg, 99mg, 100mg, 101mg, 102mg, 103mg, 104mg, 105mg, 106mg, 107mg, 108mg, 109mg, 110mg, 111mg, 112mg, 113mg, 114mg, 115mg, 116mg,

117mg, 118mg, 119mg, 120mg, 121mg, 122mg, 123mg, 124mg, 125mg, 126mg, 127mg,
128mg, 129mg, 130mg, 131mg, 132mg, 133mg, 134mg, 135mg, 136mg, 137mg, 138mg,
139mg, 140mg, 141mg, 142mg, 143mg, 144mg, 145mg, 146mg, 147mg, 148mg, 149mg,
150mg, 151mg, 152mg, 153mg, 154mg, 155mg, 156mg, 157mg, 158mg, 159mg, 160mg,
5 161mg, 162mg, 163mg, 164mg, 165mg, 166mg, 167mg, 168mg, 169mg, 160mg, 171mg,
172mg, 173mg, 174mg, 175mg, 176mg, 177mg, 178mg, 179mg, 170mg, 181mg, 182mg,
183mg, 184mg, 185mg, 186mg, 187mg, 188mg, 189mg, 180mg, 191mg, 192mg, 193mg,
194mg, 195mg, 196mg, 197mg, 198mg, 199mg, or 200mg.

In one embodiment, the therapeutic dose level(s) and dosing interval(s) are determined by
10 the pharmacokinetic:pharmacodynamic (PK/PD) correlation of the anti-myostatin Adnectin in
the target population, as determined in a controlled clinical trial. Desired pharmacodynamic
changes include, but are not limited to, reduction in free (unbound) myostatin, improvements in
body composition and total body weight.

The frequency of dosing depends upon the pharmacokinetic:pharmacodynamic
15 parameters of the binding agent molecule in the formulation used. Typically, a composition is
administered until a dosage is reached that achieves the desired effect. The composition can,
therefore, be administered as a single dose or as multiple doses (at the same or different
concentrations/dosages) over time. Further refinement of the appropriate dosage is routinely
made. Appropriate dosages can be ascertained through use of appropriate dose-response data.
20 For example, the anti-myostatin Adnectin can be administered less frequently (*e.g.*, bi-weekly, or
monthly). In addition, as is known in the art, adjustments for age as well as the body weight,
general health, sex, time of administration, drug interaction, and the severity of the disease may
be necessary, and re ascertainable with routine experimentation by those skilled in the art. The
anti-myostatin Adnectin can be suitably administered to the patient at one time or over a series of
25 treatments.

III. Methods

Provided herein are methods for improving glycemic control in a human patient, methods
for treating, preventing, or reducing obesity and related comorbidities, and methods of treating or
preventing type II diabetes, by administering to the patient a polypeptide comprising a

fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the is administered (or is for administration) according to a particular clinical dosage regimen (*e.g.*, at particular doses and according to a specific schedule).

As used herein, the term “subject” or “patient” is a human patient (*e.g.*, an obese patient, patient having obesity-related comorbidities, and/or a patient having type II diabetes).

The notations "mpk", "mg/kg", or "mg per kg" refer to milligrams per kilogram. All notations are used interchangeably throughout the present disclosure.

The terms "individual," "subject," and "patient," used interchangeably herein, refer to an animal, preferably a mammalian (including a non-primate and a primate) or avian species, including, but not limited to, murines, simians, humans, mammalian farm animals (*e.g.*, bovine, porcine, ovine), mammalian sport animals (*e.g.*, equine), and mammalian pets (*e.g.*, canine and feline); preferably the term refers to humans. The term also refers to avian species, including, but not limited to, chickens and turkeys.

The term "therapeutically effective amount" refers to at least the minimal dose, but less than a toxic dose, of an agent which is necessary to impart a therapeutic benefit to a subject.

As used herein, the term “pediatric” patient is a human patient that has been classified by a physician or caretaker as belonging to a non-adult category and can include, *e.g.*, newborn (both preterm and of term), infants, children, and adolescents. Typically, pediatric patients are patients under 18 years of age (<18 years of age).

As used herein, the term “adult” patient is a human patient that has been classified by a physician or caretaker as such, *e.g.*, one who is not a newborn, infant, child or adolescent, *e.g.*, based on age, developmental status, physiological features, *etc.* Typically, adult patients are patients who are 18 years of age or older (≥18 years of age).

As used herein, the phrase “Body Mass Index (BMI)” refers to a person’s weight in kilograms (or pounds) divided by the square of height in meters (or feet). A high BMI can indicate high body fatness. BMI screens for weight categories that may lead to health problems, but it does not diagnose the body fatness or health of an individual.

The term "obesity" is based on Body Mass Index (BMI) for both youth and adults, but the definitions are not directly comparable. Among adults, there is a set cut point based on health

risk, while among children the definition is statistical and is based on a comparison to a reference population. BMI is calculated as weight in kilograms divided by height in meters squared, rounded to one decimal place. Obesity in adults is defined as a BMI of greater than or equal to 30 kg/m². Obesity in youth is defined as a BMI of greater than or equal to the age- and sex-specific 5 95th percentile of the 2000 CDC growth charts.

The terms "overweight" or "overweight condition" are based on a BMI ≥ 25 - < 30 kg/m². Overweight condition can also be associated with at least one additional risk factor for fatal diseases (e.g., stroke, heart failure, sudden death), such as diabetes, hypertension, family history of premature coronary artery disease, etc.

10 Because different subjects can have same BMI, but different percentage of fat and muscle mass, BMI is not always a good index to classify overweight and obesity. A high percentage of muscle mass can lead to a high BMI even with a small percentage of fat. In this case the subject can be wrongly considered overweight or obese, based on the BMI classification. Other indexes in addition to BMI are used, namely waist circumference and a body shape index. Imaging, by 15 DXA and MRI, is often used in clinical trials to quantify the percentage of muscle, fat and the fat distribution.

The term "body composition" is used herein to describe the percentages of fat and muscle in human bodies. Because muscular tissue takes up less space in our body than fat tissue, body composition, as well as our weight, determines leanness. Two people of same sex and body 20 weight may look completely different from each other because they have a different body composition.

"Lean body mass" (LBM) is a component of body composition, calculated by subtracting body fat weight from total body weight. Total body weight is lean plus fat. Lean body mass equals body weight minus body fat. Lean body mass plus body fat equals body weight.

25 The percentage of total body mass that is lean is usually not quoted, but would typically be 60-90%. Instead, the body fat percentage, which is the complement, is computed, and is typically 10-40%. The lean body mass (LBM) has been described as an index superior to total body weight for prescribing proper levels of medications and for assessing metabolic disorders, as body fat is less relevant for metabolism.

The term "fat mass" refers to that portion of the human body that is composed strictly of fat. It can be measured with dual energy absorptiometry DXA, MRI or bioelectrical impedance techniques.

The term "central adiposity refers to the following:

5 Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue. The amount of excess fat in absolute terms, and its regional distribution between different fat depots both play an important role in determining the health impact of obesity. Obesity can be categorized into central/android obesity and peripheral/gynoid obesity, android obesity being more typical for men while gynoid obesity being more characteristic for women.

