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(54) **Title**: ANTI-FLT-1 ANTIBODIES FOR TREATING DUCHENNE MUSCULAR DYSTROPHY

(57) **Abstract**: The present invention provides, among other things, anti-Flt-1 antibodies and methods for treating muscular dystrophy, in particular, Duchenne muscular dystrophy (DMD). In some embodiments, a method according to the present invention includes administering to an individual who is suffering from or susceptible to DMD an effective amount of an anti-Flt-1 antibody or antigen-binding protein thereof such that at least one symptom or feature of DMD is reduced in intensity, severity, or frequency, or has delayed onset.

ANTI-FLT-1 ANTIBODIES FOR TREATING DUCHENNE MUSCULAR DYSTROPHY

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/144,251, filed April 7, 2015 and U.S. Provisional Application Serial No. 62/307,645, filed March 14, 2016, the disclosure of each of which is hereby incorporated by reference.

BACKGROUND

[0002] Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder affecting an estimated 1:3600 male births with an estimated 50,000 affected individuals worldwide. The disorder is marked by a progressive wasting of the muscles and affected children are wheelchair dependent by the time they reach 13 years of age. Affected individuals usually present with symptoms at 3 years of age with the median survival for such individuals being between 25 and 30 years of age. Respiratory failure due to diaphragmatic weakness and cardiomyopathy are common causes of death.

[0003] DMD is caused by a mutation in the dystrophin gene. The dystrophin gene is located on the X chromosome and codes for the protein dystrophin. Dystrophin protein is responsible for connecting the contractile machinery (actin-myosin complex) of a muscle fiber to the surrounding extracellular matrix through the dystroglycan complex. Mutations in the dystrophin gene result in either alteration or absence of the dystrophin protein and abnormal sarcolemal membrane function. While both males and females can carry a mutation in the dystrophin gene, females are rarely affected with DMD.

[0004] One characteristic of DMD is ischemia of the affected tissues. Ischemia is a restriction or decrease in blood supply to tissues or organs, causing a shortage of oxygen and nutrients need for cellular metabolism. Ischemia is generally caused by constriction or obstruction of blood vessels resulting in damage to or dysfunction of the tissue or organ.

Treatment of ischemia is directed toward increasing the blood flow to the affected tissue or organ.

[0005] Presently, there is no cure for DMD. Several therapeutic avenues have been investigated including gene therapy and corticosteroid administration, however the need for alternatives for DMD patients still exists.

SUMMARY OF INVENTION

[0006] The present invention provides, among other things, improved methods and compositions for treating muscular dystrophy, in particular, Duchenne muscular dystrophy (DMD) and/or Becker muscular dystrophy based on anti-Flt-1 antibody therapy. As described in the Examples below, the invention is, in part, based on the discovery that anti-Flt-1 antibodies, or antigen-binding fragments thereof, can inhibit VEGF and other ligands from binding to the Flt-1 receptor, thereby increasing the amount of VEGF and/or other ligands available to bind to VEGF receptors. Increased availability of VEGF promotes angiogenesis with increased blood flow to muscle to combat functional ischemia and leading to improvements in structural and functional characteristics of DMD. Indeed, as shown in the present Examples, the present inventors have demonstrated that administration of the anti-Flt-1 antibody improves measures of muscle pathology (e.g., improved angiogenesis, reduced fibrosis, reduced necrosis). Therefore, the present invention provides safe and effective antibody-based therapeutics for the treatment of DMD.

[0007] In one aspect, the invention provides antibodies or antigen-binding fragments thereof that specifically bind to human Flt-1 comprising one or more complementarity determining regions (CDR) selected from the group consisting of: a variable light (VL) chain CDR1 defined by an amino acid sequence having at least 80% identity to any one of SEQ ID NO:19 to SEQ ID NO:21, a VL CDR2 defined by an amino acid sequence having at least 80% identity to any one of SEQ ID NO:22 to SEQ ID NO:24; a VL CDR3 defined by an amino acid sequence having at least 80% identity to any one of SEQ ID NO:25 to SEQ ID NO:34; a variable heavy (VH) chain CDR1 defined by an amino acid sequence having at least 80% identity to any one of SEQ ID NO:1 to SEQ ID NO:4, a VH CDR2 defined by an amino acid sequence having at least 80% identity to any one of SEQ ID NO:5 to SEQ ID NO:14, and a VH CDR3 defined by an amino acid sequence having at least 80% identity to any one of SEQ ID NO:15 to SEQ ID NO:18.

[0008] In some embodiments, the one or more CDRs comprise the VL CDR3 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:25 to SEQ ID NO:34; and the VH CDR3 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:15 to SEQ ID NO:18.

[0009] In another embodiment, the one or more CDRs comprise the VL CDR1 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:19 to SEQ ID NO:21, the VL CDR2 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:22 to SEQ ID NO:24, and the VL CDR3 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:25 to SEQ ID NO:34. In a particular embodiment, VL chain comprises the VL CDR1, VL CDR2, and VL CDR3 defined by the amino acid sequence of SEQ ID NO:19, SEQ ID NO:22, and SEQ ID NO:25, respectively. In yet another embodiment, the VL chain comprises the VL CDR1, VL CDR2, and VL CDR3 defined by the amino acid sequence of SEQ ID NO:20, SEQ ID NO:23, and SEQ ID NO:25, respectively. In another embodiment, the VL chain comprises the VL CDR1 and VL CDR2 defined by the amino acid sequence of SEQ ID NO:21 and SEQ ID NO:24, respectively, and the VL CDR3 defined by the amino acid sequence of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, or SEQ ID NO:34. In a particular embodiment, the VL chain comprises the VL CDR1, VL CDR2, and VL CDR3 defined by the amino acid sequence of SEQ ID NO:21, SEQ ID NO:24, and SEQ ID NO:32, respectively.

[0010] In other embodiments, the one or more CDRs comprise the VH CDR1 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:1 to SEQ ID NO:4, the VH CDR2 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:5 to SEQ ID NO:14, and the VH CDR3 defined by the amino acid sequence having at least 80% identity to any one of SEQ ID NO:15 to SEQ ID NO:18. In a particular embodiment, the VH chain comprises the VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:1, SEQ ID NO:5, and SEQ ID NO:15, respectively. In another embodiment the VH chain comprises the VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:2, SEQ ID NO:6, and SEQ ID NO:16, respectively. In yet another embodiment, the VH chain comprises the VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:2, SEQ ID NO:10, and SEQ ID NO:18, respectively. In another embodiment, the VH chain comprises the VH CDR1 and the VH CDR3 defined by the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:17, respectively, and the VH CDR2 defined by the amino acid sequence of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NO:14. In another embodiment, the VH chain comprises the VH CDR1 and the VH CDR3 defined by the amino acid sequences of SEQ ID NO:3 and SEQ ID NO:17, respectively, and the VH CDR2 defined by the amino acid sequence of SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:12. In yet another embodiment, the VH chain comprises the VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:4, SEQ ID NO:9, and SEQ ID NO:17, respectively. In a particular embodiment, the VH chain comprising the VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:3, SEQ ID NO:12, and SEQ ID NO:17, respectively.

[0011] In another aspect, the invention provides antibodies or antigen-binding fragments thereof that specifically bind to human Flt-1, comprising: (i) a light chain variable (VL) region comprising an amino acid sequence having at least 80% identity to any one of SEQ ID NO:49 to SEQ ID NO:61, and/or (ii) a heavy chain variable (VH) region comprising an amino acid sequence having at least 80% identity to any one of SEQ ID NO:35 to SEQ ID NO:48. In a particular embodiment the VL region comprises the amino acid sequence of SEQ ID NO:60 and the VH region comprises the amino acid sequence of SEQ ID NO:45.

[0012] In some embodiments the antibody further comprises a heavy chain constant region comprising an amino acid sequence having at least 80% identity to any one of SEQ ID NO:87 to SEQ ID NO:89.

[0013] In another aspect,the invention provides antibodies or antigen-binding fragments thereof that specifically binds to human Flt-1, comprising: (i) a light chain comprising an amino acid sequence having at least 80% identity to any one of SEQ ID NO:75 to SEQ ID NO:86, and/or (ii) a heavy chain comprising an amino acid sequence having at least 80% identity to any one of SEQ ID NO:62 to SEQ ID NO:74. In a particular embodiment, the light chain comprises the amino acid sequence of SEQ ID NO:76 and the heavy chain comprises the amino acid sequence of SEQ ID NO:71.

[0014] In another embodiment, the antibody or antigen-binding fragment thereof is selected from the group consisting of IgG, $F(ab')_2$, $F(ab)_2$, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies. In one embodiment the antibody or antigen-binding fragment thereof is IgG. In another embodiment the antibody or antigen-binding fragment thereof is IgGl. In yet another embodiment, the antibody or antigen-binding fragment thereof is a monoclonal antibody. In a particular embodiment, the antibody is a humanized monoclonal antibody. In yet another embodiment, the humanized monoclonal antibody contains a human Fc region. In some embodiments, Fc region contains one or more mutations that enhance the binding affinity between the Fc region and the FcRn receptor such that the in vivo half-life of the antibody is prolonged. In another embodiment, the Fc region contains one or more mutations at positions corresponding to Leu 234, Leu 235 and/or Gly 237 of human IgGl.

[0015] In one embodiment, the antibody or antigen-binding fragment thereof does not bind to VEGF R2 and/or VEGF R3. In another embodiment, the antibody or antigen-binding fragment thereof does not bind to a mouse or monkey Flt- 1.

[0016] In another aspect, the invention provides an isolated antibody or antigen-binding fragment thereof that recognizes a peptide comprising an amino acid sequence corresponding to positions 139 to 148, positions 139 to 153, positions 178 to 206, positions 199 to 204 and positions 128 to 138 of SEQ ID NO:90, or a fragment thereof. In one embodiment the peptide consists of the amino acid sequence corresponding to positions 130 to 138, positions 141 to 148, positions 141 to 153 and positions 193 to 206 of SEQ ID NO:90.

[0017] In another aspect, the invention provides an isolated antibody or antigen-binding fragment thereof that competes with any anti-Flt-1 antibody or antigen-binding fragment thereof.

[0018] In another aspect, the invention provides pharmaceutical compositions comprising anti-Flt-1 antibodies or antigen-binding fragments thereof and a pharmaceutically acceptable carrier.

[0019] In yet another aspect,the invention provides polynucleotides encoding a CDR, a VL region, a VH region, a light chain, and/or a heavy chain of the antibody or antigen-binding fragment thereof of of the invention. In one embodiment, the invention provides expression vectors comprising the polynucleoties. In yet another embodiment, the invention provides a host cell comprising the polynucleotide or the expression vector. In a particular embodiment, the invention provides methods of making an antibody or antigen-binding fragment thereof that specifically binds to human Flt-1 comprising culturing the host cell. In another embodiment, a hybridoma cell produces the antibody or antigen-binding fragment thereof.

[0020] In another aspect, the invention provides methods for treating a Flt-1-mediated disease, disorder or condition comprising administering to a subject in need of treatment an anti-Flt-1 antibody or antigen-binding fragment thereof. In a particular embodiment, the Flt-1-mediated disease, disorder or condition is Duchenne muscular dystrophy, Becker muscular dystrophy, bronchopulmonary dysplasia, preeclampsia or chronic kidney disease.

[0021] In another aspect, the method provides methods of treating Duchenne Muscular Dystrophy (DMD), the method comprising administering to a subject who is suffering from or susceptible to DMD an effective amount of an anti-Flt-1 antibody or antigen-binding fragment thereof, such that at least one symptom or feature of DMD is reduced in intensity, severity, or frequency, or has delayed onset. In one embodiment the method further comprises administering to the subject one or more additional therapeutic agents. In a particular embodiment the additional therapeutic agents are selected from the group consisting of prednisone, deflazacort, follistatin, RNA modulating therapeutics, exon-skipping therapeutics and gene therapy.

[0022] In one embodiment, the antibody or antigen-binding fragment thereof is administered parenterally. In some embodiments, the parenteral administration is selected from intravenous, intradermal, intrathecal, inhalation, transdermal (topical), intraocular, intramuscular, subcutaneous, and/or transmucosal administration. In a particular embodiment, the parenteral administration is intravenous administration. In yet another embodiment, the parenteral administration is subcutaneous administration.

[0023] In some embodiments, the antibody or antigen-binding fragment thereof is administered daily, twice weekly, weekly or monthly. In a particular embodiment antibody or antigen-binding fragment thereof is administered twice weekly.

[0024] In another embodiment, the effective amount of the antibody or antigen-binding fragment thereof is a dose amount of approximately 1 mg/kg to 50 mg/kg. In a particular embodiment the dose amount is approximately 1 mg/kg, 3 mg/kg or 10 mg/kg.

[0025] In one embodiment, the administration of the antibody or antigen-binding fragment thereof results in reduced fibrosis and/or necrosis relative to a control. In another embodiment, the administration of the antibody or antigen-binding fragment thereof results in improved angiogenesis in muscle of the subject relative to a control. In another embodiment the improved angiogenesis is reflected by increased blood flow on muscle pathology, increased VEGF levels in serum, decreased creatine kinase (CK) levels in serum, increased CD31 score by IHC, and/or reduced sFlt-1 levels in serum. In yet another embodiment, the administration of the antibody or antigen-binding fragment thereof results in improved muscle function relative to a control. In yet another embodiment, the improved muscle function is reflected by improved muscle force and/or resistance to fatigue.

[0026] In another aspect, the invention provides for a method of treating tissue fibrosis comprising administering to a subject in need of treatment an effective amount of an anti-Flt-1 antibody or antigen-binding fragment thereof.

BRIEF DESCRIPTION OF FIGURES

[0027] The present teachings described herein will be more fully understood from the following description of various illustrative embodiments, when read together with the accompanying drawings. It should be understood that the drawings described below are for illustration purposes only and are not intended to limit the scope of the present teachings in any way.

[0028] **Figure 1A** shows exemplary results depicting clearance of anti-Flt-1 antibody 13B4 after mice were administered the antibody at a dose of 10 mg/kg intravenously.

[0029] **Figure 1B** shows exemplary results depicting clearance of anti-Flt-1 antibody 10G12 after mice were administered the antibody at a dose of 10 mg/kg intravenously.

[0030] **Figure 2A** shows exemplary results depicting peak serum anti-Flt-1 antibody levels. **Figure 2B** shows exemplary results depicting trough serum anti-Flt-1 antibody levels.

[0031] **Figure 3** shows exemplary results depicting a decrease in free soluble Flt-1 (sFlt-1) levels in serum following administration of an anti-Flt-1 antibody (13B4 or 10G12), isotype control antibody, commercial antibody (Angio) or vehicle alone to *mdx* mice.

[0032] **Figure 4** shows exemplary results depicting an increase in serum VEGF levels following administration of an anti-Flt-1 antibody (13B4 or 10G12), isotype control antibody, commercial antibody (Angio) or vehicle alone to *mdx* mice.

[0033] **Figures 5A-5D** show exemplary results of CD31 staining of tissue sections obtained from the diaphragm muscle of *mdx* mice administered an anti-Flt-1 antibody (13B4 or 10G12), isotype control antibody or commercial antibody (Angio).

[0034] **Figure 6A** shows exemplary results of quantification of the CD31 positive area as a percentage of the total stained area in tissue sections obtained from diaphragm muscle. **Figure 6B** shows exemplary results of quantification of the CD31 positive area as a percentage of the total stained area in tissue sections obtained from the tibialis anterior (TA) muscle.

[0035] **Figure 6B** shows exemplary results of quantification of the CD31 positive area as a percentage of the total stained area in tissue sections obtained from the tibialis anterior (TA) muscle of *mdx* mice administered an anti-Flt-1 antibody.

[0036] **Figure** 7 shows exemplary results depicting binding of anti-Flt-1 antibodies to recombinant sFlt-1 by an ELISA assay.

[0037] **Figure 8** shows exemplary results depicting inhibition of binding of sFlt-1 to VEGF by anti-Flt-1 antibodies in a competition ELISA assay.

[0038] **Figure 9** shows exemplary results depicting rescue of phosphorylation of VEGF R2 by anti-Flt-1 antibodies. Human primary vein endothelial cells (HUVECs) were treated with VEGF in the presence of sFlt-1 and anti-Flt-1 antibodies and the level of VEGF R2 phosphorylation was determined. Percent rescue indicates the level of phosphorylation of VEGF R2 relative to the level of phosphorylation of VEGF R2 when HUVECs were treated with VEGF and sFlt-1 alone (i.e., no anti-Flt-1 antibody).

[0039] **Figure 10** shows exemplary results depicting inhibition of binding of soluble Flt-1 to VEGF by anti-Flt-1 antibodies in a competition ELISA assay.

[0040] **Figure 11** shows exemplary results depicting clearance of anti-Flt-1 antibodies from the serum over 672 hours after mice were administered the anti-Flt-1 antibody at a dose of 10 mg/kg intravenously.

[0041] **Figures 12A-12C** show exemplary results of CD31 staining of tissue sections from diaphragm muscle. **Figures 12D-12F** show exemplary results of CD31 staining of tissue sections obtained from tibialis anterior muscle.

[0042] **Figures 13A-13C** show exemplary biodistribution of anti-Flt-1 antibodies 27H9, 13B4 and 21B3 in the diaphragm, tibialis and gastrocnemius muscles of mice over a 256 hour time course following administration of the antibody.

[0043] **Figure 14A** shows exemplary results depicting peak anti-Flt-1 antibody 21B3 levels at peak exposure. **Figure 14B** shows exemplary results depicting trough anti-Flt-1 antibody 21B3 levels.

[0044] **Figure 15** shows exemplary results depicting free sFlt-1 following administration of anti-Flt-1 antibody 21B3 to *mdx* mice.

[0045] **Figure 16** shows exemplary results depicting VEGF levels following administration of anti-Flt-1 antibody 21B3 to *mdx* mice.

[0046] **Figure 17A-17E** shows exemplary results of CD31 staining of tissue sections obtained from the diaphragm muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or isotype control antibody.

[0047] **Figure 18** shows exemplary results of quantification of the normalized CD31 positivity percentage in tissue sections obtained from the diaphragm muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or isotype control antibody.

[0048] **Figure 19A-19E** shows exemplary results of CD31 staining of tissue sections obtained from the tibialis anterior muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or isotype control antibody.

[0049] **Figure 20** shows exemplary results of quantification of the normalized CD31 positivity percentage in tissue sections obtained from the tibialis anterior muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or isotype control antibody.

[0050] **Figure 21A** shows exemplary results of reverse phase liquid chromatography/mass spectrometry (RP-LC/MS) analysis to determine the molecular weight of the deglycosylated 21B3 antibody. **Figure 21B** shows exemplary results of analysis of the glycosylation pattern of the heavy chain.

[0051] **Figure 22A and 22B** show exemplary results depicting rescue of phosphorylation of VEGF R2 by anti-Flt-1 antibodies. Human primary vein endothelial cells (HUVECs) were treated with VEGF in the presence of sFlt-1 and anti-Flt-1 antibodies and the level of VEGF R2 phosphorylation was determined. Percent rescue indicates the level of phosphorylation of VEGF R2 relative to the level of phosphorylation of VEGF R2 when HUVECs were treated with VEGF and sFlt-1 alone (i.e., no anti-Flt-1 antibody).

[0052] **Figure 23** shows exemplary results depicting binding of anti-Flt-1 antibodies to recombinant sFlt-1 by an ELISA assay.

[0053] **Figure 24** shows exemplary results depicting serum levels of free anti-Flt-1 antibody 21B3 and isotype control antibody in *mdx* mice.

[0054] **Figure 25** shows exemplary results depicting serum levels of free sFlt-1 in *mdx* mice treated with either anti-Flt-1 antibody 21B3 or isotype control antibody.

[0055] **Figure 26** shows exemplary results depicting serum levels of VEGF in *mdx* mice treated with either anti-Flt-1 antibody 21B3 or isotype control antibody.

[0056] **Figures 27A-27H** show exemplary results of CD31 staining of tissue sections obtained from the diaphragm muscle of *mdx* mice anti-Flt-1 antibody 21B3 or vehicle control for 6 (27A-27D) or 12 (27E-27H) weeks.

[0057] **Figures 28A-28H** show exemplary results of CD31 staining of tissue sections obtained from the gastrocnemius muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control for 6 weeks (**28A-28D**) or 12 weeks (**28E-28H**).

[0058] **Figures 29A-29H** show exemplary results of CD31 staining of tissue sections obtained from the tibialis muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control for 6 weeks (**29A-29D**) or 12 weeks (**29E-29H**).

[0059] **Figures 30A-30C** show exemplary results of quantification of the percent positivity of CD31 staining in tissue sections obtained from the diaphragm, gastrocnemius and tibialis muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control antibody for 6 or 12 weeks.