10 There is substantial evidence in the literature arguing that not all obesity is associated with adverse metabolic profile and increases in cardiovascular risk. In fact, body fat distribution (*i.e.*, relative presence of abdominal versus peripheral fat mass) was deemed as a better indicator of the metabolic and cardiovascular risks than the degree of obesity per se. In men, who tend to accumulate fat in the truncal region, increasing BMI is associated with increasing CV risk, while
15 in women BMI is a generally poor indicator/surrogate of cardiovascular risk. Trunk fat mass can be subdivided into subcutaneous (SC) fat (in the abdominal wall) and visceral adipose tissue (in the intra-abdominal cavity). Subcutaneous and visceral fat differ significantly in terms of their anatomy, cellular composition, endocrine function and cellular regulation. VAT compared with SC is more cellular, vascular, innervated and infiltrated by inflammatory and immune cells,
20 which translate to a higher metabolic activity and increased release of pro-inflammatory cytokines with direct and indirect implications for insulin resistance, type 2 diabetes, and the risk of cardiovascular disease. In contrast, subcutaneous fat mass, especially fat depots of the thigh and buttocks, were associated with constitutive secretion of adiponectin conferring insulin sensitizing, anti-inflammatory and anti-atherogenic effects. Low-grade inflammation has been
25 associated with muscle wasting, which in turn may further worsen insulin sensitivity and add to the relative risk of developing type-2 diabetes. Therefore, an imbalance between central and peripheral fat depots (central adiposity) even without manifest obesity (*i.e.* BMI <30 kg/m²) can be linked to pronounced insulin resistance, metabolic alterations and systemic low-grade inflammation collectively driving accelerated atherogenesis.

In clinical practice, anthropometric measurements such as waist circumference or the waist-to-hip ratio are widely used to estimate abdominal obesity, but these anthropometric measures are not able to distinguish between visceral and subcutaneous fat in the abdominal region and hence are associated with inaccuracies. More advanced technologies, such as
5 computed tomography (CT) or dual energy X-ray absorptiometry (DXA) can provide more direct assessment/measurement of fat mass. CT has an advantage in distinguishing between VAT and SAT, whereas DXA is useful for assessing the distribution of body fat mass between predefined anatomic regions (arms, legs, trunk) and help to distinguish android from gynoid adiposity.

The term "type II diabetes" referred as type 2 diabetes, previously referred to as "non-
10 insulin-dependent diabetes" or "adult-onset diabetes," accounts for 90-95% of all diabetes, encompasses individuals who have insulin resistance and usually relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals may not need insulin treatment to survive.

There are various causes of type 2 diabetes. Although the specific etiologies are not
15 known, autoimmune destruction of B-cells does not occur, and patients do not have any of the other known causes of diabetes. Most, but not all, patients with type 2 diabetes are overweight or obese. Excess weight itself causes some degree of insulin resistance. Patients who are not obese or overweight by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Type 2 diabetes frequently goes undiagnosed
20 for many years because hyperglycemia develops gradually and, at earlier stages, is often not severe enough for the patient to notice the classic diabetes symptoms. Nevertheless, even undiagnosed patients are at increased risk of developing macrovascular and microvascular complications.

The term "comorbidities of obesity or overweight" is associated with serious chronic
25 disorders including, but not limited to: type 2 diabetes, glucose intolerance, prediabetes, insulin resistance, hypertension, dyslipidemia, increased waist circumference, cardiovascular disease, non-alcoholic fatty liver disease, obstructive sleep apnea, physical impairment, osteoarthritis, osteoporosis, renal disease, sexual hormone(s) impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, stroke, and gallstones.

"Glucose intolerance" is defined as the inability to properly metabolize glucose.

"Insulin sensitivity" describes how sensitive the body is to the effects of insulin. Someone said to be insulin sensitive will require smaller amounts of insulin to lower blood glucose levels than someone who has low sensitivity. Insulin sensitivity varies from person to person and
5 doctors can perform tests to determine how sensitive an individual is to insulin.

"Insulin resistance" is defined as a condition of tolerance to insulin, making the hormone less effective, causing decreased glucose uptake in muscle tissue that result in impaired glucose oxidation and glycogen synthesis, and a deficient suppression of hepatic glucose production in the liver. In obese, increased visceral fat mass with elevation of plasma free fatty acid (FFA)
10 caused by the intensified lipolytic activity, worsen insulin resistance through the impairment of insulin action (Reaven, G.M., *et al.*, (1988), *Diabetes*. 37:1020-1024), a mechanism known as lipotoxicity (DeFronzo R.A. (2004), *Int. J. Clin. Pract. Suppl.*; (143):9-21). Herein the terms "improving insulin sensitivity" and "treating/lowering insulin resistance" shall be construed as equivalent.

15 High concentrations of plasma free fatty acid (FFA) in skeletal muscle cells lead to reduction in insulin-stimulated intracellular transport of glucose through the Glut4 transporter (see, *e.g.*, Dresner A., *et al.* (1999), *J. Clin. Invest.* 103(2): 253-259), in the hepatocytes lead to enhanced rate of gluconeogenesis and glucose release from the liver and augmented insulin secretion from β cells in response to a transient increase of FFA or an inhibition effect in
20 response to chronic elevated levels (see, *e.g.*, Boden G. (1997), *Diabetes*. 46(1):3-10). As a result of insulin resistance and lipotoxicity, more insulin is needed to induce glucose uptake from fat and muscle cells, and glycogen synthesis in the liver (see, *e.g.*, Boden G. (1997), *Diabetes*. 46(1):3-10). The overproduction of insulin by pancreatic β cells is the physiologic reaction to insulin resistance and can lead to decline of β cell function and, eventually to prediabetes and
25 type 2 diabetes (Donath M.Y., *et al.*, (2005), *Diabetes*, 54: S108-S113.).

The term "improving insulin sensitivity" refers to the systemic responsiveness to glucose, which can be measured by the insulin sensitivity index (which measures the ability of endogenous insulin to lower glucose in extracellular fluids by inhibiting glucose release from the liver and stimulating the peripheral consumption of glucose) and the glucose-clamp technique

(which measures the effect of changes in insulin concentration on glucose clearance-glucose uptake rate divided by plasma glucose concentration per unit of body surface area).

"Prediabetes" is the precursor stage before diabetes mellitus in which blood sugar is abnormally high (*e.g.*, 100-125 mg/dL).

5 Impaired fasting glycemia and impaired glucose tolerance are two aspects of prediabetes that are similar in clinical definition (glucose levels too high for their context) but are physiologically distinct. Insulin resistance, metabolic syndrome (or syndrome X), and prediabetes are closely related to one another and have overlapping aspects.

10 Provided herein are methods for improving glycemic control in a human patient, methods for treating, preventing, or reducing obesity and related comorbidities, and methods of treating or preventing type II diabetes, by administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin. In some embodiments, the administered (or is for administration) according to a particular clinical dosage regimen (*e.g.*, at particular doses and according to a specific schedule).

15 In one aspect, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

20 In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in
25 SEQ ID NOs: 5, 6 and 7, respectively, and wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections). In one embodiment, the ¹⁰F_n3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 11, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 78, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of improving glycemic control in a human patient is provided, the method comprising administering to the patient BHV-2000, wherein BHV-2000 is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In another aspect, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated

subcutaneous injections). In one embodiment, the ¹⁰F_n3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.

In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering
5 to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 11, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of treating, preventing, or reducing overweight or obesity
10 and related comorbidities in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 78, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

15 In one embodiment, a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient is provided, the method comprising administering to the patient BHV-2000, wherein BHV-2000 is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

20 In a further aspect, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated
25 subcutaneous injections).

In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has

0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections). In one
5 embodiment, the ¹⁰Fn3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.

In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 11, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the
10 polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient a polypeptide comprising the amino acid sequence set forth SEQ ID NO: 78, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the
15 polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, a method of treating or preventing type II diabetes in a human patient is provided, the method comprising administering to the patient BHV-2000, wherein BHV-2000 is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated
20 subcutaneous injections).