[0060] **Figures 31A-31H** show exemplary results of collagen type I immunohistochemical staining of tissue sections obtained from the diaphragm muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control for 6 weeks (**31A-31D**) or 12 weeks (**31E-31H**).

[0061] **Figures 32A-32H** show exemplary results of collagen type I immunohistochemical staining of tissue sections obtained from the gastrocnemius muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control for 6 weeks (**32A-32D**) or 12 weeks (**32E-32H**).

[0062] **Figures 33A-33H** show exemplary results of collagen type I immunohistochemical staining of tissue sections obtained from the tibialis muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control for 6 weeks (**33A-33D**) or 12 weeks (**33E-33H**).

[0063] **Figures 34A-34C** show exemplary results of quantification of the percent positivity of collagen type I staining in tissue sections obtained from the diaphragm, gastrocnemius and tibialis muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control antibody for 6 or 12 weeks.

[0064] **Figure 35A** and **35B** show exemplary results of quantification of the percent necrosis of gastrocnemius muscle of *mdx* mice administered anti-Flt-1 antibody 21B3 or vehicle control antibody for 6 or 12 weeks.

[0065] **Figure 36** shows exemplary results depicting a differential heat map comparing hydrogen/deuterium exchange of human sFlt-1 alone to that of human sFlt-1 and anti-Flt-1 antibody (21B3) mixture. Grey: no deuterium protection; blue: deuterium protection upon Fab binding.

[0066] **Figure 37** shows exemplary results depicting a differential heat map comparing hydrogen/deuterium exchange of human sFlt-1 alone to that of human sFlt-1 and anti-Flt-1 antibody (21C6) mixture. Grey: no deuterium protection; blue: deuterium protection upon Fab binding.

[0067] **Figures 38A-3E** show exemplary results depicting MS/MS spectra for identified peptides containing amino acid residues from epitope regions.

DEFINITIONS

[0068] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0069] ***Affinity***: As is known in the art, “affinity” is a measure of the tightness with a particular ligand binds to its partner. In some embodiments, the ligand or partner is Flt-1. In some embodiments, the ligand or partner is soluble Flt-1. In some embodiments, the ligand or partner is a recombinant Flt-1. In a particular embodiment the ligand or partner is human sFlt-1. In a particular embodiment, the ligand or partner is a recombinant sFlt-1. In other embodiments, the ligand or partner is an anti-Flt-1 antibody. Affinities can be measured in different ways. In some embodiments, affinity is measured by a quantitative assay. In some such embodiments, binding partner concentration may be fixed to be in excess of ligand concentration so as to mimic physiological conditions. Alternatively or additionally, in some embodiments, binding partner concentration and/or ligand concentration may be varied. In some such embodiments, affinity may be compared to a reference under comparable conditions (e.g., concentrations).

[0070] ***Affinity matured (or affinity matured antibody):*** As used herein, the term "affinity matured" or "affinity matured antibody", refers to an antibody with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some embodiments, affinity matured antibodies will have nanomolar or even picomolar affinities for a target antigen. Affinity matured antibodies may be produced by any of a variety of procedures known in the art. Marks et al., BioTechnology 10:779-783 (1992) describes affinity maturation by V_H and V_L domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. Proc. Nat. Acad. Sci. U.S.A. 91:3809-3813 (1994); Schier et al., Gene 169: 147-155 (1995); Yelton et al., J. Immunol. 155: 1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al., J. Mol. Biol. 226:889-896 (1992).

[0071] ***Amelioration***: As used herein, the term "amelioration" is meant the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes, but does not require complete recovery or complete prevention of a disease condition.

[0072] ***Animal***: As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0073] ***Antibody:*** As used herein, the term "antibody" refers to any immunoglobulin, whether natural or wholly or partially synthetically produced. All derivatives thereof which maintain specific binding ability are also included in the term. The term also covers any protein having a binding domain which is homologous or largely homologous to an immunoglobulin-binding domain. Such proteins may be derived from natural sources, or partly or wholly synthetically produced. An antibody may be monoclonal or polyclonal. An antibody may be a member of any immunoglobulin isotype, including any of the human isotypes: IgG, IgM, IgA, IgD, and IgE. In certain embodiments, an antibody may be a member of the IgG immunoglobulin class (e.g., IgG1, IgG2, IgG3, etc). In some embodiments, an antibody may be a human antibody. In some embodiments, an antibody may be a humanized antibody.

[0074] As is known by those of ordinary skill in the art, antibodies produced in nature are typically comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy and light chain is comprised of a variable region (abbreviated herein as HCVR, VH or V_H and LCVR, VL or V_L, respectively) and a constant region. The constant region of a heavy chain comprises a C_H1, C_H2 and C_H3 domain (and optionally a C_H4 domain in the case of IgM and IgE). The constant region of a light chain is comprised of one domain, C_L. The V_H and V_L regions further contain regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, which are termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The binding regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

[0075] ***Antigen-binding portion***: As used herein, the term “antigen-binding portion” or “antigen-binding fragment” refers to one or more fragments or portions of an antibody that retain the ability to specifically bind to an antigen (e.g., Flt-1). Examples of antigen-binding portions include (i) a Fab fragment, a monovalent fragment consisting of the V_H, V_L, C_H1 and C_L domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_H and V_L domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341 :544-546), which comprises a single variable domain; (vi) an isolated complementarity determining region (CDR); (vii) a Fab’ fragment, which is essentially a Fab with part of the hinge region; (viii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Furthermore, although the two domains of the Fv fragment, V_H and V_L, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_H and V_L regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). An antigen-binding fragment of an antibody may optionally comprise a single chain antibody fragment. Alternatively or additionally, an antigen-binding fragment of an antibody may comprise multiple chains which are linked together, for example, by disulfide linkages. An antigen-binding fragment of an antibody may optionally comprise a multimolecular complex. A functional antibody fragment typically comprises at least about 50 amino acids and more typically comprises at least about 200 amino acids.

[0076] In some embodiments, an antibody fragment contains sufficient sequence of the parent antibody of which it is a fragment that it binds to the same antigen as does the parent antibody; in some embodiments, a fragment binds to the antigen with a comparable affinity to that of the parent antibody and/or competes with the parent antibody for binding to the antigen.

[0077] Those skilled in the art will appreciate that the term “antibody fragment” does not imply and is not restricted to any particular mode of generation. An antibody fragment may be produced through use of any appropriate methodology, including but not limited to cleavage of an intact antibody, chemical synthesis, and recombinant production. The fragments are screened for utility in the same manner as are intact antibodies.

[0078] ***Approximately or about:*** As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0079] ***Associated with:*** Two events or entities are "associated" with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0080] ***Carrier or diluent***: As used herein, the terms "carrier" and "diluent" refer to a pharmaceutically acceptable (e.g., safe and non-toxic for administration to a human) carrier or diluting substance useful for the preparation of a pharmaceutical formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0081] ***CDR:*** As used herein, refers to a complementarity determining region within an antibody variable region. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. A "*set of CDRs*" or "*CDR set*" refers to a group of three or six CDRs that occur in either a single variable region capable of binding the antigen or the CDRs of cognate heavy and light chain variable regions capable of binding the antigen. Certain systems have been established in the art for defining CDR boundaries (e.g., Kabat, Chothia, etc.); those skilled in the art appreciate the differences between and among these systems and are capable of understanding CDR boundaries to the extent required to understand and to practice the claimed invention.

[0082] ***Chimeric antibody:*** as used herein, refers to an antibody whose amino acid sequence includes V_H and V_L region sequences that are found in a first species and constant region sequences that are found in a second species, different from the first species. In many embodiments, a chimeric antibody has murine V_H and V_L regions linked to human constant regions. In some embodiments, an antibody with human V_H and V_L regions linked to non-human constant regions (e.g., a mouse constant region) is referred to as a "*reverse chimeric antibody.*"

[0083] ***Dosage form:*** As used herein, the terms "dosage form" and "unit dosage form" refer to a physically discrete unit of a therapeutic protein (e.g., antibody) for the patient to be treated. Each unit contains a predetermined quantity of active material calculated to produce the desired therapeutic effect. It will be understood, however, that the total dosage of the composition will be decided by the attending physician within the scope of sound medical judgment.

[0084] ***Dysfunction***: As used herein, the term "dysfunction" refers to an abnormal function. Dysfunction of a molecule (e.g., a protein) can be caused by an increase or decrease of an activity associated with such molecule. Dysfunction of a molecule can be caused by defects associated with the molecule itself or other molecules that directly or indirectly interact with or regulate the molecule.

[0085] ***Epitope:*** as used herein, includes any moiety that is specifically recognized by an immunoglobulin (e.g., antibody, antibody fragment thereof, receptor) binding component. In some embodiments, an epitope is comprised of a plurality of chemical atoms or groups on an antigen. In some embodiments, such chemical atoms or groups are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some embodiments, such chemical atoms or groups are physically near to each other in space when the antigen adopts such a conformation. In some embodiments, at least some such chemical atoms are groups are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized).

[0086] ***Fc region***: As used herein, the term “Fc region” refers to a dimer of two “Fc polypeptides”, each “Fc polypeptide” comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. In some embodiments, an “Fc region” includes two Fc polypeptides linked by one or more disulfide bonds, chemical linkers, or peptide linkers. “Fc polypeptide” refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and may also include part or all of the flexible hinge N-terminal to these domains. For IgG, “Fc polypeptide” comprises immunoglobulin domains Cgamma2 (Cγ2) and Cgamma3 (Cγ3) and the lower part of the hinge between Cgamma1 (Cγ1) and Cγ2. Although the boundaries of the Fc polypeptide may vary, the human IgG heavy chain Fc polypeptide is usually defined to comprise residues starting at T223 or C226 or P230, to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Services, Springfield, VA). For IgA, Fc polypeptide comprises immunoglobulin domains Calpha2 (Cα2) and Calpha3 (Cα3) and the lower part of the hinge between Calpha1 (Cα1) and Cα2. An Fc region can be synthetic, recombinant, or generated from natural sources such as IVIG.

[0087] ***Framework* or *framework region***: As used herein, refers to the sequences of a variable region minus the CDRs. Because a CDR sequence can be determined by different systems, likewise a framework sequence is subject to correspondingly different interpretations. The six CDRs divide the framework regions on the heavy and light chains into four sub-regions (FRl, FR2, FR3 and FR4) on each chain, in which CDRl is positioned between FRl and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, FR1, for example, represents the first framework region closest to the amino terminal end of the variable region and 5' with respect to CDR1, and FRs represents two or more of the sub-regions constituting a framework region.

[0088] ***Half-Life***: As used herein, the term “half-life” is the time required for a quantity such as protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0089] ***High affinity:*** As used herein, the term "*high affinity*", when referring an IgG type antibody, refers to an antibody having a K_D of 10^{-8} M or less, more preferably 10^{-9} M or less and even more preferably 10^{-10} M or less for a domain of Flt-1. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-7} M or less, more preferably 10^{-8} M or less, even more preferably 10^{-9} M or less.

[0090] ***Human antibody:*** As used herein, is intended to include antibodies having variable and constant regions generated (or assembled) from human immunoglobulin sequences. In some embodiments, antibodies (or antibody components) may be considered to be "*human*" even though their amino acid sequences include residues or elements not encoded by human germline immunoglobulin sequences (e.g., include sequence variations, for example that may (originally) have been introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in one or more CDRs and in particular CDR3.

[0091] ***Human monoclonal antibody:*** As used herein, is intended to refer to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, e.g., a transgenic mouse, having a genome comprising a human heave chain transgene and a light chain transgene fused to an immortalized cell.

[0092] ***Humanized:*** As is known in the art, the term "*humanized*" is commonly used to refer to antibodies (or antibody components) whose amino acid sequence includes V_H and V_L region sequences from a reference antibody raised in a non-human species (e.g., a mouse, a llama), but also includes modifications in those sequences relative to the reference antibody intended to render them more "*human-like*", i.e., more similar to human germline variable sequences. In some embodiments, a "*humanized*" antibody (or antibody component) is one that immunospecifically binds to an antigen of interest and that has a framework (FR) region having substantially the amino acid sequence as that of a human antibody, and a complementary determining region (CDR) having substantially the amino acid sequence as that of a non-human antibody (e.g., a mouse, a llama). A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor immunoglobulin) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In some embodiments, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin constant region. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include a C_H1, hinge, C_H2, C_H3, and, optionally, a C_H4 region of a heavy chain constant region. In some embodiments, a humanized antibody only contains a humanized V_L region. In some embodiments, a humanized antibody only contains a humanized V_H region. In some certain embodiments, a humanized antibody contains humanized V_H and V_L regions.

[0093] ***Hypertrophy***: As used herein, the term “hypertrophy” refers to the increase in volume of an organ or tissue due to the enlargement of its component cells.

[0094] ***Improve, increase, or reduce***: As used herein, the terms “improve,” “increase” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A “control individual” is an individual afflicted with the same form of disease as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0095] ***Inhibition***: As used herein, the terms "inhibition," "inhibit" and "inhibiting" refer to processes or methods of decreasing or reducing activity and/or expression of a protein or a gene of interest. Typically, inhibiting a protein or a gene refers to reducing expression or a relevant activity of the protein or gene by at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% or more, or a decrease in expression or the relevant activity of greater than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 50-fold, 100-fold or more as measured by one or more methods described herein or recognized in the art.

[0096] ***In Vitro***: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.

[0097] ***In Vivo***: As used herein, the term "*in vivo*" refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0098] ***Isolated Antibody:*** As used herein, the term "isolated antibody" is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to Flt-1). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0099] K_a: As used herein, refers to the association rate of a particular antibody-antigen interaction, whereas the term "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of *Kd* to *Ka (i.e.,* Kd/Ka) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a BIAcore® system.

[0100] ***Light-chain reshuffling:*** As used herein, the term “light-chain reshuffling” is intended to refer to an affinity maturation step where the heavy chain sequence is kept constant and a library of light chain sequences is generated. The light chain library is screened against the heavy chain to identify antibodies with improved binding affinity. The improved binding affinity may be in the nanomolar or picomolar ranges.

[0101] ***Monoclonal antibody***: As used herein, the term “monoclonal antibody” is intended to refer to a preparation of antibody molecules of a single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0102] ***Pharmaceutically acceptable***: As used herein, the term “pharmaceutically acceptable” refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0103] ***Polypeptide***: As used herein, the term “polypeptide” refers to a sequential chain of amino acids linked together via peptide bonds. The term is used to refer to an amino acid chain of any length, but one of ordinary skill in the art will understand that the term is not limited to lengthy chains and can refer to a minimal chain comprising two amino acids linked together via a peptide bond. As is known to those skilled in the art, polypeptides may be processed and/or modified.

[0104] ***Prevent***: As used herein, the term “prevent” or “prevention”, when used in connection with the occurrence of a disease, disorder, and/or condition, refers to reducing the risk of developing the disease, disorder and/or condition. See the definition of “risk.”

[0105] ***Protein***: As used herein, the term “protein” refers to one or more polypeptides that function as a discrete unit. If a single polypeptide is the discrete functioning unit and does not require permanent or temporary physical association with other polypeptides in order to form the discrete functioning unit, the terms “polypeptide” and “protein” may be used interchangeably. If the discrete functional unit is comprised of more than one polypeptide that physically associate with one another, the term “protein” refers to the multiple polypeptides that are physically coupled and function together as the discrete unit.

[0106] ***Risk*:** As will be understood from context, a “risk” of a disease, disorder, and/or condition comprises a likelihood that a particular individual will develop a disease, disorder, and/or condition (*e.g.*, DMD). In some embodiments, risk is expressed as a percentage. In some embodiments, risk is from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 up to 100%. In some embodiments risk is expressed as a risk relative to a risk associated with a reference sample or group of reference samples. In some embodiments, a reference sample or group of reference samples have a known risk of a disease, disorder, condition and/or event (e.g., DMD). In some embodiments a reference sample or group of reference samples are from individuals comparable to a particular individual. In some embodiments, relative risk is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

[0107] ***Selective binding***: As used herein, “selective binding”, “selectively binds” “specific binding”, or “specifically binds” refers, with respect to a binding moiety and a target, preferential association of a binding moiety to a target and not to an entity that is not the target. A certain degree of non-specific binding may occur between a binding moiety and a non-target. In some embodiments, a binding moiety selectively binds a target if binding between the binding moiety and the target is greater than 2-fold, greater than 5-fold, greater than 10-fold, or greater than 100-fold as compared with binding of the binding moiety and a non-target. In some embodiments, a binding moiety selectively binds a target if the binding affinity is less than about 10^{-5} M, less than about 10^{-6} M, less than about 10^{-7} M, less than about 10^{-8} M, or less than about 10^{-9} M.

[0108] ***Striated muscle***: As used herein, the term “striated muscle” refers to multinucleated muscle tissue with regular arrangement of their intracellular contractile units, sarcomeres, leading to the appearance of striations using microscopy and under voluntary control. Typically, striated muscle can be cardiac muscle, skeletal muscle, and Branchiomeric muscles.

[0109] ***Smooth muscle***: As used herein, the term "smooth muscle" refers to involuntarily controlled, non-striated muscle, including unitary and multi-unit muscle.

[0110] ***Subject***: As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0111] ***Substantially***: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0112] ***Substantial homology***: The phrase "substantial homology" is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially homologous" if they contain homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues will appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as "hydrophobic" or "hydrophilic" amino acids, and/or as having "polar" or "non-polar" side chains. Substitution of one amino acid for another of the same type may often be considered a "homologous" substitution.

[0113] As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-

BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, et al., *Methods in Enzymology*; Altschul, et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis, et al., *Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some embodiments, two sequences are considered to be substantially homologous if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[0114] ***Substantial identity***: The phrase “substantial identity” is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be “substantially identical” if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, *et al.*, *Methods in Enzymology*; Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis *et al.*, *Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[0115] ***Surface plasmon resonance***: as used herein, refers to an optical phenomenon that allows for the analysis of specific binding interactions in real-time, for example through detection of alterations in protein concentrations within a biosensor matrix, such as by using a Biacore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson, U., et al. (1993) Ann. Biol. Clin. 51 : 19-26; Jonsson, U., et al. (1991) Biotechniques 11:620-627; Johnsson, B., et al. (1995) J. Mol. Recognit. 8: 125-131; and Johnnson, B., et al. (1991) Anal. Biochem. 198:268-277.

[0116] ***Suffering from***: An individual who is “suffering from” a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of the disease, disorder, and/or condition such as, for example DMD.

[0117] ***Susceptible to*****:** An individual who is “susceptible to” a disease, disorder, and/or condition has not been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, condition, or event (for example, DMD) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, condition, and/or event (5) having undergone, planning to undergo, or requiring a transplant. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0118] ***Target tissues***: As used herein , the term “target tissues” refers to any tissue that is affected by a disease to be treated such as DMD. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature, including but not limited to muscle wasting, skeletal deformation, cardiomyopathy, muscle ischemia, cognitive impairment, and impaired respiratory function. In some embodiment the target tissue is smooth muscle, striated muscle or cardiac muscle.

[0119] ***Therapeutically effective amount***: As used herein, the term “therapeutically effective amount” of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0120] ***Treating*:** As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition, such as for example, DMD. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

DETAILED DESCRIPTION

[0121] The present invention provides, among other things, methods and compositions for treating muscular dystrophy, including Duchenne muscular dystrophy (DMD) and/or Becker Muscular Dystrophy, based on the use of anti-Flt-1 antibodies, or antigen -binding fragments thereof, as therapeutics for treating muscular dystrophy. In some embodiments, the present invention provides methods of treating DMD including administering to an individual who is suffering from or susceptible to DMD a therapeutically effective amount of an anti-Flt-1 antibody, or antigen-binding fragment thereof, such that at least one symptom or feature of DMD is reduced in intensity, severity, or frequency, or has delayed onset.

[0122] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Duchenne muscular dystrophy (DMD)

[0123] DMD is a disease characterized by progressive deterioration of muscles and loss of muscle related functions throughout the body. It is contemplated that the present invention provides methods and compositions for slowing, delaying or preventing deterioration of muscles, regenerating muscle and reversing, eliminating, delaying, preventing, or minimizing fibrosis, inflammation and other symptoms or features associated with DMD and other muscular dystrophies in various muscle tissues.

Muscle tissues

[0124] There are two major types of muscle tissue in an animal, striated muscle and smooth muscle. As used herein, the term "striated muscle" refers to muscle tissues containing repeating sarcomeres. Striated muscle tends to be under voluntary control and attached to the skeleton. Striated muscle allows for voluntary movement of the body and includes the major muscle groups including the quadriceps, gastrocnemius, biceps, triceps, trapezius, deltoids, and many others. Striated muscle tends to be very long and, many striated muscles are able to function independently. Some striated muscle, however, is not attached to the skeleton, including those in the mouth, anus, heart, and upper portion of the esophagus.