In one embodiment, the patient has a body mass index (BMI) ≥ 30 kg/m². In another embodiment, the patient has a BMI ≥ 25 and < 30 kg/m². In another embodiment, the patient has a BMI < 25 .

In one embodiment, the patient is overweight. In another embodiment, the patient is
25 obese. In another embodiment, the patient has an obesity related comorbidity. Exemplary obesity related comorbidities include, but are not limited to, type 2 diabetes, glucose intolerance, prediabetes, insulin resistance, high triglycerides, physical impairment, osteoporosis, renal disease, obstructive sleep apnea, sexual hormones impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, osteoarthritis, gastrointestinal

cancers, dyslipidaemia, hypertension, heart failure, coronary heart disease, stroke, gallstones, and altered gonadal hormone profile. In one embodiment, the patient has type II diabetes.

The peptide or formulation comprising the peptide can be administered to a patient by any suitable means. In one embodiment, the peptide is formulated for intravenous administration. In one embodiment, the peptide is formulated for subcutaneous administration.

IV. Outcomes

The efficacy of the treatment methods provided herein can be assessed using any suitable means.

In one aspect, methods for improving glycemic control in a human patient are provided. Glycemic control is a key goal in the management of patients, for example, patients with diabetes, and it remains the main therapeutic target for the prevention of organ damage and other complications arising from diabetes (see, *e.g.*, Imran SA, *et al.*, *Can. J. Diabetes*. 2018;42:S42–S46 and American Diabetes Association. Classification and diagnosis of diabetes standards of medical care in diabetes, *ADA Diabetes Care J. Clin. Appl. Res. Educ.* 2018;41 (Supplement 1):S13–S27). Hemoglobin A1c (HbA1c) is the gold standard for monitoring glycemic control and serves as a surrogate for diabetes-related complications. Poor glycemic control is a major public health issue among patients with type 2 diabetes mellitus and a significant risk factor for the progression of diabetic complications (see, *e.g.*, Koro CE, *et al.*, *Diabetes Care*. 2004;27(1):17–20; Yakubu A, *et al.*, *Int. J. Sci. Health Res.* 2020;5(4):207–214; and Digssie A, *et al.*, *Metab Open*. 2020;8:100056). To minimize diabetic complications, strictly maintaining a patient's blood glucose level in the normal or close to the normal range is crucial (see, *e.g.*, Mariye T, *et al.*, *Endocrinol. Metab. Open Access*. 2020;4(1):1–7). Accordingly, patients treated according to the methods disclosed herein experience improvement in at least one sign of glycemic control, for example, as assessed by HbA1c.

In another aspect, methods for treating, preventing, or reducing obesity and related comorbidities in a patient are provided. Symptoms of obesity include, but are not limited to, difficulty in sleeping, sleep apnea, daytime drowsiness, back and/or joint pains, excessive sweating, intolerance to heat, infections in skin folds, fatigue, depression, and feeling of

shortness of breath (dyspnea). Signs of obesity include, but are not limited to, acanthosis nigricans (i.e., a skin disorder characterized by the presence of hyperkeratosis and hyperpigmentation in the skin folds and armpits), stretch marks (due to distension and rupture of the elastic fibers of the skin), vinous in the case of obesity due to endocrinological alteration (Cushing), swelling and varicose veins in the lower limbs, Body Mass Index (BMI) greater than or equal to 30 kg/m², waist circumference greater than 94 cm in men and 88 cm in women, and high blood pressure level > 140/90 mmHg. Accordingly, patients treated according to the methods disclosed herein experience improvement in at least one or more symptoms or signs of obesity.

In a further aspect, methods of treating or preventing type II diabetes are provided. Symptoms of type II diabetes include, but are not limited to, increased thirst, frequent urination, increased hunger, unintended weight loss, fatigue, blurred vision, slow-healing sores, frequent infections, numbness or tingling in the hands or feet, areas of darkened skin, usually in the armpits and neck. Factors that increase the risk of type 2 diabetes include, but are not limited to: being overweight or obese, fat distribution mainly in the abdomen (as opposed to hips and thighs), inactivity, family history, race and ethnicity, blood lipid levels, age, prediabetes, pregnancy-related risks, and polycystic ovary syndrome. Potential complications of diabetes and frequent comorbidities include heart and blood vessel disease, nerve damage (neuropathy) in limbs, other nerve damage, kidney disease, eye damage, skin conditions, slow healing, hearing impairment, sleep apnea, and dementia. Accordingly, patients treated according to the methods disclosed herein experience improvement in at least one or more symptoms of type II diabetes.

In one embodiment, the method results in an improvement in glycemic control. In one embodiment an improvement in glycemic control is achieved by improving insulin sensitivity.

In one embodiment, the treatment results in an improvement in a Diabetes Treatment Satisfaction Questionnaire (DTSQ) score. In another embodiment, the treatment results in an improvement in an Impact of Weight on Quality of Life (IWQOL) score.

In one embodiment, central adiposity is reduced.

In one embodiment, the treatment results in a decrease in total body fat mass (FM) of at least 5 kg compared to baseline. For example, in one embodiment, the treatment results in a

decrease in total body FM of at least 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11 kg or more. In one embodiment, the treatment results in a decrease in total body
 5 FM of at least 5 kg by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an at least 5% decrease in total body FM compared to baseline. For example, in one embodiment, the treatment results in an at least 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5 % or more
 10 decrease in total body FM. In one embodiment, the treatment results in an at least 5% decrease in total body FM by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an at least 5% decrease in body weight compared to baseline. For example, in one embodiment, the treatment results in an at least 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17,
 15 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5 % or more decrease in body weight. In one embodiment, the treatment results in an at least 5% decrease in body weight by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an at least 2% increase in body lean mass (LM) compared to baseline. For example, in one embodiment, the treatment results in at least 2,
 20 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 % or more increase in body LM compared to baseline. In one embodiment, the treatment results in an at least 2% increase in body LM by week 6, week 8, week 10, week 12, week 16, week 20, 24, week 36, week 48, or week 60 compared to baseline.
 25 In another embodiment, the treatment results in an at least 25% increase in body LM by week 8, week 10, week 12, week 16, week 20, 24, week 36, week 48, or week 60 compared to baseline.

FM, LM, and body fat can be assessed by any suitable means. In one embodiment, FM is assessed by dual-energy x-ray absorptiometry (DXA). In one embodiment, LM is assessed by DXA. In one embodiment, body fat is assessed by skinfold calipers, body circumference

measurements, DXA, hydrostatic weighing, air displacement plethysmography (Bod Pod), bioelectrical impedance analysis (BIA), bioimpedance spectroscopy (BIS), or electrical impedance myography (EIM), a 3-D body scanner, a multi-compartment model, and/or magnetic resonance spectroscopy (MRI).

5 In one embodiment, the treatment results in a decrease in waist circumference (WC) and/or waist-to-hip ratio compared to baseline. For example, in one embodiment, the treatment results in a decrease in WC by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline. In another embodiment, the treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more compared to baseline. In another embodiment, the
10 treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more by week 12, week 15, week 18, week 24, week 36, week 48, or week 60 compared to baseline.

 In one embodiment, the treatment results in a decrease in WC and a decrease in total body weight compared to baseline. For example, in one embodiment, the treatment results in a
15 decrease in WC by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline and a decrease in total body weight by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline. In another embodiment, the treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more compared to baseline and a decrease in total body weight by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% compared to baseline. In another
20 embodiment, the treatment results in a decrease in WC by 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, 10 cm or more and a decrease in total body weight by at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5% by week 12, week 15, week 18, week 24, week 36, week 48, or week 60 compared to baseline.