[0125] Smooth muscle, on the other hand, has very different structure. Rather than a series of long muscles with separate skeletal attachments, smooth muscle tends to be organized into continuous sheets with mechanical linkages between smooth muscle cells. Smooth muscle is often located in the walls of hollow organs and is usually not under voluntary control. Smooth muscles lining a particular organ must bear the same load and contract concurrently. Smooth muscle functions, at least in part, to handle changes in load on hollow organs caused by movement and/or changes in posture or pressure. This dual role means that smooth muscle must not only be able to contract like striated muscle, but also that it must be able to contract tonically to maintain organ dimensions against sustained loads. Examples of smooth muscles are those lining blood vessels, bronchioles, bladder, and gastrointestinal tract such as rectum.

[0126] The strength of a muscle depends on the number and sizes of the muscle's cells and on their anatomic arrangement. Increasing the diameter of a muscle fiber either by synthesis of new myofibrils (hypertrophy) and/or the formation of more muscle cells (hyperplasia) will increase the force-generating capacity of the muscle.

[0127] Muscles may also be grouped by location or function. In some embodiments, an Flt-1 antibody or antigen-binding fragment thereof is targeted to one or more muscles of the face, one or more muscles for mastication, one or more muscles of the tongue and neck, one or more muscles of the thorax, one or more muscles of the pectoral girdle and arms, one or more muscles of the arm and shoulder, one or more ventral and dorsal forearm muscles, one or more muscles of the hand, one or more muscles of the erector spinae, one or more muscles of the pelvic girdle and legs, and/or one or more muscles of the foreleg and foot.

[0128] In some embodiments, muscles of the face include, but are not limited to, intraocular muscles such as ciliary, iris dilator, iris sphincter; muscles of the ear such as auriculares, temporoparietalis, stapedius, tensor tympani; muscles of the nose such as procerus, nasalis, dilator naris, depressor septi nasi, levator labii superioris alaeque nasi; muscles of the mouth such as levator anguli oris, depressor anguli oris,orbicularis oris, Buccinator, Zygomaticus Major and Minor, Platysma, Levator Labii Superioris, Depressor Labii Inferioris, Risorius, Mentalis, and/or Corrugator Supercilii.

[0129] In some embodiments, muscles of mastication include, but are not limited to, Masseter, Temporalis, Medial Pterygoid, Lateral Pterygoid. In some embodiments, muscles of the tongue and neck include, but are not limited to, Genioglossus, Styloglossus, Palatoglossus, Hyoglossus, Digastric, Stylohyoid, Mylohyoid, Geniohyoid, Omohyoid, Sternohyoid, Sternothyroid, Thyrohyoid, Sternocleidomastoid, Anterior Scalene, Middle Scalene, and/or Posterior Scalene.

[0130] In some embodiments, muscles of the thorax, pectoral girdle, and arms include, but are not limited to, Subclavius Pectoralis major, Pectoralis minor, Rectus abdominis, External abdominal oblique, Internal abdominal oblique, Transversus Abdominis, Diaphragm, External Intercostals, Internal Intercostals, Serratus Anterior, Trapezius, Levator Scapulae, Rhomboideus Major, Rhomboideus Minor, Latissimus dorsi, Deltoid, subscapularis, supraspinatus, infraspinatus, Teres major, Teres minor, and/or Coracobrachialis.

[0131] In some embodiments, muscles of the arm and shoulder include, but are not limited to, Biceps brachii-Long Head, Biceps brachii-Short Head, Triceps brachii-Long Head, Triceps brachii Lateral Head, Triceps brachii-Medial Head, Anconeus, Pronator teres, Supinator, and/or Brachialis.

[0132] In some embodiments, muscles of the ventral and dorsal forearm include, but are not limited to, Brachioradialis, Flexor carpi radialis, Flexor carpi ulnaris, Palmaris longus, Extensor carpi ulnaris, Extensor carpi radialis longus, Extensor carpi radialis brevis, Extensor digitorum, Extensor digiti minimi.

[0133] In some embodiments, muscles of the hand include, but are not limited to intrinsic muscles of the hand such as thenar, abductor pollicis brevis, flexor pollicis brevis, opponens pollicis, hypothenar, abductor digiti minimi, the flexor digiti minimi brevis, opponens digiti minimi, palmar interossei, dorsal interossei and/or lumbricals.

[0134] In some embodiments, muscles of the erector spinae include, but are not limited to, cervicalis, spinalis, longissimus, and/or iliocostalis.

[0135] In some embodiments, muscles of the pelvic girdle and the legs include, but are not limited to, Psoas Major, Iliacus, quadratus femoris, Adductor longus, Adductor brevis, Adductor magnus, Gracilis, Sartorius, Quadriceps femoris such as, rectus femoris, vastus lateralis, vastus medialis, vastus intermedius, Gastrocnemius, Fibularis (Peroneus) Longus, Soleus, Gluteus maximus, Gluteus medius, Gluteus minimus, Hamstrings: Biceps Femoris: Long Head, Hamstrings: Biceps Femoris: Short Head, Hamstrings: Semitendinosus, Hamstrings: Semimembranosus, Tensor fasciae latae, Pectineus, and/or Tibialis anterior.

[0136] In some embodiments, muscles of the foreleg and foot include, but are not limited to, Extensor digitorum longus, Extensor hallucis longus, peroneus brevis, plantaris, Tibialis posterior, Flexor hallucis longus, extensor digitorum brevis, extensor hallucis brevis, Abductor hallucis, flexor hallucis brevis, Abductor digiti minimi, flexor digiti minimi, opponens digiti minimi, extensor digitorum brevis, lumbricales of the foot, Quadratus plantae or flexor accessorius, flexor digitorum brevis, dorsal interossei, and/or plantar interossei.

[0137] Exemplary muscle targets are summarized in **Table 1**.

Table 1.

ORBICULARIS OCULI			
Intraocular: ciliary, iris dilator, iris sphincter			
Ear: auriculares, temporoparietalis, stapedius, tensor tympani			
Nose: procerus, nasalis, dilator naris, depressor septi nasi, levator labii superioris alaeque nasi			
Mouth: levator anguli oris, depressor anguli oris,orbicularis oris			
Buccinator	Zygomaticus Major and Minor	Platysma	Levator Labii Superioris
Depressor Labii Inferioris	Risorius	Mentalis	Corrugator Supercilii
Anconeus	Pronator teres	Supinator	Brachialis
MUSCLES OF MASTICATON			
Masseter	Temporalis	Medial Pterygoid	Lateral Pterygoid

MUSCLES OF THE TONGUE AND NECK			
Genioglossus	Styloglossus	Palatoglossus	Hyoglossus
Digastric	Stylohyoid	Mylohyoid	Geniohyoid
Omohyoid	Sternohyoid	Sternothyroid	Thyrohyoid
Sternocleidomastoid	Anterior Scalene	Middle Scalene	Posterior Scalene
MUSCLES OF THE THORAX, PECTORAL GIRDLE AND ARMS			
Subclavius	Pectoralis major	Pectoralis minor	Rectus abdominis
External abdominal oblique	Internal abdominal oblique	Transversus Abdominis	Diaphragm
External Intercostals	Internal Intercostals	Serratus Anterior	Trapezius
Levator Scapulae	Rhomboideus Major	Rhomboideus Minor	Latissimus dorsi
Deltoid	subscapularis	supraspinatus	infraspinatus
Teres major	Teres minor	Coracobrachialis	
ARM AND SHOULDER			
Biceps brachii-Long Head	Biceps brachii-Short Head	Triceps brachii-Long Head	Triceps brachii-Lateral Head
Triceps brachii-Medial Head	Anconeus	Pronator teres	Supinator
Brachialis			
FOREARM MUSCLES: Ventral and Dorsal			
Brachioradialis	Flexor carpi radialis	Flexor carpi ulnaris	Palmaris longus
Extensor carpi ulnaris	Extensor carpi radialis longus	Extensor carpi radialis brevis	Extensor digitorum
Extensor digiti minimi	erector spinae: cervicalis	erector spinae: spinalis	erector spinae: longissimus

erector spinae: iliocostalis			
Intrinsic Muscles of the Hand: thenar, abductor pollicis brevis, flexor pollicis brevis, and the opponens pollicis			
Intrinsic Muscles of the Hand: hypothenar, abductor digiti minimi, the flexor digiti minimi brevis, and the opponens digiti minimi			
Intrinsic Muscles of the Hand: palmar interossei, dorsal interossei and lumbricals			
MUSCLES OF THE PELVIC GIRDLE AND THE LEGS			
Iliopsoas: Psoas Major	Iliopsoas: Iliacus	quadratus femoris	Adductor longus
Adductor brevis	Adductor magnus	Gracilis	Sartorius
Quadriceps femoris: rectus femoris	Quadriceps femoris: vastus lateralis	Quadriceps femoris: vastus medialis	Quadriceps femoris: vastus intermedius
Gastrocnemius	Fibularis (Peroneus) Longus	Soleus	Gluteus maximus
Gluteus medius	Gluteus minimus	Hamstrings: Biceps Femoris: Long Head	Hamstrings: Biceps Femoris: Short Head
Hamstrings: Semitendinosus	Hamstrings: Semimembranosus	Tensor fasciae latae	Pectineus
Tibialis anterior			
MUSCLES OF THE FORELEG AND FOOT			
Extensor digitorum longus	Extensor hallucis longus	peroneus brevis	plantaris
Tibialis posterior	Flexor hallucis longus	extensor digitorum brevis	extensor hallucis brevis
Abductor hallucis	flexor hallucis brevis	Abductor digiti minimi	flexor digiti minimi
opponens digiti minimi	extensor digitorum brevis	lumbricales of the foot	Quadratus plantae or flexor accessorius

Flexor digitorum brevis	dorsal interossei	plantar interossei	

Muscular Dystrophy

[0138] Muscular dystrophies are a group of inherited disorders that cause degeneration of muscle, leading to weak and impaired movements. A central feature of all muscular dystrophies is that they are progressive in nature. Muscular dystrophies include, but are not limited to: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophies, and myotonic dystrophy Types 1 and 2, including the congenital form of Myotonic dystrophy Type 1. Symptoms may vary by type of muscular dystrophy with some or all muscles being affected. Exemplary symptoms of muscular dystrophies include delayed development of muscle motor skills, difficulty using one or more muscle groups, difficulty swallowing, speaking or eating, drooling, eyelid drooping, frequent falling, loss of strength in a muscle or group of muscles as an adult, loss in muscle size, problems walking due to weakness or altered biomechanics of the body, and/or cognitive or behavioral impairment/mental retardation.

[0139] While there are no known cures for muscular dystrophies, several supportive treatments are used which include both symptomatic and disease modifying therapies. Corticosteroids, ACE inhibitors, Angiotensin receptor blockers, physical therapy, orthotic devices, wheelchairs, or other assistive medical devices for ADLs and pulmonary function are commonly used in muscular dystrophies. Cardiac pacemakers are used to prevent sudden death from cardiac arrythmias in Myotonic dystrophy. Anti-myotonic agents which improve the symptoms of myotonia (inability to relax) include mexilitine, and in some cases phenytoin, procainamide and quinine.

Duchenne muscular dystrophy

[0140] Duchenne muscular dystrophy (DMD) is a recessive X-linked form of muscular dystrophy which results in muscle degeneration and eventual death. DMD is characterized by weakness in the proximal muscles, abnormal gait, hypertrophy in the gastrocnemius (calf) muscles, and elevated creatine kinase. Many DMD patients are diagnosed around the age of 5, when symptoms/signs typically become more obvious. Affected individuals typically stop walking around age 10-13 and die in or before their mid to late 20's due to respiratory complications and cardiomyopathy.

[0141] In individuals affected with DMD, serum creatine kinase levels may be increased by greater than 10-fold as compared to unaffected individuals. In some embodiments, administering the provided composition to an affected individual results in a reduced serum creatine kinase level as compared to the baseline level before treatment. Typically, the baseline level is measured immediately before treatment. In some embodiments, administering the provided composition results in a reduced serum creatine kinase level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% as compared to the baseline serum creatine kinase level immediately before treatment. In some embodiments, administering the provided composition results in a reduced serum creatine kinase level to less than about 3500 IU/L, 3000 IU/L, 2500 IU/L, 2000 IU/L, 1500 IU/L, 1000 IU/L, 750 IU/L, 500 IU/L, 250 IU/L, 100 IU/L, 90 IU/L, 80 IU/L, 70 IU/L or 60 IU/L. In some embodiments, administering the provided composition results in a reduced serum creatine kinase level as compared to the serum creatine kinase level in subjects who are not treated.

[0142] The disorder DMD is caused by a mutation in the dystrophin gene, located on the human X chromosome, which codes for the protein dystrophin, an important structural component within muscle tissue that provides structural stability to the dystroglycan complex (DGC) of the cell membrane. Dystrophin links the internal cytoplasmic actin filament network and extracellular matrix, providing physical strength to muscle fibers. Accordingly, alteration or absence of dystrophin results in abnormal sarcolemnal membrane tearing and necrosis of muscle fibers. While both sexes can carry the mutation, females rarely exhibit severe signs of the disease.

[0143] A main symptom of DMD is muscle weakness associated with muscle wasting with the voluntary muscles being first affected typically, especially affecting the muscles of the hips, pelvic area, thighs, shoulders, and calf muscles. Muscle weakness also occurs in the arms, neck, and other areas. Calves are often enlarged. Signs and symptoms usually appear before age 6 and may appear as early as infancy. Cardiomyopathy occurs in individuals with DMD usually after the age of 18. Other physical symptoms include, but are not limited to, delayed ability to walk independently, progressive difficulty in walking, stepping, or running, and eventual loss of ability to walk (usually by the age of 12); frequent falls; fatigue; difficulty with motor skills (running, hopping, jumping); increased lumbar lordosis, leading to shortening of the hip-flexor muscles; impaired functionality of achilles tendon and hamstrings, fibrosis in connective tissue; muscle fiber deformities; pseudohypertrophy (enlarging) of tongue and calf muscles caused by replacement of muscle tissue by fat and connective tissue; higher risk of neurobehavioral disorders (e.g., ADHD), learning disorders (dyslexia), and non-progressive weaknesses in specific cognitive skills (in particular short-term verbal memory); skeletal deformities (including scoliosis in some cases).

[0144] The changes in muscle seen in DMD are accompanied by an increase in connective tissue, i.e., the development of fibrosis, and result from either reactive or reparative processes involving mechanical, humoral and or cellular factors. Lack of functional dystrophin leads to instability of muscle fiber membranes, and as a result, the cells are less resistant to mechanical shear and prone to excess influx of electrolytes resulting in tissue damage. As muscle tissue in DMD is damaged, recovery is limited by the ability of satellite cells to proliferate. This leads to necrosis, inflammation, fibrosis and fatty cell replacement. The increase in connective tissue occurs early in the disease process as areolar connective tissue that ensheaths each myocyte and overlays the sarcolemma (i.e., the endomysium) increases prior to observable muscle damage. The increase in collagenous connective tissue is a factor in muscle pathology in DMD, adversely affecting the supply of nutrients to the affected myocyte, and secondarily affecting muscle strength and age of loss of ambulation.

[0145] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof, *in vivo* results in decreased fibrosis of muscle tissue. In some embodiments the muscle is skeletal muscle. In particular embodiments, the muscle is the cardiac muscle, diaphragm muscle, gastrocnemius muscle and/or tibialis anterior (TA) muscle. In some embodiments, the decreased fibrosis is demonstrated by decreased collagen staining. In some embodiments, the collagen in Type I collagen. In some embodiments, the decreased fibrosis may be measured, for example, by measuring the percent collagen positive area in muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof. For example, the percent collagen positive area in the diaphragm muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof, may be at least about 3.0%, about 3.5%, about 4.0%, about 4.5%, about 5.0%, about 5.5%, about 6.0%, about 6.5%, about 7.0%, about 7.5%, about 8.0%, about 8.5% or about 9.0% of the total tissue area. In a particular embodiment, the percent collagen positive area in the diaphragm muscle of mice administered the anti-Flt-1 antibody may be significantly lower than the percent collagen positive area in the diaphragm muscle of mice administered an isotype control antibody.

[0146] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof, *in vivo* results in decreased necrosis of muscle tissue. In some embodiments the muscle is skeletal muscle. In particular embodiments, the muscle is the cardiac muscle, diaphragm muscle, gastrocnemius muscle and/or tibialis anterior (TA) muscle. In some embodiments, the decreased necrosis may be measured, for example, by measuring the percent necrosis positive area in muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof. For example, the percent necrosis positive area in the gastrocnemius muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof may be at least about 0.5%, about 0.45%, about 0.4%, about 0.35%, about 0.3%, about 0.25%, about 0.2%, about 0.15%, about 0.1%, about 0.05%, or about 0.025% of the total tissue area. In a particular embodiment, the percent necrosis positive area in the gastrocnemius muscle of mice administered the anti-Flt-1 antibody may be significantly lower than the percent necrosis positive area in the gastrocnemius muscle of mice administered an isotype control antibody.

[0147] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof, *in vivo* results in increased muscle force and/or resistance to fatigue.

Flt-1 receptor

[0148] Flt-1 receptor, also known as vascular endothelial growth factor receptor 1 (VEGFR-1) or Flt-1, is a receptor that is encoded by the FLT1 gene and expressed on endothelia cell and monocyte cell membranes. The vascular endothelial growth factor (VEGF) family of signal glycoproteins act as potent promoters of angiogenesis during embryogenesis and postnatal growth. Specifically, the binding of the VEGF-A ligand with the VEGF receptors has been shown to promote vascular permeability and also trigger endothelial cell migration, proliferation, and survival, and the newly formed endothelial cells provide the basic structure of new vasculatures. The dominant VEGF signal molecule for angiogenesis, VEGF-A, mediates its signal through VEGF receptor-1 (VEGFR-1, also known as Flt-1) and VEGF receptor-2 (VEGFR-2, also known as Flk-1). A soluble form of Flt-1 (sFlt-1) also exists, but lacks an intracellular signaling domain and thus is believed to only serve in a regulatory capacity by sequestering VEGF-A or other ligands that bind to it. sFlt-1 and other molecules containing Flt-1 binding sites that are not linked to an intracellular signal transduction pathway are referred to as “decoy receptors”. Flt-1 and Flk-1 receptors contain an extracellular VEGF-A-binding domain and an intracellular tyrosine kinase domain, and both show expression during the developmental stage and tissue regeneration in hemangioblasts and endothelial cell lineages. Flt-1 has about 10 times greater binding affinity for VEGF-A (K_d ~2^{-10} pM) compared to Flk-1, but weaker tyrosine kinase activity indicates that angiogenic signal transduction following VEGF-A binding to Flt-1 is comparably weaker than that resulting from VEGF-A binding to Flk-l. As such, homozygous Flt-1 gene knockout mice die in the embryonic stage from endothelial cell overproduction and blood vessel disorganization. Inversely, homozygous Flk-1 gene knockout mice die from defects in the development of organized blood vessels due to lack of yolk-sac blood island formation during embryogenesis. Both the Flt-1 and Flk-1 receptors are needed for normal development, but selective augmentation in VEGF-A concentration may allow for greater binding to the Flk-1 receptor and induce a pro-angiogenic effect that increases capillary density and facilitates regeneration of muscle, reduction of fibrosis and inflammation, and mitigation of symptoms and features associated with DMD and other muscular dystrophies in various muscle tissues.

[0149] As used herein, the term "Flt-1 receptor" refers to both soluble and membrane associate Flt-1 receptors, or functional fragments thereof.

Anti-Flt-1 antibodies

[0150] As used herein, the term "anti-Flt-1 antibodies" refers to any antibodies, or antigen-binding fragments thereof, that bind to a Flt-1 receptor (e.g., soluble or membrane associated Flt-1 receptor). In some embodiments, anti-Flt-1 antibodies are produced that bind with high affinity to Flt-1 receptors. Without wishing to be bound by theory, it is believed that anti-Flt-1 antibody binding to Flt-1 receptors inhibits one or more endogenous ligands from binding to Flt-1 and thereby allowing a greater amount of available ligand to associate with other VEGF receptors, such as the Flk-1 receptor. Increased availability of VEGF promotes angiogenesis with increased blood flow to muscle to combat functional ischemia and leading to improvements in structural and functional characteristics of DMD. In some embodiments, antibody binding to Flt-1 receptors increases the amount of VEGF available to bind to other VEGF receptors.

[0151] In some embodiments the anti-Flt-1 antibody or antigen-binding fragment thereof comprises the sequences provided in **Table 2**.

Table 2.

Heavy Chain Variable Region	**CDR1**	**CDR2**	**CDR3**
IGHV3-23*01	SYAMS (SEQ ID NO:1)	AISGSGGSTYYADSVKG (SEQ ID NO:5)	---------------DY (SEQ ID NO:15)
IGHV3-23*04	SYAMS (SEQ ID NO:1)	AISGSGGSTYYADSVKG (SEQ ID NO:5)	---------------DY (SEQ ID NO:15)
13B4_VH	DYSMS (SEQ ID NO:2)	AISWNGDSTYYAESMKG (SEQ ID NO:6)	SWATPIESLYYYGMDY (SEQ ID NO:16)
27H4_VH(97.6_ 1.0	DYSMS (SEQ ID NO:2)	AISWNGDSTYYAESLKG (SEQ ID NO:7)	SWATPIESLYYYGSDY (SEQ ID NO:17)
27H9_VH(97.5_ 1.1	DYSMS (SEQ ID NO:2)	AISWNGDSTYYAESAKG (SEQ ID NO:8)	SWATPIESLYYYGSDY (SEQ ID NO:17)
25D4_VH(97.0_ 0.9	DYSAS (SEQ ID NO:3)	AISWNGDSTYYAESVKG (SEQ ID NO:9)	SWATPIESLYYYGSDY (SEQ ID NO:17)
25G9_VH(97.0_ 0.9	DYSMS SEQ ID NO:2)	AITWSGDSTYYAESVKG (SEQ ID NO:10)	SWATPIESLYYYGTDY (SEQ ID NO:18)
25F11_VH(97.5 _1.	DYSMS (SEQ ID NO:2)	AISWNGDSTYYAESAKG (SEQ ID NO:8)	SWATPIESLYYYGSDY (SEQ ID NO:17)
29E2_VH(96.3_ 1.1	DYSLS (SEQ ID NO:4)	AISWNGDSTYYAESVKG (SEQ ID NO:9)	SWATPIESLYYYGSDY (SEQ ID NO:17)
27G9_VH(96.3_ 1.3	DYSAS (SEQ ID NO:3)	AISWSGDSTYYAESLKG (SEQ ID NO:11)	SWATPIESLYYYGSDY (SEQ ID NO:17)
27H6_VH(97.5	DYSAS (SEQ ID NO:3)	AISWSGDSTYYAESVKG (SEQ ID NO:12)	SWATPIESLYYYGSDY (SEQ ID NO:17)
27H9_NG/QG	DYSMS (SEQ ID NO:2)	AISWQGDSTYYAESAKG (SEQ ID NO:13)	SWATPIESLYYYGSDY (SEQ ID NO:17)
27H9_NG/NA	DYSMS (SEQ ID NO:2)	AISWNADSTYYAESAKG (SEQ ID NO:14)	SWATPIESLYYYGSDY (SEQ ID NO:17)
27H9_NA_+_AAA	DYSMS (SEQ ID NO:2)	AISWNADSTYYAESAKG (SEQ ID NO:14)	SWATPIESLYYYGSDY (SEQ ID NO:17)
Light Chain Variable Region	**CDR1**	**CDR2**	**CDR3**

IGLV3-9*01	GGNNIGSKNVH (SEQ ID NO:19)	RDSNRPS (SEQ ID NO:22)	QV-------VV (SEQ ID NO:25)
IGLV3-9*02	GGNNLGYKSVH (SEQ ID NO:20)	RDNNRPS (SEQ ID NO:23)	QV-------VV (SEQ ID NO:25)
LC_21B3	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWDGSTQAIV (SEQ ID NO:26)
VL_27H4	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWEDSTQAIV (SEQ ID NO:27)
VL_27H9	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWDESTQAIV (SEQ ID NO:28)
VL_25D4	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWAASTQAIV (SEQ ID NO:29)
VL_25G9	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWDDSTQAIV (SEQ ID NO:30)
VL_25F11	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWEASTQAIV (SEQ ID NO:31)
VL_29E2	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWDASTQAIV (SEQ ID NO:32)
VL_27G9	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWEESTQAIV (SEQ ID NO:33)
VL_27H6	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWDGSTQAIV (SEQ ID NO:26)
VL_27H6(DA)	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWDASTQAIV (SEQ ID NO:32)
VL_27H6(EG)	GGNNIGSQTAQ (SEQ ID NO:21)	ANNRRPS (SEQ ID NO:24)	QVWEGSTQAIV (SEQ ID NO:34)
Heavy Chain Variable Region	**VH**		
IGHV3-23*01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMS WVRQAPGKGLEWVS AISGSGGSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK --------------DY WGQGTLVTVSS (SEQ ID NO:35)		

IGHV3-23*04	EVQLVESGGGLVQPGGSLRLSCAASGFTFS SYAMS WVRQAPGKGLEWVS AISGSGGSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK --------------DY WGQGTLVTVSS (SEQ ID NO:36)
13B4_VH	ELQLVESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWNGDSTYYAESMKG RFTISRDNAKNTLYLQMNSLKSEDTAVYYCAK SWATPIESLYYYGMDY WGKGTLVTVSS (SEQ ID NO:37)
27H4_VH(97.6_1.0	ELQLVESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWNGDSTYYAESLKG RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:38)
27H9_VH(97.5_1.1	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWNGDSTYYAESAKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:39)
25D4_VH(97.0_0.9	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSAS WVRQAPGKGLEWVS AISWNGDSTYYAESVKG RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:40)
25G9_VH(97.0_0.9	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AITWSGDSTYYAESVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGTDY WGKGTLVTVSS (SEQ ID NO:41)
25F11_VH(97.5_1.	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWNGDSTYYAESAKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:42)
29E2_VH(96.3_1.1	EVQLVESGGGLVQPGGSLRLSCAASGFTFR DYSLS WVRQAPGKGLEWVS AISWNGDSTYYAESVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGKGTLVTVSS (SEQ ID NO:43)

27G9_VH(96.3_ 1.3	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSAS WVRQAPGKGLEWVS AISWSGDSTYYAESLKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGKGTLVTVSS (SEQ ID NO:44)
27H6_VH(97.5	ELQLVESGGGLVQPGGSLRLSCAASGFTFS DYSAS WVRQAPGKGLEWVS AISWSGDSTYYAESVKG RFTIFRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:45)
27H9_NG/QG	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWQGDSTYYAESAKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:46)
27H9_NG/NA	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWNADSTYYAESAKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:47)
27H9_NA_+_AAA	EVQLLESGGGLVQPGGSLRLSCAASGFTFR DYSMS WVRQAPGKGLEWVS AISWNADSTYYAESAKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDY WGQGTLVTVSS (SEQ ID NO:48)
Light Chain Variable Region	**VL**
IGLV3-9*01	SYELTQPLSVSVALGQTARITC GGNNIGSKNVH WYQQKPGQAPVLVIY RDSNRPS GIPERFSGSNSGNTATLTISRAQAGDEADYYC QV-------VV FGGGTKLTVL (SEQ ID NO:49)
IGLV3-9*02	SYELTQPLSVSVALGQAARITC GGNNLGYKSVH WYQQKPGQAPVLVIY RDNNRPS GIPERFSGSNSGNTATLTISRAQAGDEADYYC QV-------VV FGGGTKLTVL (SEQ ID NO:50)
LC_21B3	SYELTQSPSVSVALRQTAKITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISGAQAEDEADYYC QVWDGSTQAIV FGGGTHLTVL (SEQ ID NO:51)
VL_27H4	SYELTQPLSVSVALGQTARITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS

	GIPERFSGSKSGNTATLTISRAQAEDEADYYC QVWEDSTQAIV FGGGTKLTVL (SEQ ID NO:52)
VL_27H9	SYELTQPLSVSVALRQTARITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAEDEADYYC QVWDESTQAIV FGGGTKLTVL (SEQ ID NO:53)
VL_25D4	SYELTQPLSVSVALGQTARITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISGAQAEDEADYYC QVWAASTQAIV FGGGTKLTVL (SEQ ID NO:54)
VL_25G9	SYELTQPLSVSVALRQAARITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAEDEADYYC QVWDDSTQAIV FGGGTKLTVL (SEQ ID NO:55)
VL_25F11	SYELTQPLSVSVALRQAARITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAEDEADYYC QVWEASTQAIV FGGGTKLTVL (SEQ ID NO:56)
VL_29E2	SYELTQSPSVSVALRQTAKITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISGAQAGDEADYYC QVWDASTQAIV FGGGTKLTVL (SEQ ID NO:57)
VL_27G9	SYELTQPLSVSVALGQTAKITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAEDEADYYC QVWEESTQAIV FGGGTHLTVL (SEQ ID NO:58)
VL_27H6	SYELTQPLSVSVALRQAAKITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAGDEADYYC QVWDGSTQAIV FGGGTKLTVL (SEQ ID NO:59)
VL_27H6(DA)	SYELTQPLSVSVALRQAAKITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAGDEADYYC QVWDASTQAIV FGGGTKLTVL (SEQ ID NO:60)
VL_27H6(EG)	SYELTQPLSVSVALRQAAKITC GGNNIGSQTAQ WYQQKPGQAPVLVIY ANNRRPS GIPERFSGSKSGNTATLTISRAQAGDEADYYC QVWEGSTQAIV FGGGTKLTVL (SEQ ID NO:61)
Heavy Chain	**Heavy Chain Sequence (VH+CH1/2/3)**
VH_27H6_DG/EG	ELQLVESGGGLVQPGGSLRLSCAASGFTFSDYSASWVRQAPGKGLEWVSAISWSGDSTY YAESVKGRFTIFRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG

	TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:62)
VH_27H6_DG/DA	ELQLVESGGGLVQPGGSLRLSCAASGFTFSDYSASWVRQAPGKGLEWVSAISWSGDSTY YAESVKGRFTIFRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:63)
VH_27H9_NG/QG	EVQLLESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWQGDSTY YAESAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:64)
VH_27H9_NG/NA	EVQLLESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNADSTY YAESAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF

	LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:65)
VH_27H4_NG/QG	ELQLVESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWQGDSTY YAESLKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:66)
VH_27H4_NG/NA	ELQLVESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNADSTY YAESLKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:67)
VH_27H4_NA_+_ AAA	ELQLVESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNADSTY YAESLKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:68)
VH_27H9_NA_+_ AAA	EVQLLESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNADSTY YAESAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT

	FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:69)
VH_21B3_AAA	ELQLVESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNGDSTY YAESMKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKSWATPIESLYYYGMDYWGKG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:70)
VH_27H6	ELQLVESGGGLVQPGGSLRLSCAASGFTFSDYSASWVRQAPGKGLEWVSAISWSGDSTY YAESVKGRFTIFRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:71)
VH_27H4	ELQLVESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNGDSTY YAESLKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

	(SEQ ID NO:72)
VH_27H9	EVQLLESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNGDSTY YAESAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:73)
HC_13B4	ELQLVESGGGLVQPGGSLRLSCAASGFTFRDYSMSWVRQAPGKGLEWVSAISWNGDSTY YAESMKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKSWATPIESLYYYGMDYWGKG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKSYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMNEALHNHYTQKSLSLSPGK (SEQ ID NO:74)
Light Chain	**Light Chain Sequence (VL+CL)**
LC_27H6_DG/EG	SYELTQPLSVSVALRQAAKITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAGDEADYYCQVWEGSTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:75)
LC_27H6_DG/DA	SYELTQPLSVSVALRQAAKITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAGDEADYYCQVWDASTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:76)
LC_27H9_NG/QG	SYELTQPLSVSVALRQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWDESTQAIVFGGGTKLTVLGQPKAAPSVT

	LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:77)
LC_27H9_NG/NA	SYELTQPLSVSVALRQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWDESTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:78)
LC_27H4_NG/QG	SYELTQPLSVSVALGQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWEDSTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:79)
LC_27H4_NG/NA	SYELTQPLSVSVALGQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWEDSTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:80)
LC_27H4_NA_+_AAA	SYELTQPLSVSVALGQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWEDSTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:81)
LC_27H9_NA_+_AAA	SYELTQPLSVSVALRQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWDESTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:82)
LC_21B3_AAA	SYELTQSPSVSVALRQTAKITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISGAQAEDEADYYCQVWDGSTQAIVFGGGTHLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

	(SEQ ID NO:83)
LC_27H6	SYELTQPLSVSVALRQAAKITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAGDEADYYCQVWDGSTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:84)
LC_27H4	SYELTQPLSVSVALGQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWEDSTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:85)
LC_27H9	SYELTQPLSVSVALRQTARITCGGNNIGSQTAQWYQQKPGQAPVLVIYANNRRPSGIPE RFSGSKSGNTATLTISRAQAEDEADYYCQVWDESTQAIVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:86)
Constant Region	**CH1-CH2-CH3**
27H6_DG/EG	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H6_DG/DA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H9_NG/QG	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE

	QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H9_NG/NA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H4_NG/QG	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H4_NG/NA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H4_NA_+_AAA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:88)
27H9_NA_+_AAA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV

	DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:88)
21B3_AAA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:88)
27H6	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H4	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
27H9	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:87)
13B4	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKSYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMNEALHNHYTQKSLSLSPGK (SEQ ID NO:89)

[0152] In some embodiments, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises one or more complementarity determining regions (CDR) selected from the group consisting of a VL CDR1 defined by an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:19 to SEQ ID NO:21, a VL CDR2 defined by an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:22 to SEQ ID NO:24, a VL CDR3 defined by an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:25 to SEQ ID NO:34, a VH chain CDR1 defined by an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:1 to SEQ ID NO:4, a VH CDR2 defined by an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:5 to SEQ ID NO:14, and a VH CDR3 defined by an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:15 to SEQ ID NO:18. In some embodiments, the VL CDR3 is not SEQ ID NO:25. In some embodiments, the VH CDR3 is not SEQ ID NO:15.

[0153] In some embodiments, the one or more CDRs comprise the VL CDR3 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:25 to SEQ ID NO:34; and the VH CDR3 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:15 to SEQ ID NO:18. In some embodiments, the VL CDR3 is not SEQ ID NO:25. In some embodiments, the VH CDR3 is not SEQ ID NO:15.

[0154] In some embodiments, the one or more CDRs comprise the VL CDR1 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:19 to SEQ ID NO:21, the VL CDR2 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:22 to SEQ ID NO:24, and the VL CDR3 defined by amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:25 to SEQ ID NO:34. In some embodiments, the VL CDR3 is not SEQ ID NO:25.

[0155] In some embodiments, the one or more CDRs comprise the VH CDR1 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:1 to SEQ ID NO:4, theVH CDR2 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:5 to SEQ ID NO:14, and the VH CDR3 defined by the amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:15 to SEQ ID NO:18. In some embodiments, the VH CDR3 is not SEQ ID NO:15.

[0156] In some embodiments, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VL chain comprising the VL CDR1, VL CDR2, and VL CDR3 defined by the amino acid sequence of SEQ ID NO:19, SEQ ID NO:22, and SEQ ID NO:25, respectively. In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VL chain comprising the VL CDR1, VL CDR2, and VL CDR3 defined by the amino acid sequence of SEQ ID NO:20, SEQ ID NO:23, and SEQ ID NO:25, respectively. In yet another embodiment the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VL chain comprising the VL CDR1 and VL CDR2 defined by the amino acid sequence of SEQ ID NO:21, SEQ ID NO:24, respectively, and the VL CDR3 defined by the amino acid sequence of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 or SEQ ID NO:34. In a particular embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VL chain comprising the VL CDR1 defined by the amino acid sequence of SEQ ID NO:21, the VL CDR2 defined by the amino acid sequence of SEQ ID NO:24, and the VL CDR3 defined by the amino acid sequence of SEQ ID NO:32. In some embodiments, the VL CDR3 is not SEQ ID NO:25.

[0157] In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VH chain comprising theVH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:1, SEQ ID NO:5, and SEQ ID NO:15, respectively. In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VH chain comprising a VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:2, SEQ ID NO:6, and SEQ ID NO:16, respectively. In another embodiment, the anti-Flt-1 antibody or antigen-binding fragment thereof, comprises a VH chain comprising a VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:2, SEQ ID NO:10, and SEQ ID NO:18, respectively. In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VH chain comprising the VH CDR1 and the VH CDR3 defined by the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:17, respectively, and the VH CDR2 defined by the amino acid sequence of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NO:14. In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VH chain comprising the VH CDR1 and the VH CDR3 defined by the amino acid sequences of SEQ ID NO:3 and SEQ ID NO:17, respectively, and a VH CDR2 defined by the amino acid sequence of SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:12. In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VH chain comprising the VH CDR1, VH CDR2, and VH CDR3 defined by the amino acid sequences of SEQ ID NO:4, SEQ ID NO:9, and SEQ ID NO:17, respectively. In a particular embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a VH chain comprising the VH CDR1 defined by the amino acid sequence of SEQ ID NO:3, the VH CDR2 defined by the amino acid sequence of SEQ ID NO:12 and the VH CDR3 defined by the amino acid sequence of SEQ ID NO:17. In some embodiments, the VH CDR3 is not SEQ ID NO:15.

[0158] In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a light chain VL region comprising an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:49 to SEQ ID NO:61, and/or a heavy chain VH region comprising an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:35 to SEQ ID NO:48. In a particular embodiment, the VL region comprises the amino acid sequence of SEQ ID NO:60 and the VH region comprises the amino acid sequence of SEQ ID NO:45. In another embodiment, the antibody further comprises a heavy chain constant region comprising an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:87 to SEQ ID NO:89. In some embodiments, the VL region is not SEQ ID NO:49 or SEQ ID NO:50. In some embodiment, the VH region is not SEQ ID NO:35 or SEQ ID NO:36.

[0159] In another embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a light chain comprising an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:75 to SEQ ID NO:86, and/or a heavy chain comprising an amino acid sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity to any one of SEQ ID NO:62 to SEQ ID NO:74. In a particular embodiment, the light chain comprises the amino acid sequence of SEQ ID NO:76 and the heavy chain region comprises the amino acid sequence of SEQ ID NO:71.

[0160] In some embodiments, the heavy chain of the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises the amino acid sequence MGWSCIILFLVATATGVHSELQLVESGGGLVQPGGSLRLSCAASGFTFSDYSASWVRQA PGKGLEWVSAISWSGDSTYYAESVKGRFTIFRDNSKNTLYLQMNSLRAEDTAVYYCAK SWATPIESLYYYGSDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKX (SEQ ID NO:108). In some embodiments, the heavy chain of the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises the amino acid sequence ELQLVESGGGLVQPGGSLRLSCAASGFTFSDYSASWVRQAPGKGLEWVSAISWSGDST YYAESVKGRFTIFRDNSKNTLYLQMNSLRAEDTAVYYCAKSWATPIESLYYYGSDYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:109).

[0161] In some embodiments, the light chain of the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises the amino acid sequence MGWSCIILFLVATATGVHSSYELTQPLSVSVALRQAAKITCGGNNIGSQTAQWYQQKPG QAPVLVIYANNRRPSGIPERFSGSKSGNTATLTISRAQAGDEADYYCQVWDASTQAIVF GGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK

AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECSX (SEQ ID NO:110).

[0162] In some embodiments, the anti-Flt-1 antibody, or antigen-binding fragment thereof, comprises a heavy chain of SEQ ID NO:108 or SEQ ID NO:109 and a light chain of SEQ ID NO:110 or SEQ ID NO:76.

[0163] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, binds human Flt-1 with an affinity greater than about 10^{-7} M, greater than about 0.5 x 10^{-7}, greater than about 10^{-8}, greater than about 0.5 x 10^{-8}, greater than about 10^{-9} M, greater than about 0.5 x 10^{-9}, greater than about 10^{-10} M, greater than about 0.5 x 10^{-10} M, greater than about 10^{-11} M, greater than about 0.5 x 10^{-11} M, greater than about 10^{-12} M, or greater than about 0.5 x 10^{-12} M. In other embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, binds to mouse Flt-1 with an affinity greater than about 10^{-7} M, greater than about 0.5 x 10^{-7}, greater than about 10^{-8}, greater than about 0.5 x 10^{-8}, greater than about 10^{-9} M, greater than about 0.5 x 10^{-9}, greater than about 10^{-10} M, greater than about 0.5 x 10^{-10} M, greater than about 10^{-11} M, greater than about 0.5 x 10^{-11} M, greater than about 10^{-12} M, or greater than about 0.5 x 10^{-12} M. The affinity of an Flt-1 antibody may be measured, for example, in a surface plasmon resonance assay, such as a BIACORE assay.