 In one embodiment, the treatment results in a decrease in subcutaneous and abdominal
25 visceral adipose tissue compared to baseline. For example, in one embodiment, the treatment results in at least 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5 % or more decrease in subcutaneous and abdominal visceral adipose tissue compared to baseline. In one embodiment, the results in a decrease in subcutaneous and abdominal visceral adipose tissue by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an improvement in diabetes status. For example, in one embodiment, the treatment results in an improvement in the patient's HbA1c. In another embodiment, the treatment results in an improvement as assessed by Homeostatic Model Assessment (HOMA). In another embodiment, the treatment results in an improvement as
5 assessed by quantitative insulin-sensitivity check index (QUICKI). In another embodiment, the treatment results in an improvement as assessed by Matsuda Index. In one embodiment, the treatment results in an improvement in diabetes status by week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in a shift toward normal levels of one or more
10 biomarkers selected from the group consisting of serum lipids, high-sensitivity C-reactive protein (hs-CRP), interleukin 6, leptin, and adiponectin. In one embodiment, the treatment results in a shift toward normal levels of one or more biomarkers selected from the group consisting of serum lipids, high-sensitivity C-reactive protein (hs-CRP), interleukin 6, leptin, and adiponectin by week 24, week 36, week 48, or week 60 compared to baseline.

15 In one embodiment, the treatment results in a reduction in insulin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in insulin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,
20 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in insulin level by week 8, week 10, week 12, week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in a reduction in leptin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
25 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50% reduction in leptin level compared to baseline. In one embodiment, the treatment results in a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,

48, 49 or 50% reduction in leptin level by week 8, week 10, week 12, week 24, week 36, week 48, or week 60 compared to baseline.

In one embodiment, the treatment results in an improvement in hand grip strength, for example, as assessed by dynamometry. In one embodiment, the treatment results in an improvement in hand grip strength by week 24, week 36, week 48, or week 60 compared to baseline.

V. Kits

Further provided are kits that include a pharmaceutical formulation containing a polypeptide which comprises a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, such as BHV-2000, in a therapeutically effective amount adapted for use in the methods described herein. The kits optionally also can include instructions, *e.g.*, comprising administration schedules, to allow a practitioner (*e.g.*, a physician, nurse, or patient) to administer the formulation to a patient in need thereof. The kit also can include a syringe.

In one embodiment, a kit for improving glycemic control in a human patient is provided, wherein the kit comprises:

- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and
- (b) instructions for using the polypeptide in the method of any one of the preceding claims.

In another embodiment, a kit for treating, preventing, or reducing obesity and related comorbidities in a human patient is provided, wherein the kit comprises:

- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3

amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and

- (b) instructions for using the polypeptide in the method of any one of the preceding claims.

5 In another embodiment, a kit for treating or preventing type II diabetes in a human patient is provided, wherein the kit comprises:

- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3
10 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and
- (b) instructions for using the polypeptide in the method of any one of the preceding claims.

In one embodiment, the ¹⁰Fn3 domain comprises the amino acid sequence set forth in
15 SEQ ID NO:8. In one embodiment, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO:11. In one embodiment, the polypeptide is for administration as a unit dosage form comprising about 1.0 mL or less of a formulation comprising, (i) about 10-75 mg/mL of the polypeptide; (ii) about 5-25% trehalose dihydrate; (iii) about 20-30 mM histidine; (iv) about 0.02-0.06 mM DTPA; (v) about 0.01-0.05% polysorbate 80; and (vi) a pharmaceutically
20 acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

Also provided are uses of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin for improving glycemic control in a human patient, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated
25 subcutaneous injections).

Further provided are uses of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin for treating, preventing, or reducing obesity and related comorbidities in a human patient, wherein the polypeptide is administered at a dose of 5-200mg

once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

Further provided are uses of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin for treating or preventing type II diabetes in a human patient, wherein the polypeptide is administered at a dose of 5-200mg once every week or once every two weeks. In one embodiment, the polypeptide is administered a subcutaneous injection (*e.g.*, repeated subcutaneous injections).

In one embodiment, the polypeptide comprises a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively. In another embodiment, the ¹⁰Fn3 domain comprises the amino acid sequence set forth in SEQ ID NO:8. In another embodiment, the polypeptide comprises the amino acid sequence set forth SEQ ID NO:11. In another embodiment, the polypeptide is for administration as a unit dosage form comprising about 1.0 mL or less of a formulation comprising (i) about 10-75 mg/mL of the polypeptide; (ii) about 5-25% trehalose dihydrate; (iii) about 20-30 mM histidine; (iv) about 0.02-0.06 mM DTPA; (v) about 0.01-0.05% polysorbate 80; and (vi) a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

The following example is merely illustrative and should not be construed as limiting the scope of this disclosure in any way as many variations and equivalents will become apparent to those skilled in the art upon reading the present disclosure. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1: Clinical Development Program

A clinical development program, including a series of pre-clinical and clinical trials, is
5 conducted to assess the potential of the anti-myostatin Adnectin to treat overweight, obesity, and
related health conditions. This development plan adheres to internationally recognized standards
around the proper conduct of animal and human trials in accordance with Good Clinical Practice
and Good Laboratory Practice standards. Trials are conducted in a manner which looks to satisfy
10 current regulatory guidelines on the development of investigational products intended for use in
weight management.

Clinical trials are designed to assess the effect of the anti-myostatin Adnectin on
individuals living with overweight, obesity, and related health conditions. The trials are generally
conducted in a double-blind, placebo-controlled manner. Trials include primary treatment
periods lasting between 6 and 24-months, during which blinded study medication or matching
15 placebo is administered at regular intervals. Study candidates are screened (by medical history,
physical examination, laboratory and clinical evaluation, etc.) for eligibility to participate in the
treatment phase. Periodically, throughout the trial, participants return to their study sites to
complete pre-specified and/or emergent evaluation of clinical, pharmacologic, quality, and other
measures. Commonly, participants receive counseling on physical activity and healthy diet as
20 part of the clinical trial. Trials are developed, organized, and implemented by trained
professionals with validated credentials in the conduct of human trials and the care for
individuals living with overweight, obesity, and related health conditions.

Key objectives for the program include safety, tolerability, and pharmacokinetic
assessment of the investigational agent across a range of doses (in the general range of 5–200 mg
25 every one to two weeks), dosing intervals, treatment duration, and possibly varied modes of
administration (e.g., subcutaneous or intramuscular injections, etc.). Important efficacy
outcomes utilized within the program include endpoints consistent with the current standard
measures of beneficial effect as defined by sources such as: expert opinion, guidance from key
regulatory bodies and other competent authorities, and current precedence.

Major efficacy endpoints in the treatment of weight-related diseases such as obesity and overweight have included things such as: change in total body weight, changes in body composition (*e.g.*, fat mass, lean mass, etc.), and changes in glycemic control. These outcomes are measured by validated laboratory and imaging techniques (*e.g.*, magnetic resonance imaging [MRI] and dual x-ray absorptiometry [DEXA]). In addition, beneficial effects are measured by the employment of patient-reported outcomes measures (*e.g.*, questionnaires) validated to inform changes in quality of life in the target population.

The intended target population is individuals living with overweight, obesity, and/or related health conditions. The program includes adults ≥ 18 years-of-age, but may also expand to include individuals living with overweight, obesity, and related health conditions younger than 18-years as data and regulations permit.

EXAMPLE 2: Taldefgrobep Alfa Reduces Fat and Increases Muscle in an Obese Mouse Model

Obesity is a disease of excess or abnormal adipose tissue, the key driver of its pathogenic process. Current approved anti-obesity medications (AOMs) achieve reductions in total body weight (TBW) based on a composite loss of fat mass and loss of lean muscle mass. However, the loss of lean muscle mass with AOMs may have adverse long-term health consequences.