[0164] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, is characterized by an IC_{50} below about 500 pM, below about 400 pM, below about 300 pM, below about 200 pM, below about 100 pM, below about 50 pM, below about 25 pM, below about 10 pM, below about 5 pM or below about 1 pM in a competition assay with human Flt-1. In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, is characterized by an IC_{50} below about 500 pM, below about 400 pM, below about 300 pM, below about 200 pM, below about 100 pM, below about 50 pM, below about 25 pM, below about 10 pM, below about 5 pM or below about 1 pM in a competition assay with mouse Flt-1.

[0165] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof inhibits the binding and/or activity of VEGF at the Flt-1 receptor. In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, is characterized by an IC_{50} below about

500 pM, below about 400 pM, below about 300 pM, below about 200 pM, below about 100 pM, below about 50 pM, below about 25 pM, below about 10 pM, below about 5 pM or below about 1 pM for inhibition of binding of VEGF to human Flt-1 in a competition assay.

[0166] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof completes with, and or inhibits VEGF binding to soluble Flt-1. In other embodiments, the competition, and or inhibition, is in a dose dependent manner. In particular embodiments, the inhibition of binding of VEGF to Flt-1 results in increased phosphorylation of VEGF R2. Without intending to be bound by theory, binding of the anti-Flt-1 antibody, or antigen-binding fragment thereof to Flt-1 inhibits the binding of VEGF to Flt-1. Unbound VEGF binds VEGF R2 which may be demonstrated by measuring phosphorylation of VEGF R2. In particular embodiments, the anti-Flt-1 antibody, or antigen-binding fragment rescues VEGF R2 phosphorylation in a dose dependent manner. For example, VEGF R2 phosphorylation may be rescued by at least about 100%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35% about 30%, about 25%, about 20%, about 15%, about 10% or about 5%.

[0167] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof provide greater than about 95%, greater than about 90%, greater than about 85%, greater than about 80%, greater than about 75%, greater than about 70%, greater than about 65%, greater than about 60%, greater than about 55%, greater than about 50%, greater than about 45%, greater than about 40%, greater than about 35%, greater than about 30%, greater than about 25%, greater than about 20%, greater than about 15%, or greater than about 10% rescue in a bioassay. In a particular embodiment the bioassay comprises human primary vein endothelial cells (HUVECs) stimulated with VEGF in the presence of sFlt-1 and an anti-Flt-1 antibody or antigen-binding fragment thereof. VEGF induced activation of cells may be assayed by determining the phosphorylation status of the VEGF R2 receptor. Data may be expressed as a percent rescue of the phosphorylation of the VEGF R2 receptor relative to the phosphorylation of the VEGF R2 receptor in the presence of sFlt-1 alone (e.g., without anti-Flt-1 antibodies).

[0168] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, has a half-life of greater than about 200 hours, greater than about 150 hours, greater than about 100 hours, greater than about 95 hours, greater than about 90 hours, greater than about 85 hours, greater than about 80 hours, greater than about 75 hours, greater than about 70 hours, greater than about 65 hours, greater than about 60 hours, greater than about 55 hours, greater than about 50 hours or greater than about 45 hours, and ranges therein. In some embodiments, the half-life is measured in a mouse.

[0169] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, has a maximum serum concentration of greater than about 400 ug/mL, greater than 375 ug/mL, greater than about 350 ug/mL, greater than about 325 ug/mL, greater than about 300 ug/mL, greater than about 275 ug/mL, greater than about 250 ug/mL, greater than about 225 ug/mL, greater than about 200 ug/mL, greater than about 175 ug/mL, greater than about 150 ug/mL, greater than about 125 ug/mL, greater than about 100 ug/mL, greater than about 75 ug/mL or greater than about 50 ug/mL, and ranges therein. In some embodiments, the maximum serum concentration is measured in a mouse.

[0170] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof selectively binds Flt-1 and has minimal or no appreciable binding to other VEGF receptors. In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof selectively binds Flt-1 and has minimal or no appreciable binding to VEGF R2 (Flk-1) and/or VEGF R3 (Flt-4).

[0171] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof has a *ka* of greater than about 1×10^{-3} M^{-1} sec^{-1}, greater than about 1×10^{-4} M^{-1} sec^{-1}, greater than about 1×10^{-5} M^{-1} sec^{-1}, greater than about 1×10^{-6} M^{-1} sec^{-1}, or greater than about 1×10^{-7} M^{-1} sec^{-1} when binding to human Flt-1.

[0172] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof has a *kd* of greater than about 1×10^{-3} sec^{-1}, greater than about 1×10^{-4} sec^{-1}, greater than about 1×10^{-5} sec^{-1} or greater than about 1×10^{-6} sec^{-1} when binding to human Flt-1.

[0173] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof has a K_D of greater than about 1×10^{-8} M, greater than about 1×10^{-9} M, greater than about 1×10^{-10} M, greater than about 1×10^{-11} M or greater than about 1×10^{-12} M when binding to human Flt-1.

[0174] In some embodiments an anti-Flt-1 antibody, or antigen-binding fragment thereof binds to soluble Flt-1. In particular embodiments, the binding is dose-dependent wherein higher concentrations of antibody, or antigen-binding fragment thereof, bind greater amounts of soluble Flt-1.

[0175] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof has a percent human identify of greater than about 99%, greater than about 98%, greater than about 97%, greater than about 96%, greater than about 95%, greater than about 94%, greater than about 93%, greater than about 92%, greater than about 91%, greater than about 90% or greater than about 80%.

[0176] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof has a percent human homology of greater than about 99%, greater than about 98%, greater than about 97%, greater than about 96%, greater than about 95%, greater than about 94%, greater than about 93%, greater than about 92%, greater than about 91%, greater than about 90% or greater than about 80%.

[0177] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof binds to Flt-1 protein. In some embodiment the Flt-1 protein is a recombinant protein, for example recombinant sFlt-1. In a particular embodiment, the anti-Flt-1 antibody, or antigen-binding fragment thereof binds human Flt-1 isoform 1 (NP_002010.2 GI:156104876; SEQ ID NO:90) (**Table 13**). In another embodiment the anti-Flt-1 antibody, or antigen-binding fragment thereof binds to human Flt-1 isoform X1 (XP_011533316.1 GI:767977511; SEQ ID NO:91). In another embodiment the anti-Flt-1 antibody, or antigen-binding fragment thereof binds to human Flt-1 isoform 2 precursor (NP_001153392.1 GI:229892220; SEQ ID NO:92). In yet another embodiment the anti-Flt-1 antibody, or antigen-binding fragment thereof binds to human Flt-1 isoform 3 precursor (NP_001153502.1 GI:229892300; SEQ ID NO:93). In another embodiment the anti-Flt-1 antibody, or antigen-binding fragment thereof binds to human Flt-1 isoform 4 precursor (NP_001153503.1 GI:229892302; SEQ ID NO:94).

[0178] In some embodiments the anti-Flt-1 antibody, or antigen-binding fragment thereof binds to a particular a epitope of the Flt-1 protein. For example, the anti-Flt-1 antibody or antigen-binding portion thereof binds to amino acids sequences as provided in **Table 3**.

Table 3.

amino acid position based on SEQ ID NO:90	Amino acid sequence	SEQ ID NO
141-153	EIPEIIHMTEGRE	SEQ ID NO:95
193-206	IISNATYKEIGLLT	SEQ ID NO:96
130-138	DTGRPFVEM	SEQ ID NO:97
141-148	EIPEIIHM	SEQ ID NO:98
139-148	YSEIPEIIHM	SEQ ID NO:99
139-153	YSEIPEIIHMTEGRE	SEQ ID NO:100
178-206	IPDGKRIIWDSRKGFIISNATYKEIGLLT	SEQ ID NO:101
199-204	YKEIGL	SEQ ID NO:102
128-138	ISDTGRPFVEM	SEQ ID NO:103

[0179] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof *in vivo* results in peak serum antibody levels of at least about 700 ug/mL, at least about 650 ug/mL, at least about 600 ug/mL, at least about 550 ug/mL, at least about 500 ug/mL, at least about 450 ug/mL, at least about 400 ug/mL, at least about 350 ug/mL, at least about 300 ug/mL, at least about 250 ug/mL, at least about 200 ug/mL, at least about 150 ug/mL, at least about 100 ug/mL, at least about 50 ug/mL, at least about 40 ug/mL, at least about

30 ug/mL, at least about 20 ug/mL, at least about 10 ug/mL or at least about 5 ug/mL, and ranges therein. In some embodiments, the peak serum antibody level is dose dependent.

[0180] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof *in vivo* results in trough serum antibody levels of at least about 450 ug/mL, at least about 400 ug/mL, at least about 350 ug/mL, at least about 300 ug/mL, at least about 250 ug/mL, at least about 200 ug/mL, at least about 150 ug/mL, at least about 100 ug/mL, at least about 50 ug/mL or at least about 25 ug/mL, and ranges therein. In some embodiments, the trough serum antibody level is dose dependent.

[0181] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof *in vivo* results in a decreased serum level of soluble Flt-1 as compared to a baseline level or as compared to a level in subjects administered vehicle alone. Typically, the baseline level is measured immediately before administration. In some embodiments, administration of the anti-Flt-1 antibody or antigen-binding fragment thereof results in a decreased serum level of soluble Flt-1 by at least about 95%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20% or about 10% as compared to the baseline serum level of soluble Flt-1 immediately before administration. In some embodiments, administration of the anti-Flt-1 antibody, or antigen-binding fragment thereof results in a decreased serum level of soluble Flt-1 to less than about 4000 pg/mL, about 3500 pg/mL, about 3000 pg/mL, about 2500 pg/mL, about 2000 pg/mL, about 1750 pg/mL, about 1500 pg/mL, about 1250 pg/mL, about 1000 pg/mL, about 900 pg/mL, about 800 pg/mL, about 700 pg/mL, about 600 pg/mL, about 500 pg/mL, about 450 pg/mL, about 400 pg/mL, about 350 pg/mL, about 300 pg/mL, about 250 pg/mL, about 200 pg/mL, about 150 pg/mL, about, 100 pg/mL, about 50 pg/mL or about 10 pg/mL, and ranges therein. In some embodiments, administration of the anti-Flt-1 antibody, or antigen-binding fragment thereof results in a decreased serum level of soluble Flt-1 as compared to the serum level of soluble Flt-1 in a subject who is not administered the antibody or antigen-binding fragment thereof. In some embodiments, the decreased serum level of soluble Flt-1 is dose dependent.

[0182] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof *in vivo* results in an increased serum level of VEGF as compared to a baseline level or as compared to a level in subjects treated with vehicle alone. Typically, the baseline level is measured immediately before treatment. In some embodiments, administration of the anti-Flt-1 antibody or antigen-binding fragment thereof results in an increased serum level of VEGF by at least about 95%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20% or about 10% as compared to the baseline serum level of VEGF immediately before administration. In some embodiments, administration of the anti-Flt-1 antibody, or antigen-binding fragment thereof results in an increased serum level of VEGF to more than about 500 pg/mL, about 450 pg/mL, about 400 pg/mL, about 350 pg/mL, about 300 pg/mL, about 250 pg/mL, about 200 pg/mL, about 150 pg/mL, about 100 pg/mL about 50 pg/mL or about 25 pg/mL and ranges therein. In some embodiments, administration of the anti-Flt-1 antibody, or antigen-binding fragment thereof results in an increased serum level of VEGF as compared to the serum level of VEGF in a subject who is not treated. In some embodiments, the increased serum level of VEGF is dose dependent.

[0183] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof *in vivo* results in increased angiogenesis in muscle tissue. In some embodiments the muscle is skeletal muscle. In particular embodiments, the muscle is the diaphragm muscle, the gastrocnemius muscle and/or the tibialis anterior (TA) muscle. In some embodiments, the increased angiogenesis is demonstrated by increased CD31 staining of an endothelial cell marker, for example, CD31. In some embodiments, the increased staining may be measured, for example, by measuring the percent CD31 positive area in muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof. For example, the percent CD31 positive area in the diaphragm muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof may be at least about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4% or about 2.5% of the total tissue area. In a further example, the percent CD31 positive area in the TA muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof may be at least about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9% or about 1.0% pf the total tissue area. In a particular embodiment, the percent

CD31 positive area in the diaphragm muscle or TA muscle of mice administered the anti-Flt-1 antibody may be significantly higher than the percent CD31 positive area in the diaphragm muscle or TA muscle of mice administered an isotype control antibody.

[0184] In some embodiments, the increased staining of an endothelial cell marker may be measured, for example, by measuring the normalized CD31 percent positivity in the muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof. In particular embodiments the increased CD31 staining the muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof, is relative to CD31 staining measured in the muscle of mice administered an isotype control antibody. For example, the normalized CD31 percent positivity in the diaphragm muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof may be at least about 200%, about 190%, about 180%, about 170%, about 160%, about 150%, about 140%, about 130%, about 120% or about 110%. In a further example, the normalized CD31 percent positivity in the TA muscle of mice administered the anti-Flt-1 antibody, or antigen-binding fragment thereof may be at least about 300%, about 290%, about 280%, about 270%, about 260%, about 250%, about 240%, about 230%, about 220%, about 210%, about 200%, about 190%, about 180%, about 170%, about 160%, about 150%, about 140%, about 130%, about 120% or about 110%, and ranges therein.

[0185] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof selectively binds human Flt-1, and has minimal or no appreciable binding to other mammalian Flt-1 receptors (e.g., with a binding affinity less than 10^{-7} M or 10^{-6} M). In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof selectively binds human Flt-1 and does not bind to monkey Flt-1. In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof selectively binds human Flt-1 and does not bind to mouse Flt-1.

[0186] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof binds human Flt-1 as well as monkey Flt-1. In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof binds to cynomolgus Flt-1. In some embodiments an anti-Flt-1 antibody, or antigen-binding fragment thereof binds human Flt-1 as well as mouse Flt-1.

[0187] In some embodiments, an anti-Flt-1 antibody, or antigen-binding fragment thereof, is selected from the group consisting of IgG, $F(ab')_2$, $F(ab)_2$, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies.

[0188] In some embodiments an anti-Flt-1 antibody, or antigen-binding fragment thereof, is IgG. In some embodiments an anti-Flt-1 antibody, or antigen-binding fragment thereof, is IgG1.

Engineered Constant Regions

[0189] In some embodiments, a suitable anti-Flt-1 antibody contains an Fc domain or a portion thereof that binds to the FcRn receptor. As a non-limiting example, a suitable Fc domain may be derived from an immunoglobulin subclass such as IgG. In some embodiments, a suitable Fc domain is derived from IgG1, IgG2, IgG3, or IgG4. Particularly suitable Fc domains include those derived from human or humanized antibodies.

[0190] It is contemplated that improved binding between Fc domain and the FcRn receptor results in prolonged serum half-life. Thus, in some embodiments, a suitable Fc domain (SEQ ID NO:104) comprises one or more amino acid mutations that lead to improved binding to FcRn. Various mutations within the Fc domain that effect improved binding to FcRn are known in the art and can be adapted to practice the present invention. In some embodiments, a suitable Fc domain comprises one or more mutations at one or more positions corresponding to Leu 234, Leu 235, Gly 237, Thr 250, Met 252, Ser 254, Thr 256, Thr 307, Glu 380, Met 428, His 433, and/or Asn 434 of human IgG1.

[0191] Some mutations in the Fc domain lead to reduced binding of the IgG with the FcRn receptor and thereby inhibit effector function. In some embodiments, a suitable Fc domain (SEQ ID NO:104) comprises one or more mutations at one or more positions corresponding to Leu 234, Leu 235 and Gly 237 of human IgG1. In a particular embodiment Leu 234 is mutated to Ala. In another embodiment Leu 235 is mutated to Ala. In yet another embodiment, Gly 237 is mutated to Ala.

[0192] In some embodiments, an anti-FLT-1 antibody or antigen-binding fragment contains a spacer and/or is linked to another entity. In some embodiments, the linker or spacer comprises a sequence at least 50% (e.g., at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to GAPGGGGGAAAAAGGGGGGAP (SEQ ID NO:105) (GAG linker). In some embodiments, the linker or spacer comprises a sequence at least 50% (e.g., at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to GAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAP (SEQ ID NO:106) (GAG2 linker). In some embodiments, the linker or spacer comprises a sequence at least 50% (e.g., at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to GAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGG GAP (SEQ ID NO:107) (GAG3 linker).

Production of Anti-Flt-1 antibodies and antigen-binding fragments

[0193] A recombinant anti-Flt-1 antibody or antigen-binding fragment thereof, suitable for the present invention may be produced by any available means. For example, a recombinant anti-Flt-1 antibody or antigen-binding fragment may be recombinantly produced by utilizing a host cell system engineered to express a recombinant anti-Flt-1 antibody or antigen-binding fragment-encoding nucleic acid.

[0194] Thus, the present invention further provides polynucleotide sequences encoding the various amino acid sequences described herein. In some embodiments, the present invention provides a polynucleotide sequence encoding an anti-Flt-1 antibody heavy chain or light chain amino acid sequence described herein, for example, any one of SEQ ID NOs:62-86 or SEQ ID NOs:108-110. In some embodiments, the present invention provides a polynucleotide sequence encoding a variable region of an anti-Flt-1 antibody heavy chain or light chain amino acid sequence described herein, for example, any one of SEQ NOs:35-61. In some embodiments, the present invention provides a polynucleotide sequence encoding a CDR region of an anti-Flt-1 antibody heavy chain or light chain amino acid sequence described herein, for example, any one of SEQ ID NOs:1-34. In some embodiments, the present invention provides a polynucleotide sequence encoding an anti-Flt-1 antibody constant region amino acid sequence described herein, for example, any one of SEQ ID NO:87-89. In some embodiments, the present invention provides a polynucleotide sequence encoding an anti-Flt-1 antibody Fc region amino acid sequence described herein, for example, SEQ ID NO:104. In some embodiments, the present invention provides a polynucleotide sequence encoding an anti-Flt-1 antibody linker amino acid sequence described herein, for example, SEQ ID NOs:105-107.

[0195] In some embodiments, a polynucleotide sequence encoding an anti-Flt-1 antibody heavy chain, light chain, variable region, CDR region, Fc region or linker region amino acid sequence further includes a sequence encoding a signal peptide. As a non-limiting example, a suitable signal peptide includes amino acid sequence MGWSCIILFLVATATGVHS (SEQ ID NO:111).

[0196] Various polynucleotide sequences described herein may be embodied in various vector systems for expression of recombinant anti-Flt-1 antibodies or antigen-binding fragment thereof.

[0197] Where antibodies are recombinantly produced, any expression system can be used. To give but a few examples, known expression systems include, for example, egg, baculovirus, plant, yeast, or mammalian cells.

[0198] In some embodiments, a recombinant anti-Flt-1 antibody or antigen-binding fragment thereof suitable for the present invention are produced in mammalian cells. Non-limiting examples of mammalian cells that may be used in accordance with the present invention include BALB/c mouse myeloma line (NSO/l, ECACC No: 85110503); human retinoblasts (PER.C6, CruCell, Leiden, The Netherlands); and monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651).

[0199] In some embodiments, the present invention provides a recombinant anti-Flt-1 antibody or antigen-binding fragment thereof produced from human cells. In some embodiments, the present invention provides an anti-Flt-1 antibody or antigen-binding fragment thereof produced from CHO cells.

Pharmaceutical Compositions Containing the Antibodies of the Invention

[0200] The present invention further provides pharmaceutical compositions comprising therapeutically active ingredients in accordance with the invention (e.g. anti-Flt-1 antibody, or antigen-binding fragment thereof), together with one or more pharmaceutically acceptable carrier or excipient. Such pharmaceutical compositions may optionally comprise one or more additional therapeutically-active substances.

[0201] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation.

[0202] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a diluent or another excipient or carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0203] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0204] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient or carrier, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0205] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient or carrier, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium or carrier is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[0206] In some embodiments, a pharmaceutically acceptable excipient or carrier is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient or carrier is approved for use in humans and for veterinary use. In some embodiments, an excipient or carrier is approved by United States Food and Drug Administration. In some embodiments, an excipient or carrier is pharmaceutical grade. In some embodiments, an excipient or carrier meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0207] Pharmaceutically acceptable excipients or carriers used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients or carriers may optionally be included in pharmaceutical formulations. Excipients or carriers such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

[0208] Suitable pharmaceutically acceptable excipients or carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, sugars such as mannitol, sucrose, or others, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents (e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like) which do not deleteriously react with the active compounds or interfere with their activity. In a preferred embodiment, a water-soluble carrier suitable for intravenous administration is used.

[0209] A suitable pharmaceutical composition or medicament, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. A composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. A composition can also be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[0210] A pharmaceutical composition or medicament can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, in some embodiments, a composition for intravenous administration typically is a solution in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0211] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

Routes of Administration

[0212] An anti-Flt-1 antibody or antigen-binding fragment thereof described herein (or a composition or medicament containing an anti-Flt-1 antibody or antigen-binding fragment thereof described herein) is administered by any appropriate route. In some embodiments, an anti-Flt-1 antibody or antigen-binding fragment protein or a pharmaceutical composition containing the same is administered parenterally. Parenteral administration may be intravenous, intradermal, intrathecal, inhalation, transdermal (topical), intraocular, intramuscular, subcutaneous, intramuscular, and/or transmucosal administration. In some embodiments, an anti-Flt-1 antibody or antigen-binding fragment thereof or a pharmaceutical composition containing the same is administered subcutaneously. As used herein, the term “subcutaneous tissue”, is defined as a layer of loose, irregular connective tissue immediately beneath the skin. For example, the subcutaneous administration may be performed by injecting a composition into areas including, but not limited to, the thigh region, abdominal region, gluteal region, or scapular region. In some embodiments, an anti-Flt-1 antibody or antigen-binding fragment thereof or a pharmaceutical composition containing the same is administered intravenously. In some embodiments, an anti-Flt-1 antibody or antigen-binding fragment thereof or a pharmaceutical composition containing the same is administered orally. More than one route can be used concurrently, if desired.

[0213] In some embodiments, administration results only in a localized effect in an individual, while in other embodiments, administration results in effects throughout multiple portions of an individual, for example, systemic effects. Typically, administration results in delivery of an anti-Flt-1 antibody or antigen-binding fragment to one or more target tissues including but not limited to kidney, liver, brain, spinal cord, intestinal tract, eye, lung, spleen, heart, including cardiac muscle, striated muscle, and smooth muscle.

[0214] In some embodiments, striated muscle is selected from the group consisting of triceps, tibialis anterior, soleus, gastrocnemius, quadriceps, and diaphragm.

[0215] In some embodiments, the smooth muscle is the muscles lining blood vessels, bronchioles, bladder, and gastrointestinal tract such as rectum.

Dosage Forms and Dosing Regimen

[0216] In some embodiments, a composition is administered in a therapeutically effective amount and/or according to a dosing regimen that is correlated with a particular desired outcome (e.g., with treating or reducing risk for a muscular dystrophy, such as Duchenne muscular dystrophy).

[0217] Particular doses or amounts to be administered in accordance with the present invention may vary, for example, depending on the nature and/or extent of the desired outcome, on particulars of route and/or timing of administration, and/or on one or more characteristics (e.g., weight, age, personal history, genetic characteristic, lifestyle parameter, severity of cardiac defect and/or level of risk of cardiac defect, etc., or combinations thereof). Such doses or amounts can be determined by those of ordinary skill. In some embodiments, an appropriate dose or amount is determined in accordance with standard clinical techniques. Alternatively or additionally, in some embodiments, an appropriate dose or amount is determined through use of one or more *in vitro* or *in vivo* assays to help identify desirable or optimal dosage ranges or amounts to be administered.

[0218] In various embodiments, an anti-Flt-1 antibody or antigen-binding fragment thereof is administered at a therapeutically effective amount. Generally, a therapeutically effective amount is sufficient to achieve a meaningful benefit to the subject (e.g., treating, modulating, curing, preventing and/or ameliorating the underlying disease or condition). In some particular embodiments, appropriate doses or amounts to be administered may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0219] In some embodiments, a provided composition is provided as a pharmaceutical formulation. In some embodiments, a pharmaceutical formulation is or comprises a unit dose amount for administration in accordance with a dosing regimen correlated with achievement of the reduced incidence or risk of a muscular dystrophy, such as Duchenne muscular dystrophy.

[0220] In some embodiments, a formulation comprising an anti-Flt-1 antibody or antigen-binding fragment described herein administered as a single dose. In some embodiments, a formulation comprising an anti-Flt-1 antibody or antigen-binding fragment described herein is administered at regular intervals. Administration at an "interval," as used herein, indicates that the therapeutically effective amount is administered periodically (as distinguished from a one-time dose). The interval can be determined by standard clinical techniques. In some embodiments, a formulation comprising an anti-Flt-1 antibody or antigen-binding fragment described herein is administered bimonthly, monthly, twice monthly, triweekly, biweekly, weekly, twice weekly, thrice weekly, daily, twice daily, or every six hours. The administration interval for a single individual need not be a fixed interval, but can be varied over time, depending on the needs of the individual. In a particular embodiment the anti-Flt-1 antibody, or antigen-binding fragment thereof is administered twice weekly.

[0221] As used herein, the term "bimonthly" means administration once per two months (i.e., once every two months); the term "monthly" means administration once per month; the term "triweekly" means administration once per three weeks (*i.e.*, once every three weeks); the term "biweekly" means administration once per two weeks (*i.e.*, once every two weeks); the term "weekly" means administration once per week; and the term "daily" means administration once per day.

[0222] In some embodiments, a formulation comprising an anti-Flt-1 antibody or antigen-binding fragment described herein is administered at regular intervals indefinitely. In some embodiments, a formulation comprising an anti-Flt-1 antibody or antigen-binding fragment described herein is administered at regular intervals for a defined period.

[0223] As described herein, the term "therapeutically effective amount" is largely determined based on the total amount of the therapeutic agent contained in the pharmaceutical compositions of the present invention. A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular composition, a therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration or on combination with other pharmaceutical agents.

[0224] In some embodiments, the anti-Flt-1 antibody, or antigen-binding fragment thereof is administered at a dose ranging from about 0.1 mg/kg to about 50 mg/kg. In other embodiments the anti-Flt-1 antibody, or antigen-binding fragment thereof is administered at a dose ranging from about 0.1 mg/kg to about 40 mg/kg, from about 0.1 mg/kg to about 30 mg/kg, from about 0.1 mg/kg to about 20 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 5 mg/kg, from about 0.1 mg/kg to about 3 mg/kg, from about 0.1 mg/kg to about 1 mg/kg, from about 1.0 mg/kg to about 40 mg/kg, from about 1.0 mg/kg to about 30 mg/kg, from about 1.0 mg/kg to about 20 mg/kg, from about 1.0 mg/kg to about 10 mg/kg, from about 1.0 mg/kg to about 5 mg/kg or from about 1.0 mg/kg to about 3 mg/kg. In particular embodiments, the anti-Flt-1 antibody, or antigen-binding fragment thereof is administered at a dose of about 1.0 mg/kg, about 3.0 mg/kg, about 10 mg/kg or about 20 mg/kg.

[0225] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof reduces the intensity, severity, or frequency, or delays the onset of at least one DMD sign or symptom. In some embodiments administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof reduces the intensity, severity, or frequency, or delays the onset of at least one DMD sign or symptom selected from the group consisting of muscle wasting, skeletal deformation, cardiomyopathy, muscle ischemia, cognitive impairment, and impaired respiratory function.

[0226] In some embodiments, administration of an anti-Flt-1 antibody, or antigen-binding fragment thereof improves clinical outcome as measured by a 6 minute walk test, quantitative muscle strength test, timed motor performance test. Brooke and Vignos limb function scales, pulmonary function test (forced vital capacity, forced expiratory volume in 1 second, peak expiratory flow rate, maximal inspiratory and expiratory pressures), health-related quality of life, knee and elbow flexors, elbow extensors, shoulder abduction, grip strength, time to rise from supine position, North Start Ambulatory Assessment, timed 10 meter walk/run, Egen-Klassification scale, Gowers score, Hammersmith motor ability, hand held myometry, range of motion, goniometry, hypercapnia, Nayley Scales of Infant and Toddler Development, and/or a caregiver burden scale.

Combination Therapy

[0227] In some embodiments, an anti-Flt-1 antibody or antigen-binding fragment thereof is administered in combination with one or more additional therapeutic agents. In one embodiment the additional therapeutic agent is a corticosteroid, e g., prednisone. In another embodiment, the additional therapeutic agent is a glucocorticoid, e.g., deflazacort. In another embodiment, the additional therapeutic agent is follistatin or a recombinant protein thereof. In another embodiment the additional therapeutic agent is an RNA modulating therapeutic. The RNA modulating therapeutic may be an exon-skipping therapeutic or gene therapy. The RNA modulating therapeutic may be, for example, Drispersen, PRO044, PRO045, Eteplirsen (AVI-4658), SRP-4053, SRP-4045, SRP-4050, SRP-4044, SRP-4052, SRP-4055 or SRP-4008. In some embodiments the additional therapeutic agent is currently used for treatment of a muscular dystrophy. In other embodiments the additional therapeutic agent may also be used to treat other diseases or disorders. In some embodiments, the known therapeutic agent(s) is/are administered according to its standard or approved dosing regimen and/or schedule. In some embodiments, the known therapeutic agent(s) is/are administered according to a regimen that is altered as compared with its standard or approved dosing regimen and/or schedule. In some embodiments, such an altered regimen differs from the standard or approved dosing regimen in that one or more unit doses is altered (e.g., reduced or increased) in amount, and/or in that dosing is altered in frequency (e.g., in that one or more intervals between unit doses is expanded, resulting in lower frequency, or is reduced, resulting in higher frequency).

EXAMPLES

Example 1. Generation and characterization of high affinity anti-Flt-1 antibodies

Generation of antibodies

[0228] Monoclonal antibodies were generated against soluble Flt-1 using llama monoclonal antibody methodology. Briefly, llamas were immunized with recombinant human soluble Flt-1 (purchased from ABCAM) and the serum was collected.

Antibody characterization

[0229] Antibodies that bound to human and mouse Flt-1 were further characterized for 1) VH family; 2) affinity for Flt-1; 3) IC50; 4) off-rate screening by Biacore assay, 5) cross-reactivity to cynomolgus Flt-1 and 6) binding to VEGF R2 and VEGF R3. Candidate antibodies against human Flt-1 (hFl-1) and mouse Flt-1 (mFlt-1) were characterized as shown in **Table 4**.

Table 4.

Antibody	VH family	Affinity (nM)		IC50 by ELISA (pM)		Bioassay (% rescue)		Human identity (%)	
		hFlt-1	mFlt-1	hFlt-1	mFlt-1	hFlt-1	mFlt-1	VH	VL
13B4	15	0.6	1.4	13.3	500	>90	72.2	94.3	93.7
10G12	17	0.29	0.33	400	300	44	87.5	90.8	81
11A11	20	0.2		33.3		>90		90.8	91.1

[0230] Pharmacokinetic properties of antibody 13B4 and 10G12 were studied in mice by intravenous administration of 10 mg/kg of each antibody (**Table 5**). The data demonstrated that antibody 10G12 cannot be detected beyond 288 hours, while antibody 13B4 can be detected at 672 hours (**Figures 1A-1B**).

Table 5.

Antibody	**$t_{1/2}$ (h)**	**Cmax (ng/ml)**	**Tmax (hr)**	**AUC $_{0\text{-last}}$ (hr*ng/ml)**	**AUC $_{0\text{-inf}}$ (hr*ng/ml)**	**CL (ml/hr/kg)**	**R^2**
13B4	87.7	293675	1	31669464	31926743	0.31	0.960
10G12	99.3	359268	0.5	15999900	19120025	0.52	0.944

In vivo efficacy of antibodies

[0231] *Mdx* mice (i.e., a mouse model of Duchenne muscular dystrophy) were treated with 20 mg/kg of either antibody 13B4 or antibody 10G12 by intravenous administration twice a week for one month beginning at 4 weeks of age. Control mice were treated with vehicle only, an isotype control antibody that does not bind to Flt-1 or a commercial anti-Flt-1 antibody known as Flt-1:VEGF antagonist (Angio Proteomie, catalog number AP-MAB0702). To assess serum antibody concentration at a trough exposure point, blood was collected 4 days after the fifth intravenous dose. To assess serum antibody concentration at a peak exposure point, blood was collected 24 hours after the last dose. Peak and trough concentration of antibodies 13B4 and 10G12 are shown in **Figures 2A** and **2B**. The concentration of free antibody 13B4 and free antibody 10G12 in blood was higher than that of the isotype control antibody and the commercial control antibody at both the peak and trough exposure time points.

[0232] To assess serum free sFlt-1 levels and VEGF levels, blood was collected 24 hours after the fifth intravenous dose and prior to sacrifice. Administration of antibody 13B4 and antibody 10G12, as well as the commercial control antibody, significantly decreased the serum concentration on sFlt-1 ($p<0.0001$) as compared to the isotype control antibody (**Figure 3**). Administration of antibody 13B4 and antibody 10G12, resulted in a significant increase in serum levels of VEGF as compared to the isotype control antibody ($p<0.001$) (**Figure 4**).

Administration of the commercial control antibody also resulted in a significant increase in blood levels of VEGF as compared to the isotype control antibody ($p<0.05$) (**Figure 4**).

Histopathology

[0233] Mice were sacrificed at the end of the 30 day treatment period and the diaphragm muscle and tibialis anterior (TA) muscle were collected and sectioned to determine if the anti-Flt-1 antibodies induced angiogenesis in skeletal muscle. Sections of muscle were stained with the endothelial cell marker CD31. A significant increase in capillary density was seen in the diaphragms of mice treated with antibody, 13B4, 10G12 or the commercial control antibody as compared to the diaphragms of mice treated with the isotype control antibody (**Figures 5A-5D**). The data were quantified using automated quantitative imaging software as shown in **Figures 6A-6B**. There was a significant increase in the CD31 positive area in diaphragms of mice treated with the commercial control antibody ($p<0.05$), antibody 13B4 ($p<0.01$) and antibody 10G12 ($p<0.0001$) as compared to the diaphragms of mice treated with the isotype control antibody. A significant increase in the CD31 positive area of the tibialis anterior (TA) muscle of mice treated with antibody 10G12 ($p<0.01$) as compared to the tibialis anterior muscle of mice treated with the isotype control antibody was also demonstrated.

[0234] This studies demonstrated that administration of an Flt-1 antibody (e.g., 10G12 and 13B4) to *mdx* mice resulted in a significant increase in endothelial cell proliferation as well as a decrease in soluble Flt-1 in serum and an increase in serum VEGF concentrations. These antibodies demonstrated binding affinity for the Flt-1 target in the pM range (see **Table 4**), IC50 for Flt-1 binding of less than 100 pM (see Table 4) and greater than 50% rescue of VEGF signaling in a bioassay.

Example 2. Generation and characterization of high affinity anti-Flt-1 antibodies

[0235] Additional anti-Flt-1 monoclonal antibodies were generated as described above. These antibodies were further characterized for binding affinity to sFlt-1 antigen (by ELISA and Biacore), competition for VEGF in a sFlt-1:VEGF competition ELISA; and performance in a cell based assay.

Antibody characterization – Binding to target

[0236] The monoclonal anti-Flt-1 antibodies were assayed for binding to recombinant sFlt-1 antigen in an ELISA assay (**Figure 7**). All antibodies demonstrated a dose-dependent increase in binding. Binding affinity of the anti-Flt-1 antibodies to mouse and human Flt-1 antigen was measured by surface plasmon resonance methodology (i.e., Biacore) (**Table 6**). The antibodies bound to human Flt-1 in the nanomolar range with antibody 11A11 demonstrating the highest binding affinity for human Flt-1. Biacore analysis also demonstrated that the antibodies did not cross-react with VEGF R2 or VEGF R3 (**Table 6**), however all antibodies did cross react with cynomolgus Flt-1.

Table 6.

	Family	Affinity (nM)		Binding	
		Human	Mouse	VEGFR2	VEGFR3
13B4	15	0.58	1.4	-	-
10G2		1.5	1.4	-	-
10G5		1.6	4.2	-	-
18H2		1.8	3.2	-	-
18A10		1.2	2	-	-
18C5		2.7	9.2	-	-
18C12		1.4	2	-	-
18B6	21	1.7	1.2	-	-
16B3	19	2.7	3	-	-
10G12	17	3.1	6.9	-	-
16B12	18	2.7	5.7	-	-
Angio		2.3	8.2		
11A11	20	0.16		-	-

Antibody characterization – Competition/Antagonism

[0237] To estimate the potency of the antibodies, the antibodies were assay in a competition ELISA using human sFlt-1 and VEGF. Antibody concentrations tested ranged from 0.1 mg/mL to 10,000 ng/mL. The commercial anti-Flt-1 antibody served as a control. With the exception of 11A11, all antibodies were able to prevent binding of VEGF to sFlt-1 (**Figure 8**).

Antibody characterization – Cell based assay

[0238] Human primary vein endothelial cells (HUVECs) were stimulated with VEGF in the presence of sFlt-1 and monoclonal antibodies 02G07, 11A11 and 13B4. VEGF induced activation of cells was assayed by determining the phosphorylation status of the VEGF R2 receptor. The data are expressed as a percent rescue of the phosphorylation of the VEGF R2 receptor relative to the phosphorylation of the VEGF R2 receptor in the presence of sFlt-1 alone (e.g., without anti-Flt-1 antibodies). The monoclonal antibodies rescued cell activation (i.e., phosphorylation) by antagonizing soluble Flt-1 (**Figure 9**).

Example 3. Characterization of high affinity anti-Flt-1 antibodies generated by light chain shuffling

[0239] Light chain shuffling of antibodies 18B6, 11A11 and 13B4 described in Example 2 was performed to increase the affinity and potency of the candidate antibodies.

Antibody characterization – Binding to target

[0240] The resulting antibodies displayed increased affinity for the Flt-1 antigen. For instance the K_D of antibody 21C6 increased approximately 10-fold over that of the parent antibody, 11A11. Similarly, the K_D of antibody 21B3 increased approximately 5-fold over that of the parent antibody, 13B4 (**Table 7**).

Table 7.

	ka (1/Ms)	kd (1/s)	KD (M)
18B6	3.95E+05	3.25E-04	8.22E-10
24A8	2.24E+05	1.35E-04	6.05E-10
21F9	2.84E+05	2.42E-04	8.52E-10
11A11	3.17E+05	5.32E-05	1.68E-10
21C6	1.31E+06	2.62E-05	2.00E-11
21B6	7.59E+05	6.50E-05	8.57E-11
13B4	4.16E+05	1.62E-04	3.89E-10
21B3	5.58E+05	4.73E-05	8.48E-11
21A1	2.23E+05	1.15E-04	5.16E-10
21D1	8.44E+05	1.44E-04	1.71E-10
21B4	4.96E+05	1.58E-04	3.19E-10
19H6	3.32E+05	1.09E-04	3.29E-10

Antibody characterization – Competition/Antagonism

[0241] To estimate the potency of the antibodies following the light chain shuffling, the antibodies were assay in a competition ELISA using human sFlt-1 and VEGF. Antibody concentrations tested ranged from 0.2 mg/mL to 200 ng/mL. The ability of parent antibody 13B4 to competitively bind sFlt-1 was compared to that of antibodies generated by light chain shuffling. All antibodies demonstrated a dose dependent inhibition of binding of VEGF to human sFlt-1 with clone 21B3 the most effective competitor (**Figure 10**).

In vivo efficacy

[0242] To determine the serum half-life and pharmacokinetic characteristics of the light chain shuffled antibodies, mice were administered a single 10 mg/kg dose of light chain shuffled antibodies 27H9 and 21B3 and parent antibody 13B4 each labeled with I^{125}. Serum was collected at 0.083, 0.25, 0.5, 1, 4, 8, and 24 hours and at 3, 5, 7, 14, 21 and 28 days and the serum concentration of the antibodies determined. The serum half-life was reduced in the light shuffled antibodies as compared to the parent antibody (**Figure 11**). However, the light chain shuffled antibodies showed improved pharmacodynamic characteristics as compared to the parent. For instance, antibody 27H9 reached a maximum concentration of 222.4 ug/mL by 0.083

hours whereas the parent antibody 13B4 reached a maximum concentration of 217 ug/mL by 0.5 hours (**Table 8**).

Table 8.

TA	T1/2 (hr)	Cmax (ug/ml)	Tmax (hr)	AUC0-last (hr*ug/ml)	AUC 0-inf (hr*ug/ml)	Vss (ml/kg)	Cl (ml/hr/kg)	V0 (ml/kg)	R2
13B4	129.4	217.3	0.5	27044	27798	71	0.36	48.7	0.97
21B3	56.5	215.8	0.083	12590	12602	73	0.79	42.9	0.95
27H9	62.8	222.4	0.083	14318	14329	68	0.7	44.3	0.96

Histopathology

[0243] To determine if the light chain shuffled and parent antibodies could induce endothelial cell proliferation, *mdx* mice were treated with 20 mg/kg of antibody biweekly for 4 weeks by intravenous administration. Mice were sacrificed at the end of the treatment period and the diaphragm and tibialis anterior muscles were collected and sectioned to determine if the antibodies induced angiogenesis in skeletal muscle. Sections of muscle were stained with the endothelial cell marker CD31. A significant increase in capillary density in the diaphragm was seen in the mice treated with antibody 13B4 and 21B3 as compared to the diaphragms of mice treated with the isotype control antibody (**Figures 12A-12C**). In addition, there was a significant increase in capillary density in the tibialis anterior from mice treated with the light chain shuffled antibody 21B3 as compared to the tibialis anterior muscle from mice treated with the isotype control antibody (**Figures 12D-12F**).