The objective of this study was to evaluate the ability of the anti-myostatin adnectin, taldefgrobep alfa (also known as BHV-2000, RO7239361, and BMS-986089[™]), to improve body composition in a high-fat diet (HFD)-induced obese mouse model. In brief, 8-week-old C57BL/6J male mice were assigned into groups based on diet, *i.e.*, either standard (STD) or HFD, and timing of intervention for mice on HFD (*i.e.*, 0 weeks (HFD), 4 weeks (HFD4), or 8 weeks (HFD8) into HFD). **FIG. 1** sets forth the study schematic. Taldefgrobep (100 mg/kg) or vehicle was administered subcutaneously (SC) twice weekly for 8 weeks across all dose groups. Assessments at baseline and posttreatment included (A) body composition with EchoMRI[™] and (B) measurement of metabolic markers, including glucose tolerance test (GTT), insulin, and leptin levels.

As evidenced by **FIG. 2**, consistent differences were observed in fat mass and lean mass change over time between vehicle and taldefgrobep arms. Specifically, after 8 weeks of treatment, all taldefgrobep dose groups demonstrated lower fat mass and greater lean mass relative to vehicle. Change in TBW was generally similar over time between vehicle and taldefgrobep arms (**FIG. 2**). 8-week-old black mice are in late adolescence/early adulthood and not fully grown. Therefore, some increases in TBW, fat mass, and lean mass over time can be attributed to the normal maturation process, as illustrated by TBW and body composition changes seen in Group 1 (vehicle on STD) (**FIG. 2**). At 8 weeks of dosing, HFD8 mice in Group 9 (taldefgrobep) had significantly reduced fat mass and significantly increased lean mass relative to mice in Group 8 (HFD8 on vehicle). Specifically, after 8 weeks of treatment, Group 9 mice achieved an 11% reduction in baseline fat mass and a 25% increase in lean mass, significantly greater than body composition changes in Group 8 mice (+31% and +8%, respectively; $P \leq 0.001$) (see **FIG. 2**, **FIG. 3**, and **FIG. 4**). Insulin and leptin levels were consistently reduced more in taldefgrobep-treated mice than vehicle-treated mice (**FIG. 5**). Glucose tolerance was not improved in the intervention arms relative to vehicle after 7 weeks of dosing (data not shown). Food intake remained generally consistent between taldefgrobep- and vehicle-treated mice over time (data not shown).

In conclusion, in an era of unprecedented reductions in TBW with AOMs, careful consideration of changes in body composition, including implications of loss of lean muscle mass, is increasingly important. In this diet-induced obese mouse model, taldefgrobep monotherapy significantly reduced adipose tissue while increasing lean muscle relative to vehicle. These data support further development of taldefgrobep as a drug candidate with the potential for differentiated benefit in individuals living with overweight and obesity.

25 **EXAMPLE 3: Correlation Between Reduction in Total Body Weight and Change in Waist Circumference**

Obesity is a disease of excess or abnormal adipose tissue, the key driver of its pathogenic process. Current guidance from the U.S. Food & Drug Administration (FDA) and European Medicines Agency recommends changes in baseline TBW as the primary efficacy measure in registrational clinical trials for overweight and obesity. Visceral adipose tissue

volume and anthropometric measures of central obesity (*e.g.*, waist circumference and waist-to-height ratio) have proven to be better predictors of cardiometabolic risk than TBW and body mass index (BMI). In addition, changes in anthropometric measures of central obesity have been shown to correlate with changes in visceral adipose tissue. The objective of this study was to investigate the relationship between changes in TBW and WC in adults receiving AOMs in pivotal trials of the medications most recently approved by the FDA or those under review by the FDA. This analysis included 6 phase 3 clinical trials conducted in adults living with overweight and obesity, comprising 10 dose groups from: (1) Semaglutide STEP 1 trial (NCT03548935) (see Wilding JPH, *et al. N. Engl. J. Med.* 2021;384(11):989-1002), (2) Naltrexone/bupropion COR-I trial (NCT00532779) (Greenway FL, *et al. Lancet.* 2010;376(9741):595-605), (3) Lorcaserin BLOOM trial (NCT00395135) (Smith SR, *et al. N. Engl. J. Med.* 2010;363(3):245-256), (4) Phentermine/topiramate CONQUER trial (NCT00553787) (Gadde KM, *et al. Lancet.* 2011;377(9774):1341-1352), (5) Liraglutide SCALE trial (NCT01272219) (Pi-Sunyer X, *et al. N. Engl. J. Med.* 2015;373(1):11-22), and (6) Tirzepatide SURMOUNT-1 trial (NCT04184622) (Jastreboff AN, *et al. N. Engl. J. Med.* 2022;387(3):205-216)). Placebo results were not included in the analysis. A correlation was estimated with a weighted Pearson correlation coefficient, using weights proportional to the number of subjects in each dose group.

The analysis included a total of 9938 participants from 6 clinical trials with study durations between 52 and 72 weeks (Table 2).

Table 2: Change in TBW and WC Across Adult AOM Trials

Drug	Dose	Sample Size (n)	Study Duration (wk)	Mean Age	Women (%)	TBW Loss (%)	Reduction in WC (cm)
Naltrexone SR/bupropion SR	16mg/360 mg BID	471	56	44.4	85	5.0	5.0
Lorcaserin	10 mg BID	1538	52	43.8	82.9	5.8	6.8
Naltrexone SR/bupropion SR	32 mg/360 mg BID	471	56	44.4	85	6.1	6.2

Phentermine/topiramate	7.5mg/46 mg QD	488	56	51.1	70	7.8	7.6
Liraglutide	3 mg SC QD	2437	56	45.2	78.7	8.0	8.2
Phentermine/topiramate	15 mg/92 mg QD	981	56	51.0	70	9.8	9.2
Semaglutide	2.4 mg SC QW	1306	68	46	73.1	14.9	13.5
Tirzepatide	5 mg SC QW	630	72	45.6	67.6	15.0	14.0
Tirzepatide	10 mg SC QW	636	72	44.7	67.1	19.5	17.7
Tirzepatide	15 mg SC QW	630	72	44.9	67.5	20.9	18.5

*Includes participants with data available for TBW and WC endpoints. BID, twice daily; QD, every day; QW, every week; SC, subcutaneous; SR, sustained release.

Minimum BMI was 27 kg/m² across all studies, per eligibility criteria. Participants were mostly female, comprising 67.1% to 85% of participants. Mean age was between 43.8 and 51.1 years; minimum age across all studies was 18 years, per eligibility criteria. Mean TBW loss ranged from 5.0% to 20.9%; corresponding mean reduction in WC ranged from 5.0 to 18.5 cm. There was a strong linear relationship between changes in TBW and WC (**FIG. 6**). Specifically, a 5% reduction in TBW was associated with an approximately 5 cm reduction in WC ($r=0.997$).

In conclusion, in an era of unprecedented reductions in TBW with AOMs, careful consideration of changes in body composition, including changes in lean muscle mass, is increasingly important. The present analysis suggests that changes in WC strongly correlate with AOM-induced changes in TBW in adults who participated in phase 3 obesity trials. Anthropometric measures of central obesity, such as WC, which are good estimators of visceral adipose tissue volume, may serve as important indicators of therapeutic response to AOMs.