[0244] The biodistribution of antibodies 27H9, 13B4 and 21B3 in the diaphragm, tibialis and gastrocnemius muscles was determined using ^{125}I labeled antibodies. The diaphragm showed the highest exposure for all antibodies over the time course (**Figures 13A-13C**).

[0245] These studies demonstrated that administration of an Flt-1 antibody (i.e., 13B4 and 21B3) to *mdx* mice resulted in a significant increase in endothelial cell proliferation.

Example 4. *In vivo* efficacy of high affinity anti-Flt-1 antibody 21B3

[0246] *Mdx* mice were treated with 1, 3, 10 or 20 mg/kg of antibody 21B3, or 20 mg/kg of an isotype control antibody, by intravenous administration twice a week for one month beginning at 4 weeks of age. To assess serum antibody concentration at a trough exposure point, blood was collected 4 days after the fifth intravenous dose. To assess blood antibody concentration at a peak exposure point, blood was collected 24 hours after the last dose. Peak and trough serum concentrations of antibody 21B3 and the isotype control antibody are shown in **Figure 14A** and **14B**. The peak and trough levels of antibody 21B3 were dose dependent and higher than the isotype control antibody.

[0247] To assess serum levels of free sFlt-1, blood was collected on days 0, 14 and 28. Administration of antibody 21B3 induced a dose dependent decrease in serum free sFlt-1 levels. The response was more durable at the 10 and 20 mg/kg doses as seen at both day 14 and day 28. However, a statistically significant decrease was observed is free sFlt-1 levels at the 3, 10 and 20 mg/kg doses as compared to the free sFlt-1 level in mice treated with vehicle alone (**Figure 15**).

[0248] To assess serum levels of VEGF, blood was collected on days 0, 14 and 28. Administration of antibody 21B3 induced a dose dependent increase in serum VEGF levels. As observed for the free sFlt-1 levels, the response was more durable at the 10 and 20 mg/kg doses and at both day 14 and day 28. In fact, a statistically significant increase in serum VEGF was observed at the 10 and 20 mg/kg doses of antibody 21B3 as compared to serum VEGF levels in mice treated with vehicle alone (**Figure 16**).

Histopathology

[0249] Mice were sacrificed at the end of the 30 day treatment period and the diaphragm and tibialis anterior muscles were collected and sectioned to determine if the anti-Flt-1 antibody induced angiogenesis in skeletal muscle. Sections of muscle were stained for the endothelial cell marker CD31. A significant increase in capillary density in the diaphragm muscles of mice treated with antibody 21B3 was seen as compared to the capillary density in the diaphragm muscles of mice treated with the isotype control antibody (**Figures 17A-17E**). The data were quantified using automated quantitative imaging software as shown in **Figure 18**. There was a significant increase in the CD31 positive area in the diaphragm muscles of mice treated with 10 mg/kg or 20 mg/kg ($p<0.0001$) as compared to the CD31 positive area in the diaphragm muscles of mice treated isotype control antibody.

[0250] A significant increase in the capillary density in the tibialis anterior muscle was also seen in the mice treated with antibody 21B3 as compared to the tibialis anterior muscle in mice treated with the isotype control antibody (**Figures 19A-19E**). The data were quantified using automated quantitative imaging software as shown in **Figure 20**. There was a significant increase in the CD31 positive area in the tibialis anterior muscle of mice treated with 10 mg/kg or 20 mg/kg ($p<0.0001$) as compared to the CD31 positive area in the tibialis anterior muscle of mice treated with isotype control antibody.

RP-LC/MS Characterization

[0251] The molecular weight of the deglycosylated 21B3 antibody was determined by reverse phase liquid chromatography/mass spectrometry (RP-LC/MS) (**Figure 21A**). Following a reduction reaction, the molecular weight of the light chain and the heavy chain were determined. The glycosylation pattern of the heavy chain was also determined (**Figure 21B**).

[0252] These results demonstrated that administration of an Flt-1 antibody (i.e., 21B3) to *mdx* mice resulted in a significant increase in endothelial cell proliferation as well as a decrease in soluble Flt-1 in serum and an increase in VEGF concentrations in serum.

Example 5. Characterization of humanized high affinity anti-Flt-1 antibodies

[0253] Light chain shuffled antibodies described in Example 3 were further modified to introduce sequence variation in the CDR regions and or Fc effector regions. These antibodies were evaluated by a surface plasmon resonance methodology (e.g., Biacore) to determine the binding characteristics (**Table 9**). Antibody 27H9 NG/NA AAA demonstrated an approximately 2 fold reduced binding affinity for Flt-1.

Table 9.

	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)	Chi^2	EXP

21B3 WT	5.4E+05	1.7E-04	292	3.2E-10	290	2
21B3 AAA	5.2E+05	1.1E-04	305	2.1E-10	371	2
27H9 old 07/05	3.5E+05	7.9E-05	306	2.3E-10	235	1
27H9 WT 23/07	3.3E+05	8.4E-05	323	2.6E-10	236	1
27H9 NG/QG	3.7E+05	1.3E-04	315	3.6E-10	280	1
27H9 NG/NA	2.9E+05	1.1E-04	298	3.9E-10	158	1
27H9 NG/NA	2.9E+05	1.5E-04	240	5.1E-10	96.4	2
27H9 NG/NA AAA	2.9E+05	1.5E-04	236	5.1E-10	72.5	2

[0254] The antibodies were also evaluated in the cell based assay for ability to rescue cell activation by antagonizing sFlt-1. Human primary vein endothelial cells (HUVECs) were stimulated with VEGF in the presence of sFlt-1 and the monoclonal antibodies. VEGF induced activation of cells was assayed by determining the phosphorylation status of the VEGF R2 receptor. The monoclonal antibodies rescued cell activation (*i.e.*, phosphorylation) by antagonizing soluble Flt-1 (**Figure 22A**) and antibody 27H9 NG/NA AAA (NA+AAA) had similar potency as compared to the non-mutated parent antibody (wt).

Example 6. Antibody optimization

[0255] Candidate antibodies were analyzed to identify the closest human VH and VL germline sequences and to identify differing residues within the framework regions and oxidation/isomerization sites within the CDRs. A Fab library containing human and wild-type residues was constructed and fused to human constant domains. Phage display was applied to identify Fabs with identical or better off rate (i.e., no loss of affinity) than the parent antibody, 21B3. Fabs with the desired off-rate were sequenced and compared to human germline sequence and those with the highest identity (e.g., VH+VL identity >95%) and homology (e.g., >96% homology) were selected for conversion into human monoclonal antibodies. The Fabs are also analyzed for unwanted amino acids. **Table 10** provides Fabs ranked by percent human identity with some clones having up to 97.6% human identity and 98.8% homology.

Table 10.

SORTED BY IDENTITY				
FAB IDENTITY				
	%Ident	%Homol	kd IMPROVEMENT	kd
27H4_VH	97,6%	98,8%	1,06	5,1E-04
27H9_VH	97,5%	98,2%	1,12	4,8E-04
25F11_VH	97,5%	98,2%	1,00	5,3E-04
27H6_VH	97,5%	97,5%	0,96	5,6E-04
25A2_VH	97,0%	98,8%	0,97	5,5E-04
27D3_VH	97,0%	98,2%	0,89	6,0E-04
27B4_VH	97,0%	98,2%	0,84	6,4E-04
25D4_VH	97,0%	98,8%	0,94	5,7E-04
25D6_VH	97,0%	98,2%	1,17	4,6E-04
25G9_VH	97,0%	98,2%	0,97	5,5E-04
27C5_VH	97,0%	98,2%	0,96	5,5E-04
25D11_VH	97,0%	98,2%	0,90	6,0E-04
25C8_VH	97,0%	97,5%	0,84	6,3E-04
25G2_VH	96,9%	98,2%	0,81	6,6E-04
27A1_VH	96,4%	98,2%	0,97	5,5E-04
25H3_VH	96,4%	97,5%	0,94	5,7E-04
27F5_VH	96,3%	98,2%	1,18	4,5E-04
25B9_VH	96,3%	98,2%	1,03	5,2E-04
27H1_VH	96,3%	98,2%	0,91	5,9E-04
27G9_VH	96,3%	97,5%	1,30	4,1E-04
27D9_VH	96,3%	97,5%	0,94	5,7E-04
25G8_VH	96,3%	97,5%	0,82	6,5E-04
21B3	93,3%	96,3%		5,3E-04

Example 7. Characterization of anti-Flt-1 monoclonal antibodies

[0256] The thermo-tolerance of the monoclonal antibodies was analyzed using a Biacore method. At a concentration of 100 µg/mL, each monoclonal antibody was incubated for 1 hour in phosphate buffered saline at different temperatures. Following the 1 hour incubation, the antibody was slowly cooled to 25°C over two hours then incubated overnight at 4°C. The percentage of functional antibody was then measured by determining the binding to human Flt-1 by Biocore (see **Table 11**). The thermo-tolerance for the wild-type antibodies was consistent with previous experiments. However, with the exception of the 27H6 DG/DA clone, mutations in the VH or VL regions reduced the melting temperature by approximately 2°C. The AAA mutation in the Fc region had no effect on the thermo-tolerance of the antibody.

Table 11.

Tm (°C)						
MAB	Exp 140421	Exp 140507	Exp 140514	Exp 140613	Exp 140723	Exp 140902
21B3 WT	70.1	70.4	70.9	69.1		70.5
21B3 AAA						˜70.8
27H4 WT	67	67.8		~ 66.6	67.5	
27H4 NG/NA (VH)					~ 64.1	
27H4 NG/QG (VH)					~ 64.1	
27H4 NA + AAA						64.9
27H6 WT					61.9	
27H6 DG/DA (VL)					~ 63.4	
27H6 DG/EG (VL)					58.8	
27H9 WT			73.8	68.6	69.0	
27H9 NG/NA (VH)					66.8	
27H9 NG/QG (VH)					66.7	
27H9 NA + AAA						67.3

[0257] The binding affinity of the humanized clones were analyzed by Biacore (**Table 12**). The ability to rescue VEGF signaling in a VEGF:sFlt-1 cell based assay was determined for antibodies 27H4, 27H6 and 27H9 (**Figure 22B**). Briefly, human primary vein endothelial cells (HUVECs) were stimulated with VEGF in the presence of sFlt-1 and monoclonal antibodies 27H4, 27H6 and 27H9. VEGF induced activation of cells was assayed by determining the phosphorylation status of the VEGF R2 receptor. The data are expressed as a percent rescue of the phosphorylation of the VEGF R2 receptor relative to the phosphorylation of the VEGF R2 receptor in the presence of sFlt-1 alone (e.g., without anti-Flt-1 antibodies). The ability to antagonize binding of VEGF and sFlt-1 (**Figure 23**) was also determined for antibodies 27H4, 27H6 and 27H9 by ELISA.

Table 12.

	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)
21B3	5.4E+05	1.7E-04	292	3.2E-10
27H4	3.4E+05	8.2E-05	305	2.5E-10
27H9	3.3E+05	8.4E-05	323	2.6E-10
27H6	9.1E+05	1.4E-04	436	1.6E-10

Example 8. *In vitro* study of anti-Flt-1 antibodies on muscle pathology

[0258] *Mdx* mice were treated with 1, 3 or 10 mg/kg of anti-Flt-1 antibody 21B3 or 10 mg/kg of IgG1 isotype control antibody by intravenous administration twice a week for 6 or 12 weeks beginning at 3 weeks of age.

[0259] To assess serum levels of free antibody concentration, blood was collected from the mice 4 days after the intravenous dose administered at 2, 4, 7 and 10 weeks. The sacrifice sample was collected 24 hours after the last dose (**Figure 24**). At the 10 mg/kg dose, there was a statistically significant difference in serum levels of free antibody at all time points relative to the serum levels of free antibody in mice receiving the isotype control antibody. At the 3 mg/kg dose, there was a statistically significant difference in serum levels of free antibody at weeks 4, 7 and 10 and at sacrifice relative to the serum levels of free antibody in mice receiving the isotype control antibody. At the 1 mg/kg dose, there was a statistically significant difference in serum levels of free antibody at sacrifice relative to the serum levels of free antibody in mice receiving the isotype control antibody.

[0260] To assess serum levels of free sFlt-1, blood was collected from the mice 4 days after the intravenous dose administered at 2, 4, 7 and 10 weeks. The sacrifice sample was collected 24 hours after the last dose (**Figure 25**). At the 10 mg/kg dose, there was a statistically significant difference in serum levels of free sFlt-1 at all time points relative to the serum levels of free sFlt-1 in mice receiving the isotype control antibody. At the 3 mg/kg dose, there was a statistically significant difference in serum levels of free sFlt-1 at weeks 4, 7 and 10 and at sacrifice relative to the serum levels of free sFlt-1 in mice receiving the isotype control antibody. At the 1 mg/kg dose, there was a statistically significant difference in serum levels of free sFlt-1 at sacrifice relative to the serum levels of free sFlt-1 in mice receiving the isotype control antibody.

[0261] To assess serum levels of VEGF, blood was collected from the mice 4 days after the intravenous dose administered at 2, 4, 7 and 10 weeks. The sacrifice sample was collected 24 hours after the last dose (**Figure 26**). Administration of antibody 21B3 induced a dose dependent increase in serum VEGF levels. At the 10 mg/kg dose, there was a statistically significant difference in serum levels of VEGF at all time points relative to the serum levels of VEGF in mice receiving the isotype control antibody. At the 3 mg/kg dose, there was a statistically significant difference in serum levels of VEGF at week 7 and at sacrifice relative to the serum levels of VEGF in mice receiving the isotype control antibody. At the 1 mg/kg dose, there was a statistically significant difference in serum levels of VEGF at sacrifice relative to the serum levels of VEGF in mice receiving the isotype control antibody.

Histopathology

[0262] Mice were sacrificed at weeks 6 and 12 of the treatment period and the diaphragm, gastrocnemius and tibialis anterior muscles were collected and sectioned to determine if treatment with the anti-Flt-1 antibody induced angiogenesis and prevented fibrosis and necrosis in skeletal muscle.

Angiogenesis

[0263] Sections of muscle were stained for the endothelial cell marker CD31 (**Figures 27A-27H, 28A-28H and 29A-29H**). A significant increase in capillary density was seen in all muscle groups studied in the mice treated with antibody 21B3 as compared to the muscles of mice treated with the isotype control antibody. The data were quantified using automated quantitative imaging software. There was a statistically significant increase in the CD31 positive area in the diaphragm muscle of mice treated with 3 mg/kg ($p<0.01$) and 10 mg/kg ($p<0.0001$) of antibody 21B3 at 6 and 12 weeks as compared to the CD31 positive area in the diaphragm muscle of mice treated with the isotype control antibody. There was a statistically significant increase in the CD31 positive area in the gastrocnemius muscle of mice treated with 10 mg/kg ($p<0.05$) of antibody 21B3 at 6 and 12 weeks as compared to the CD31 positive area in the gastrocnemius muscle of mice treated with isotype control antibody. There was a statistically significant increase in the CD31 positive area in the tibialis anterior muscle of mice treated with 3 mg/kg of antibody 21B3 at 6 weeks ($p<0.05$) and 12 weeks ($p<0.0001$) and of mice treated with 10 mg/kg ($p<0.0001$) of antibody 21B3 at 6 and 12 weeks as compared to the CD31 positive area in the tibialis anterior muscle of mice treated with the isotype control antibody. (**Figures 30A-30C**).

Fibrosis

[0264] Sections of muscle were also stained by immunohistochemistry for type I collagen (**Figures 31A-31H, 32A-32H and 33A-33H**). A significant decrease in type I collagen staining was seen in the diaphragm and gastrocnemius muscle of mice treated with antibody 21B3 as compared to the diaphragm and gastrocnemius muscle of mice treated with the isotype control antibody. There was a statistically significant decrease in the type I collagen staining in the diaphragm muscle of mice treated with 1 mg/kg ($p<0.0001$), 3 mg/kg ($p<0.001$) and 10 mg/kg ($p<0.0001$) of antibody 21B3 at 12 weeks as compared to the type I collagen staining in the diaphragm muscle of mice treated with the isotype control antibody. There was a statistically significant decrease in type I collagen staining in the gastrocnemius muscle of mice treated with 1 mg/kg ($p<0.01$), 3 mg/kg ($p<0.05$) and 10 mg/kg ($p<0.001$) of antibody 21B3 at 12 weeks as compared to the type I collagen staining in the gastrocnemius muscle of mice treated with the isotype control antibody. (**Figures 34A-34C**).

Necrosis

[0265] The percentage of necrosis present in the gastrocnemius muscle of mice treated with the 21B3 antibody was determined relative to the percentage of necrosis present in the gastrocnemius muscle of mice treated with the isotype control antibody. A trend toward improvement in necrosis was seen (**Figures 35A-35B**).

Example 9. Mapping of the epitope on human sFlt-1 targeted by anti-Flt-1 antibodies 21B3 and 21C6

[0266] The peptide level epitopes on human sFlt-1 targeted by anti- human sFlt-1 monoclonal antibodies (mAbs) 21B3 and 21C6 were established by hydrogen deuterium exchange (HDX) mass spectrometry.

Pepsin digestion and LC-MS

[0267] For pepsin digestion, 10 µg of sFlt-1 or sFlt-1 and antibody (21B3) mixture (10µg:20µg) or sFlt-1 and antibody (21C6) mixture (10 µg:20 µg) was denatured in 0.365 M TCEP and 1.7 M guanidine hydrochloride (pH 2.5). The mixture was subjected to online pepsin digestion and the resultant peptides were analyzed using an UPLC-MS system comprised of a Waters Acquity UPLC coupled to a MicroTOF-Q2 mass spectrometer (Bruker). The peptides were separated on a 50 mm x 1 mm C8 column with a 19 min gradient from 5-28.5% solvent B (0.1% formic acid in acetonitrile). Solvent A is 0.1% formic acid in water. The solvent mixture valve, injection valve, C8 column and all the connecting stainless steel tubings were immersed in a chilled circulating water bath maintained at 0 °C. Peptide identification was done through searching MS/MS data against the sFlt-1 sequence with Mascot. The mass tolerance for the precursor and product ions was 0.1 Da and 0.2 Da, respectively.

Deglycosylation treatment

[0268] 200 µg of human sFlt-1 recombinant protein was incubated with 10 µl of PNGase F at 37 °C for 4 hrs.

Fab preparation

[0269] Fabs were prepared from two anti-sFlt-1 mAbs (21B3 and 21C6) with papain digestion and Protein A capture using Pierce Fab Preparation Kit.

Size exclusion chromatography (SEC)

[0270] To check the binding between either native or deglycosylated human sFlt-1 and two anti-human sFlt-1 mAbs (21B3 and 21C6) on SEC, 10 µg of sFlt-1 (either native or deglycosylated) was mixed with 40 µg of anti-sFlt-1 mAbs. Native or deglycosylated sFlt-1 alone, anti-sFlt-1 mAb alone or the complexes were injected to a SEC column at flow rate of 0.35 ml/min with PBS as the mobile phase and the proteins were monitored at 280 nm. Fabs generated from anti-sFlt-1 mAbs (21B3 and 21C6), and the binding between anti-sFlt-1 Fabs and sFlt-1 were also assessed using SEC.

HDX

[0271] 10 µL human sFlt-1 (10 µg) or sFlt-1 and mAb (21B3) mixture (10 µg:20 µg) or sFlt-1 and mAb (21C6) mixture (10 µg:20 µg) was incubated with 90 µL deuterium oxide labeling buffer (50 mM phosphate, 100 mM sodium chloride at pH 7.4) for 0 s, 30 s, 2 min, 10 min, 1 hr or 4 hr. Deuterium exchange was quenched by adding 100 µL of 3.4 M guanidine hydrochloride, 0.73 M TCEP buffer with a final pH of 2.5, and then subjected to pepsin digestion and LC-MS analysis described above. The mass spectra were recorded in MS only mode. Raw MS data was processed using HDExaminer software (Sierra Analytics, CA). The deuterium levels were calculated using the average mass difference between the deuteriated peptide and its native form (t_0).