SEQUENCE SUMMARY**SEQ ID NO:1****Human prepromyostatin**

5 MQKLQLCVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACTWRQNTKSSRIEAIKIQILSKL
 RLETAPNISKDVIRQLLPKAPPLRELIDQYDVQRDDSSDGSLEDDDYHATTETIITMPTESDFL
 MQVDGKPKCCFFKFSSKIQYNKVVKQQLWIYLRPVETPTTVFVQILRLIKPMKDGETRYTGIRSL
 KLDMPGTGIWQSIDVKTVLQNLWKQPEENLGLIEIKALDENGHDLAFTFPGPGEDGLNPFLEVK
 VTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQK
 10 YPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS

SEQ ID NO:2**Human pro-myostatin**

NENSEQKENVEKEGLCNACTWRQNTKSSRIEAIKIQILSKLRLETAPNISKDVIRQLLPKAPPL
 15 RELIDQYDVQRDDSSDGSLEDDDYHATTETIITMPTESDFLMQVDGKPKCCFFKFSSKIQYNKV
 VKQQLWIYLRPVETPTTVFVQILRLIKPMKDGETRYTGIRSLKLDMPGTGIWQSIDVKTVLQNL
 LKQPEENLGLIEIKALDENGHDLAFTFPGPGEDGLNPFLEVKVTDTPKRSRRDFGLDCDEHSTES
 RCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPT
 20 KMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS (SEQ ID NO: 2)

SEQ ID NO:3**Mature myostatin**

DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTHLVHQA
 25 NPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS

SEQ ID NO:4**Wild-type human fibronectin type III domain (¹⁰F_n3)**

VSDVPRDLEVVAATPTSL¹⁰ISWDAPAVTVRYRITYGETGGNSPVQEFTVPGSKSTATISGLKP
 30 GVDYTITVYAVTGRGDSPASSKPISINYRT
 (BC, DE, and FG loops are underlined)

SEQ ID NO:5**Anti-myostatin adnectin BC loop**

35 SWSLPHQGGKAN

SEQ ID NO:6**Anti-myostatin adnectin DE loop**

PGRGVT

SEQ ID NO:7**Anti-myostatin adnectin FG loop**

40 TVTDTGYLKYKP

SEQ ID NO:8**Anti-myostatin adnectin core**

5 EVVAATPTSLLSISWSLPHQGKANYRITYGETGGNSPVQEFTVPGRGVTATISGLKPGVDYTIIT
VYAVTVTDTGYLKYKPISINYRT

SEQ ID NO:9**Anti-myostatin adnectin core with N-terminal (AdNT1) (underlined) and C-terminal (AdCT1) (italics) terminal sequence with His6 tag (SEQ ID NO: 38)**

10 MGVS DVPRD LEVVAATPTSLLSISWSLPHQGKANYRITYGETGGNSPVQEFTVPGRGVTATISG
LKP GVDYTIITVYAVTVTDTGYLKYKPISINYRTEIDKPSQH HHHHH

SEQ ID NO:10**Anti-myostatin adnectin core sequence preceded by N-terminal extension**

15 **sequence(GVSDVPRDL (SEQ ID NO: 12)) and followed by a C-terminal tail (EI)**
GVSDVPRD LEVVAATPTSLLSISWSLPHQGKANYRITYGETGGNSPVQEFTVPGRGVTATISGL
KPGVDYTIITVYAVTVTDTGYLKYKPISINYRTEI

SEQ ID NO: 11

20 **Exemplary Leader: "AdNT1"**
MGVS DVPRDL

SEQ ID NO: 12

25 **Exemplary Leader: "AdNT2"**
GVSDVPRDL

SEQ ID NO: 13

30 **Exemplary Leader: "AdNT3"**
VSDVPRDL

SEQ ID NO: 14

35 **Exemplary Leader: "AdNT4"**
SDVPRDL

SEQ ID NO: 15

Exemplary Leader: "AdNT5"
DVPRDL

SEQ ID NO: 16

40 **Exemplary Leader: "AdNT6"**
VPRDL

SEQ ID NO: 17
Exemplary Leader: “AdNT7”
PRDL

5 **Exemplary Leader: “AdNT8”**
RDL

10 **Exemplary Leader: “AdNT9”**
DL

SEQ ID NO: 20
Exemplary Tail: “AdCT1”
15 EIDKPSQ

Exemplary Tail: “AdCT2”
EI
20

SEQ ID NO: 22
Exemplary Tail: “AdCT3”
EIEPKSS

25 **SEQ ID NO: 23**
Exemplary Tail: “AdCT4”
EIDKPC

SEQ ID NO: 24
30 **Exemplary Tail: “AdCT5”**
EIDKP

SEQ ID NO: 25
Exemplary Tail: “AdCT6”
35 EIDK

SEQ ID NO: 26
Exemplary Tail: “AdCT7”
EIDKPS

40 **SEQ ID NO: 27**
Exemplary Tail: “AdCT8”
EIEKPSQ

- 5 **SEQ ID NO: 28**
 Exemplary Tail: “AdCT9”
 EIDKPSQLE
- 10 **SEQ ID NO: 29**
 Exemplary Tail: “AdCT10”
 _EIEDEDEDEDED
- 15 **SEQ ID NO: 30**
 Exemplary Tail: “AdCT11”
 EGSGS
- 20 **SEQ ID NO: 31**
 Exemplary Tail: “AdCT12”
 EIDKPCQ
- 25 **SEQ ID NO: 32**
 Exemplary Tail: “AdCT13”
 GSGC
- 30 **SEQ ID NO: 33**
 Exemplary Tail: “AdCT14”
 EGSGC
- 35 **SEQ ID NO: 34**
 Exemplary Tail: “AdCT15”
 EIDKPCQLE
- 40 **SEQ ID NO: 35**
 Exemplary Tail: “AdCT16”
 EIDKPSQHMMMMH
- SEQ ID NO: 36**
 Exemplary Tail: “AdCT17”
 GSGCHMMMMH
- SEQ ID NO: 37**
 Exemplary Tail: “AdCT18”
 EGSGCHMMMMH
- SEQ ID NO: 38**
 Tag: “T1”

HHHHHH

SEQ ID NO: 39

Human IgG1 Immunoglobulin Fc Domain

5 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

10 **SEQ ID NO: 40**

Linker

GAGGGGSG

SEQ ID NO: 41

15 **Linker**

EPKSSD

SEQ ID NO: 42

Linker

20 ESPKAQASSVPTAQPQAEGLA

SEQ ID NO: 43

Linker

ELQLEESAAEAQDGELD
25

SEQ ID NO: 44

Linker

GQPDEPGGS

30 **SEQ ID NO: 45**

Linker

GGSGSGSGSGSGS

SEQ ID NO: 46

35 **Linker**

ELQLEESAAEAQEGELE

SEQ ID NO: 47

Linker

40 GSGSG

SEQ ID NO: 48

Linker

GSGC

SEQ ID NO: 49

Linker

5 AGGGGSG

SEQ ID NO: 50

Linker

GSGS

10

SEQ ID NO: 51

Linker

QPDPEGGS

15

SEQ ID NO: 52

Linker

GSGSGS

SEQ ID NO: 53

Linker

TVAAPS

20

SEQ ID NO: 54

Linker

KAGGGGSG

25

SEQ ID NO: 55

Linker

KGSGSGSGSGSGS

30

SEQ ID NO: 56

Linker

KQPDPEGGS

35

SEQ ID NO: 57

Linker

KELQLEESAAEAQDGELD

SEQ ID NO: 58

Linker

KTVAAPS

40

SEQ ID NO: 59

Linker

KAGGGGSGG

SEQ ID NO: 605 **Linker**

KSGSGSGSGSGSGSG

SEQ ID NO: 61**Linker**

10 KQPDEPGGSG

SEQ ID NO: 62**Linker**

KELQLEESAAEAQDGELDG

15

SEQ ID NO: 63**Linker**

KTVAAPSG

20 **SEQ ID NO: 64****Linker**

AGGGGSGG

SEQ ID NO: 6525 **Linker**

AGGGGSG

SEQ ID NO: 66**Linker**

30 GSGSGSGSGSGSGSG

SEQ ID NO: 67**Linker**

QPDEPGGSG

35

SEQ ID NO: 68**Linker**

TVAAPSG

40 **SEQ ID NO: 69****Hinge**

DKTHTCPPCPAPELLG

SEQ ID NO: 70**Hinge (core hinge region underlined)**

GSTHTCPCPCPAPELLG

5 **SEQ ID NO: 71****Hinge (core hinge region underlined)**

EPKSSDKTHTCPCPCPAPELLGGPS

10 **SEQ ID NO: 72****Hinge (core hinge region underlined)**

EPKSSDKTHTCPCPCPAPELLGGSS

15 **SEQ ID NO: 73****Hinge (core hinge region underlined)**

EPKSSGSTHTCPCPCPAPELLGGSS

20 **SEQ ID NO: 74****Hinge (core hinge region underlined)**

DKTHTCPCPCPAPELLGGPS

25 **SEQ ID NO: 75****Hinge (core hinge region underlined)**

DKTHTCPCPCPAPELLGGSS

30 **SEQ ID NO: 76**

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

35 **SEQ ID NO: 77****Leader**

METDTLLLWVLLLWVPGSTG

40 **SEQ ID NO: 78****Anti-myostatin adnectin Fc-Fusion**

DKTHTCPCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPQLLEESAAEAQEGELEGVSDVPRDLEVVA

TPTSLLISWSLPHQGKANYRITYGETGGNSPVQEFTVPGRGVTATISGLKPGVDYITITVYAVT
VTDGTGYLKYPISINYRTEI

SEQ ID NO:79**5 Anti-myostatin adnectin Fc-Fusion**

GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYRITYGETGGNSPVQEFTVPGRGVTATISGLKPGVDYITITVYAVT
VTDGTGYLKYPISINYRTEIEPKSSDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
10 SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:80

VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
15 LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSP

SEQ ID NO:81**Exemplary N-terminal leader Sequence**

20 METDTLLLWVLLLWVPGSTG

SEQ ID NO:82

TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGT
25 CAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC
AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTG
CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
AACAAC TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGC
30 TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGC
TCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCCGAGCTGCAGCTGGAGGAAAGC
GCCGCTGAGGCTCAGGAAGGAGAACTGGAAGGCGTGAGCGACGTGCCACGGGATCTAGAAGTGG
TGGCTGCTACCCCCACAAGCTTGCTGATCAGCTGGTCTCTGCCGCACCAAGGTAAAGCCAATTA
TTACCGCATCACTTACGGCGAAACAGGAGGCAATAGCCCTGTCCAGGAGTTCACTGTGCCTGGT
35 CGTGGTGTACAGCTACCATCAGCGGCCTTAAACCTGGCGTTGATTATACCATCACTGTGTATG
CTGTCACTGTACTGATACAGGGTACCTCAAGTACAAACCAATTTCCATTAATTACCGGACCGA
AATT

SEQ ID NO:83

40 GGCGTGAGCGACGTGCCCCGGGATCTAGAAGTGGTGGCTGCTACCCCCACAAGCTTGCTGATCA
GCTGGTCTCTGCCGCACCAAGGTAAAGCCAATTATTACCGCATCACTTACGGCGAAACAGGAGG
CAATAGCCCTGTCCAGGAGTTCACTGTGCCTGGTCTGGTGTACAGCTACCATCAGCGGCCTT

AAACCTGGCGTTGATTATACCATCACTGTGTATGCTGTCACTGTTACTGATACAGGGTACCTCA
AGTACAAACCAATTTCCATTAATTACCGGACCGAAATTGAGCCTAAGAGCTCCGACAAAACCCA
CACATGCCCACCTTGTCAGCCCCCGAACTGCTGGGCGGCCCTTCAGTCTTCCTCTTCCCCCA
5 AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA
GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA
GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG
CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC
10 CCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGC
CTCCCGTGTTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCCGGGAAA

CLAIMS

1. A method of improving glycemic control in a human patient, comprising administering to
5 the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin.
2. A method of treating, preventing, or reducing overweight or obesity and related
comorbidities in a human patient, comprising administering to the patient a polypeptide
10 comprising a fibronectin type III tenth (¹⁰F_n3) domain which binds to myostatin.
3. A method of treating or preventing type II diabetes in a human patient, comprising
administering to the patient a polypeptide comprising a fibronectin type III tenth (¹⁰F_n3) domain
which binds to myostatin.
- 15 4. The method of any one of the preceding claims, wherein the patient has a BMI $\geq$ 30
kg/m².
5. The method of any one of claim 1-3, wherein the patient has a BMI $\geq$ 25 and $<$ 30
20 kg/m².
6. The method of any one of claim 1-3, wherein the patient has a BMI $<$ 25.
7. The method of any one of the preceding claims, wherein the polypeptide is administered
25 as a formulation comprising
 - (i) at least 10 mg/mL of the polypeptide;
 - (ii) a disaccharide at a concentration of at least 5%;
 - (iii) a histidine buffer at a concentration of between about 20 to about 60 mM; and
 - (iv) a pharmaceutically acceptable aqueous carrier,
30 wherein the formulation has a pH range of about 6.5 to about 7.8.

8. The method of claim 7, wherein the polypeptide concentration in the formulation is between about 10 mg/mL and 200 mg/mL, between about 10 mg/mL and 150 mg/mL, or between about 10 mg/mL and 85 mg/mL.
- 5
9. The method of claim 7 or 8, wherein the disaccharide is present at weight (w/w) ratio of at least 5:1 protein to sugar.
10. The method of any one of claims 7-9, wherein the formulation comprises about 5% to about 30% of the disaccharide.
- 10
11. The method of any one of claims 7-10, wherein the concentration of the disaccharide is about 150 mM to about 800 mM, or about 300 to about 700 mM.
12. The method of any one of claims 7-11, wherein the disaccharide is trehalose, and the formulation comprises about 5 to about 30% trehalose, about 15% to about 25% trehalose, or about 20% to about 25% trehalose.
- 15
13. The method of any one of claims 7-12, wherein the disaccharide is trehalose dehydrate, and the concentration of trehalose dihydrate in the formulation is about 150 mM to about 800 mM, about 300 to about 700 mM, about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM or about 700 mM.
- 20
14. The method of any one of claims 7-13, wherein the histidine is present at a concentration of at least 20 mM.
- 25
15. The method of any one of claims 7-14, wherein the viscosity of the formulation is from about 5 to 20 cps, from about 5 to 15 cps, or from about 7 to 12 cps.

16. The method of any one of claims 7-15, wherein the pH is about 6.6 to 7.6, about 6.8 to 7.4, or about 7.0 to 7.3.
- 5 17. The method of any one of claims 7-16, wherein the formulation comprises a surfactant at a concentration of between about 0.01% and 0.5%.
18. The method of any one of claims 7-17, wherein the formulation comprises a chelator, wherein the concentration of the chelator is between about 0.01 mM and about 0.5 mM or
10 between about 0.05 mM and 0.2 mM, and wherein the chelator is selected from the group consisting of DPTA, EDTA and EGTA.
19. The method of any one of claims 7-18, wherein the formulation comprises:
- (a) about 10-140 mg/mL of the polypeptide;
15 about 5-25% trehalose dihydrate;
about 20-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;
- (b) about 10-140 mg/mL of the polypeptide;
20 about 5-25% trehalose dihydrate;
about 20-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
25 wherein the pH of the formulation is about 6.8 to 7.3;
- (c) about 10-140 mg/mL of the polypeptide;
about 600 mM trehalose dihydrate;
25-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,

wherein the pH of the formulation is about 7.0 to 7.3;

- (d) about 10-140 mg/mL of the polypeptide;
about 600 mM trehalose dihydrate;
25-30 mM histidine;
5 about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,

wherein the pH of the formulation is about 7.0 to 7.3;

- (e) about 10-75 mg/mL of the polypeptide;
10 about 5-25% trehalose dihydrate;
about 20-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,

wherein the pH of the formulation is about 6.8 to 7.3;

- (f) about 10-75 mg/mL of the polypeptide;
15 about 5-25% trehalose dihydrate;
about 20-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,

- 20 wherein the pH of the formulation is about 6.8 to 7.3; or

- (g) about 10-75 mg/mL of the polypeptide;
about 600 mM trehalose dihydrate;
about 30 mM histidine;
about 0.05 mM DTPA;

- 25 about 0.02% polysorbate 80;
a pharmaceutically acceptable aqueous carrier,

wherein the pH of the formulation is about 7.1.

20. The method of any one of claims 7-10, wherein the polypeptide is administered as a unit dosage form comprising about 1.0 mL or less of a formulation comprising,
- (i) about 10-75 mg/mL of the polypeptide;
 - (ii) about 5-25% trehalose dihydrate;
 - 5 (iii) about 20-30 mM histidine;
 - (iv) about 0.02-0.06 mM DTPA;
 - (v) about 0.01-0.05% polysorbate 80; and
 - (vi) a pharmaceutically acceptable aqueous carrier,
- wherein the pH of the formulation is about 6.8 to 7.3.
- 10
21. The method of any one of the preceding claims, wherein the ¹⁰F_n3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively.
- 15
22. The method of any one of the preceding claims, wherein the ¹⁰F_n3 domain comprises the amino acid sequence set forth in SEQ ID NO: 8.
23. The method of any of the preceding claims, wherein the polypeptide comprises the amino acid sequence set forth SEQ ID NO: 11.
- 20
24. The method of any of the preceding claims, wherein the polypeptide comprises the amino acid sequence set forth SEQ ID NO: 78.
- 25
25. The method of any of the preceding claims, wherein the polypeptide is administered as a repeated subcutaneous injection.
26. The method of any of the preceding claims, wherein the polypeptide is administered at a dosing interval of once a week.

27. The method of any one of claims 1-25, wherein the polypeptide is administered at a dosing interval of once every two weeks.
- 5 28. The method of any one of claims 1-25, wherein the polypeptide is administered at a dosing interval of once a month.
29. The method of any of the preceding claims, wherein the polypeptide is administered at a dose of approximately 5 - 200mg.
- 10 30. The method of any one of the preceding claims, wherein central adiposity is reduced.
31. The method of any one of claims 2 and 4-30, wherein the overweight or obesity related comorbidity is selected from the group consisting of type 2 diabetes, glucose intolerance, prediabetes, insulin resistance, hypertension, dyslipidemia, increased waist circumference, 15 cardiovascular disease, non-alcoholic fatty liver disease, obstructive sleep apnea, physical impairment, osteoarthritis, osteoporosis, renal disease, sexual hormone(s) impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, stroke, and gallstones.
- 20 32. The method of any one of claims 1 and 4-31, wherein an improvement in glycemic control is achieved by improving insulin sensitivity.
33. The method of any one of claims 1-2 and 4-32, wherein the patient has type II diabetes.
- 25 34. The method of any one of claims 3-33, wherein the treatment results in an improvement in a Diabetes Treatment Satisfaction Questionnaire (DTSQ) score or Impact of Weight on Quality of Life (IWQOL) score.

35. The method of any one of the preceding claims, wherein the treatment results in a decrease in total body fat mass (FM) of at least 5 kg by week 48 compared to baseline.
36. The method of any one of the preceding claims, wherein the treatment results in an at
5 least 5% decrease in total body fat mass (FM) by week 48 compared to baseline.
37. The method of any one of the preceding claims, wherein the treatment results in an at least 5% decrease in body weight by week 48 compared to baseline.
- 10 38. The method of any one of the preceding claims, wherein the treatment results in an at least 2% increase in body lean mass (LM) by week 48 compared to baseline.
39. The method of claim 35 or 36, wherein FM is assessed by dual-energy x-ray absorptiometry (DXA).
15
40. The method of claim 38, wherein LM is assessed by dual-energy x-ray absorptiometry (DXA).
41. The method of any one of the preceding claims, wherein the treatment results in a
20 decrease in waist circumference (WC) and/or waist-to-hip ratio by week 48 compared to baseline.
42. The method of any one of the preceding claims, wherein the treatment results in a decrease in subcutaneous and abdominal visceral adipose tissue by week 48 compared to
25 baseline.
43. The method of any one of the preceding claims, wherein the treatment results in an improvement in diabetes status by week 48 compared to baseline.

44. The method of claim 43, wherein the improvement in diabetes status is assessed by HbA1c, Homeostatic Model Assessment (HOMA), quantitative insulin-sensitivity check index (QUICKI), and/or Matsuda Index.
- 5 45. The method of any one of the preceding claims, wherein the treatment results in a shift toward normal levels of one or more biomarkers selected from the group consisting of serum lipids, high-sensitivity C-reactive protein (hs-CRP), interleukin 6, leptin, and adiponectin, by week 48 compared to baseline.
- 10 46. The method of any one of the preceding claims, wherein the treatment results in an improvement in hand grip strength by week 48 compared to baseline, as assessed by dynamometry.
47. A kit for improving glycemic control in a human patient, wherein the kit comprises:
- 15 (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and
- 20 (b) instructions for using the polypeptide in the method of any one of the preceding claims.
48. A kit for treating, preventing, or reducing obesity and related comorbidities in a human patient, wherein the kit comprises:
- 25 (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth in SEQ ID NOs: 5, 6 and 7, respectively, and

- (b) instructions for using the polypeptide in the method of any one of the preceding claims.
49. A kit for treating or preventing type II diabetes in a human patient, wherein the kit
5 comprises:
- (a) a dose of a polypeptide comprising a fibronectin type III tenth (¹⁰Fn3) domain which binds to myostatin, wherein the ¹⁰Fn3 domain comprises BC, DE, and FG loops, and wherein at least one loop of the BC, DE, and FG loops has 0, 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops set forth
10 in SEQ ID NOs: 5, 6 and 7, respectively, and
- (b) instructions for using the polypeptide in the method of any one of the preceding claims.
50. The kit of any one of claims 47-49, wherein the ¹⁰Fn3 domain comprises the amino acid
15 sequence set forth in SEQ ID NO:8.
51. The kit of any one of claims 47-49, wherein the polypeptide comprises the amino acid sequence set forth SEQ ID NO:11.
- 20 52. The kit of any one of claims 47-49, wherein the polypeptide comprises the amino acid sequence set forth SEQ ID NO: 78.
53. The kit of any one of claims 47-49, wherein the polypeptide is for administration as a unit dosage form comprising about 1.0 mL or less of a formulation comprising,
25 (i) about 10-75 mg/mL of the polypeptide;
(ii) about 5-25% trehalose dihydrate;
(iii) about 20-30 mM histidine;
(iv) about 0.02-0.06 mM DTPA;
(v) about 0.01-0.05% polysorbate 80; and

(vi) a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3.