Results

[0272] To verify that glycan removal does not alter the binding of human sFlt-1 to the antibodies, native and deglycosylated sFlt-1 protein were mixed with the anti-human sFlt-1 IgGs (21B3 and 21C6) and the complex formation was monitored on size exclusion chromatography. The data demonstrated that native human sFlt-1 completely binds to the two anti-human sFlt-1 IgGs (21B3 and 21C6) while deglycosylated human sFlt-1 incompletely binds to anti-human sFlt-1 mAb (21B3) or does not bind to anti-human sFlt-1 mAb (21C6), which indicated that deglycosylation perturbs the interactions between human sFlt-1 and the antibodies. Therefore, native human sFlt-1 was selected to perform HD exchange experiments. Poor sequence coverage was achieved initially for native human sFlt-1 due to the high complexity of heterogeneous glycosylation and 12 N-linked glycosyltation sites. To improve the sequence coverage, glycan masses at each glycosylation site were identified and high sequence coverage (85.2%) was achieved for native human sFlt-1.

[0273] Native human sFlt-1 was incubated in deuterium oxide either alone or in complex with either anti-human sFlt-1 mAb (21B3) or anti-human sFlt-1 mAb (21C6). The deuterium exchange was carried at room temperature for 0 sec, 30 sec, 2 min, 10 min, 60 min and 240 min. The exchange reaction was quenched by low pH and the proteins were digested with pepsin. The deuterium levels at the identified peptides were monitored from the mass shift on LC-MS. The deuterium buildup curves over exchange time for all the peptides were plotted. While most of human sFlt-1 peptides displayed identical or similar deuterium levels with and without anti-human sFlt-1 mAbs (21B3 and 21C6), several peptide segments had significantly decreased deuterium incorporation upon mAb 21B3 or mAb 21C6 binding. Residues 117-129 (corresponding to amino acids 141-153 of SEQ ID NO:90) and 169-182 (corresponding to amino acids 193-206 of SEQ ID NO:90) experienced strong deuterium protection upon binding to anti-human sFlt-1 mAb 21B3, whereas residues 106-114 (corresponding to amino acids 130-138 of SEQ ID NO:90) and 117-124 (corresponding to amino acids 141-148 of SEQ ID NO:90) experienced strong deuterium protection upon binding to anti-human sFlt-1 mAb 21C6. These strongly protected regions were assigned as the epitope peptides for anti-human sFlt-1 mAbs (21B3 and 21C6) and highlighted in blue in the differential heat map shown in **Figure 36** and **Figure 37.** The MS/MS spectra for identified peptides containing amino acid residues from epitope regions are shown in **Figure 38A-38E.** Peptide 115-124 corresponds to amino acids 139-148 of SEQ ID NO:90; peptide 115-129 corresponds to amino acids 139-153 of SEQ ID NO:90; peptide 154-182 corresponds to amino acids 178-206 of SEQ ID NO:90; peptide 175-180 corresponds to amino acids 119-204 of SEQ ID NO:90; and peptide 104-114 corresponds to amino acids 128-138 of SEQ ID NO:90.

Conclusion

[0274] 85.2% sequence coverage was achieved for human sFlt-1. Residues 117-129 and 169-180 experienced strong deuterium protection upon binding to anti-human sFlt-1 mAb 21B3, whereas residues 106-114 and 117-124 experienced strong deuterium protection upon binding to anti-human sFlt-1 mAb 21C6. These strongly protected regions were assigned as the epitope peptides for the corresponding anti-human sFlt-1 mAb.

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EQUIVALENTS AND SCOPE

[0275] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims.

[0276] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0277] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Table 13.

Human Flt-1 amino acid sequence isoform 1 (NP_002010.2 GI:156104876; SEQ ID NO: 90)
1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK
61 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET
121 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD
181 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV
241 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK
301 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK
361 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA
421 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC
481 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK
541 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM
601 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRDQEA
661 PYLLRNLSDH TVAISSSTTL DCHANGVPEP QITWFKNNHK IQQEPGIILG PGSSTLFIER

721 VTEEDEGVYH CKATNQKGSV ESSAYLTVQG TSDKSNLELI TLTCTCVAAT LFWLLLTLFI
781 RKMKRSSSEI KTDYLSIIMD PDEVPLDEQC ERLPYDASKW EFARERLKLG KSLGRGAFGK
841 VVQASAFGIK KSPTCRTVAV KMLKEGATAS EYKALMTELK ILTHIGHHLN VVNLLGACTK
901 QGGPLMVIVE YCKYGNLSNY LKSKRDLFFL NKDAALHMEP KKEKMEPGLE QGKKPRLDSV
961 TSSESFASSG FQEDKSLSDV EEEEDSDGFY KEPITMEDLI SYSFQVARGM EFLSSRKCIH
1021 RDLAARNILL SENNVVKICD FGLARDIYKN PDYVRKGDTR LPLKWMAPES IFDKIYSTKS
1081 DVWSYGVLLW EIFSLGGSPY PGVQMDEDFC SRLREGMRMR APEYSTPEIY QIMLDCWHRD
1141 PKERPRFAEL VEKLGDLLQA NVQQDGKDYI PINAILTGNS GFTYSTPAFS EDFFKESISA
1201 PKFNSGSSDD VRYVNAFKFM SLERIKTFEE LLPNATSMFD DYQGDSSTLL ASPMLKRFTW
1261 TDSKPKASLK IDLRVTSKSK ESGLSDVSRP SFCHSSCGHV SEGKRRFTYD HAELERKIAC
1321 CSPPPDYNSV VLYSTPPI

Human Flt-1 amino acid sequence, isoform X1 (XP_011533316.1 GI:767977511; SEQ ID NO:91)

1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK
61 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET
121 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD
181 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV
241 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK
301 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK
361 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA
421 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC
481 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK
541 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM
601 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRDQEA
661 PYLLRNLSDH TVAISSSTTL DCHANGVPEP QITWFKNNHK IQQEPDADPH IQKADCTFFF

Human Flt-1 amino acid sequence, isoform 2 precursor (NP_001153392.1 GI:229892220; SEQ ID NO:92)

1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK
61 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET
121 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD
181 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV
241 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK

301 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK
361 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA
421 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC
481 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK
541 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM
601 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRGEHC
661 NKKAVFSRIS KFKSTRNDCT TQSNVKH

Human Flt-1 amino acid sequence, isoform 3 precursor (NP_001153502.1 GI:229892300; SEQ ID NO:93)

1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK
61 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET
121 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD
181 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV
241 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK
301 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK
361 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA
421 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC
481 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK
541 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM
601 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRDQEA
661 PYLLRNLSDH TVAISSSTTL DCHANGVPEP QITWFKNNHK IQQEPELYTS TSPSSSSSSP
721 LSSSSSSSSS SSS

Human Flt-1 amino acid sequence, isoform 4 precursor (NP_001153503.1 GI:229892302; SEQ ID NO:94)

1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK
61 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET
121 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD
181 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV
241 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK
301 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK
361 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA
421 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC

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481 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKLPP ANSSFMLPPT SFSSNYFHFL	
541 P	

Table 14.

Fc Region Sequence (SEQ ID NO:104)
209 TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH 269 EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL 329 PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE 389 NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPG

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An anti-Flt-1 antibody or antigen-binding fragment thereof, comprising

(a) a variable light (VL) chain CDR1 defined by the amino acid sequence of SEQ ID NO:21,

a VL CDR2 defined by the amino acid sequence of SEQ ID NO:24, a VL CDR3 defined by the amino acid sequence of SEQ ID NO:34, a variable heavy (VH) chain CDR1 defined by the amino acid sequence of SEQ ID NO:2, a VH CDR2 defined by the amino acid sequence of SEQ ID NO:6, and a VH CDR3 defined by the amino acid sequence of SEQ ID NO:16;

(b) a VL CDR1 defined by the amino acid sequence of SEQ ID NO:21, a VL CDR2 defined by the amino acid sequence of SEQ ID NO:24, a VL CDR3 defined by the amino acid sequence of SEQ ID NO: 27, a VH CDR 1 defined by the amino acid sequence of SEQ ID NO: 2, a VH CDR2 defined by the amino acid sequence of SEQ ID NO: 7, and a VH CDR3 defined by the amino acid sequence of SEQ ID NO: 17;

(c) a VL CDR1 defined by the amino acid sequence of SEQ ID NO:21, a VL CDR2 defined by the amino acid sequence of SEQ ID NO:24, a VL CDR3 defined by the amino acid sequence of SEQ ID NO: 26, a VH CDR1 defined by the amino acid sequence of SEQ ID NO: 3, a VH CDR2 defined by the amino acid sequence of SEQ ID NO: 12, and a VH CDR3 defined by the amino acid sequence of SEQ ID NO: 17;

(d) a VL CDR1 defined by the amino acid sequence of SEQ ID NO:21, a VL CDR2 defined by the amino acid sequence of SEQ ID NO:24, a VL CDR3 defined by the amino acid sequence of SEQ ID NO: 28, a VH CDR1 defined by the amino acid sequence of SEQ ID NO: 2, a VH CDR2 defined by the amino acid sequence of SEQ ID NO: 8, and a VH CDR3 defined by the amino acid sequence of SEQ ID NO: 17; or

(e) a VL CDR1 defined by the amino acid sequence of SEQ ID NO:21, a VL CDR2 defined by the amino acid sequence of SEQ ID NO:24, a VL CDR3 defined by the amino acid sequence of SEQ ID NO: 32, a VH CDR1 defined by the amino acid sequence of SEQ ID NO: 3, a VH CDR2 defined by the amino acid sequence of SEQ ID NO: 12, and a VH CDR3 defined by the amino acid sequence of SEQ ID NO: 17.

2. The antibody or antigen-binding fragment thereof of claim 1, wherein the anti-Flt-1 antibody or antigen-binding fragment thereof comprises:

a VL CDR1 defined by an amino acid sequence of SEQ ID NO:21,

a VL CDR2 defined by an amino acid sequence of SEQ ID NO:24,

a VL CDR3 defined by an amino acid sequence of SEQ ID NO: 26;

a VH CDR1 defined by an amino acid sequence of SEQ ID NO: 2,

a VH CDR2 defined by an amino acid sequence of SEQ ID NO: 6, and

a VH CDR3 defined by an amino acid sequence of SEQ ID NO: 16.

3. The antibody or antigen-binding fragment thereof of claim 1, wherein the anti-Flt-1 antibody or antigen-binding fragment thereof comprises:

a VL CDR1 defined by an amino acid sequence of SEQ ID NO:21,

a VL CDR2 defined by an amino acid sequence of SEQ ID NO:24,

a VL CDR3 defined by an amino acid sequence of SEQ ID NO: 27;

a VH CDR1 defined by an amino acid sequence of SEQ ID NO: 2,

a VH CDR2 defined by an amino acid sequence of SEQ ID NO: 7, and

a VH CDR3 defined by an amino acid sequence of SEQ ID NO: 17.

4. The antibody or antigen-binding fragment thereof of claim 1, wherein the anti-Flt-1 antibody or antigen-binding fragment thereof comprises:

a VL CDR1 defined by an amino acid sequence of SEQ ID NO:21,

a VL CDR2 defined by an amino acid sequence of SEQ ID NO:24,

a VL CDR3 defined by an amino acid sequence of SEQ ID NO: 26;

a VH CDR1 defined by an amino acid sequence of SEQ ID NO: 3,

a VH CDR2 defined by an amino acid sequence of SEQ ID NO: 12, and a VH CDR3 defined by an amino acid sequence of SEQ ID NO: 17.

5. The antibody or antigen-binding fragment thereof of claim 1, wherein the anti-Flt-1 antibody or antigen-binding fragment thereof comprises:

a VL CDR1 defined by an amino acid sequence of SEQ ID NO:21,

a VL CDR2 defined by an amino acid sequence of SEQ ID NO:24,

a VL CDR3 defined by an amino acid sequence of SEQ ID NO: 28;

a VH CDR1 defined by an amino acid sequence of SEQ ID NO: 2,

a VH CDR2 defined by an amino acid sequence of SEQ ID NO: 8, and

a VH CDR3 defined by an amino acid sequence of SEQ ID NO: 17.

6. The antibody or antigen-binding fragment thereof of any one of claims 1-5, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of IgG, $F(ab')_2$, $F(ab)_2$, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies.

7. The antibody or antigen-binding fragment thereof of claim 6, wherein the antibody or antigen-binding fragment thereof is IgG.

8. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof is IgGl.

9. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody.

10. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody is a humanized monoclonal antibody.

11. The antibody or antigen-binding fragment thereof of claim 10, wherein the humanized monoclonal antibody contains a human Fc region.

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12. The antibody or antigen-binding fragment thereof of claim 11, wherein the Fc region contains one or more mutations that enhance the binding affinity between the Fc region and the FcRn receptor such that the *in vivo* half-life of the antibody is prolonged.

13. The antibody or antigen-binding fragment thereof of claim 11 or claim 12, wherein the Fc region contains one or more mutations at positions corresponding to Leu 234, Leu 235 and/or Gly 237 of human IgGl.

14. The antibody or antigen-binding fragment thereof of claim 1-13, wherein the antibody or antigen-binding fragment thereof does not bind to VEGFR2 and/or VEGFR3.

15. The antibody or antigen-binding fragment thereof of claim 1-14, wherein the antibody or antigen-binding fragment thereof does not bind to a mouse or monkey Flt-1.

16. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claim 1-15 and a pharmaceutically acceptable carrier.

17. The antibody or antigen-binding fragment thereof of claim 1, wherein the anti-Flt-1 antibody or antigen-binding fragment thereof comprises:

(a) a light chain variable (VL) region comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 60 and a heavy chain variable (VH) region comprising an amino acid sequence having at least 90% identity SEQ ID NO: 45,

(b) a light chain comprising an amino acid sequence having at least 90% identity of SEQ ID NO: 76 and a heavy chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 71,

(c) a light chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 85 and a heavy chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 72,

(d) a light chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 84 and a heavy chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 71,

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(e) a light chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 86 and a heavy chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 73, or

(f) a light chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 83 and a heavy chain comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 70.

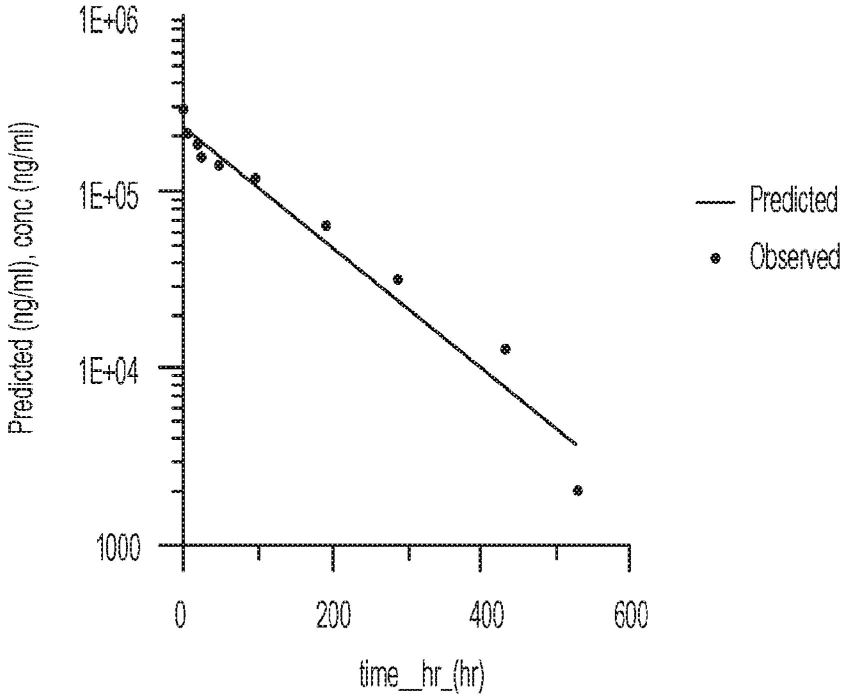


Figure 1A

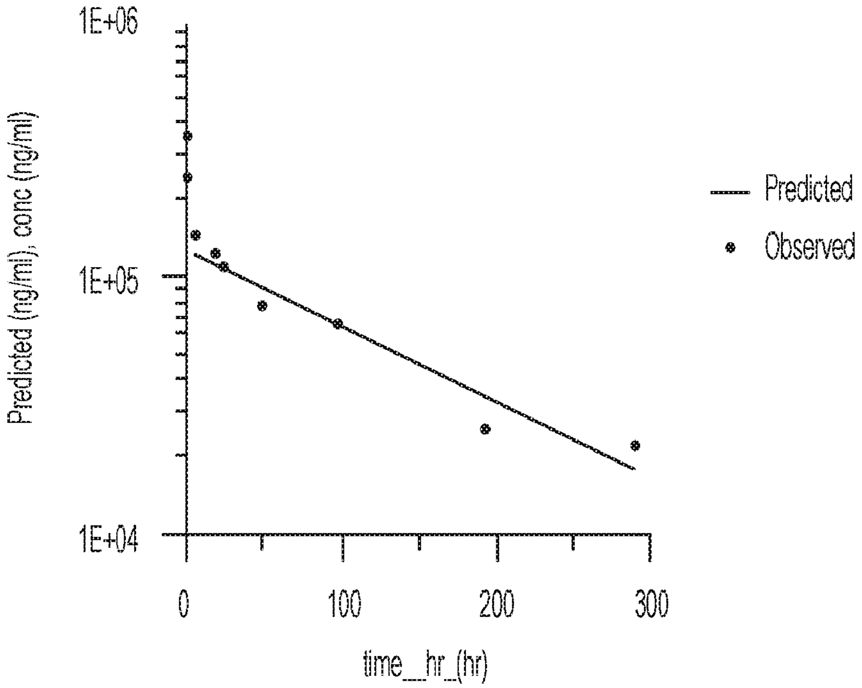


Figure 1B

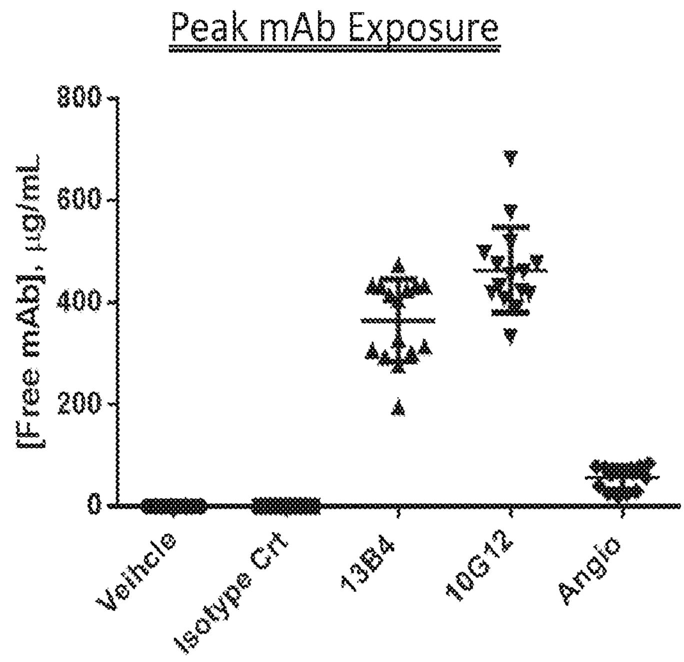


Figure 2A

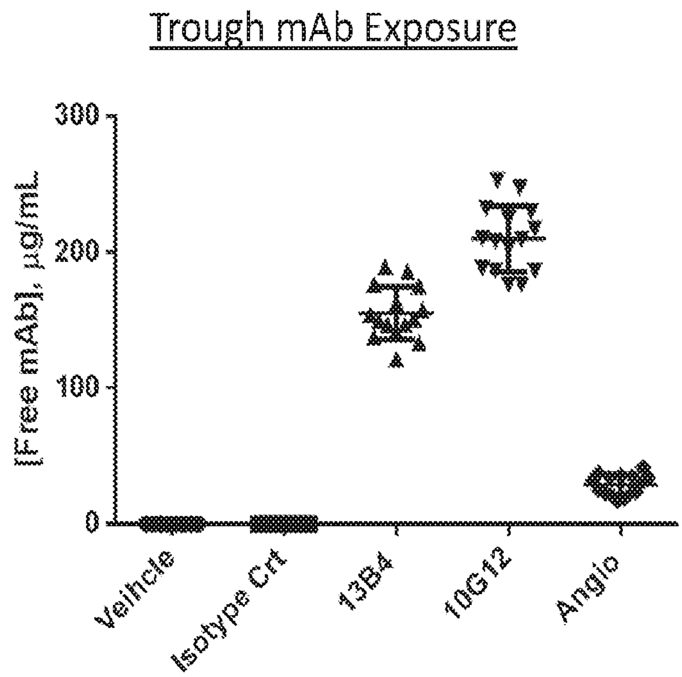


Figure 2B

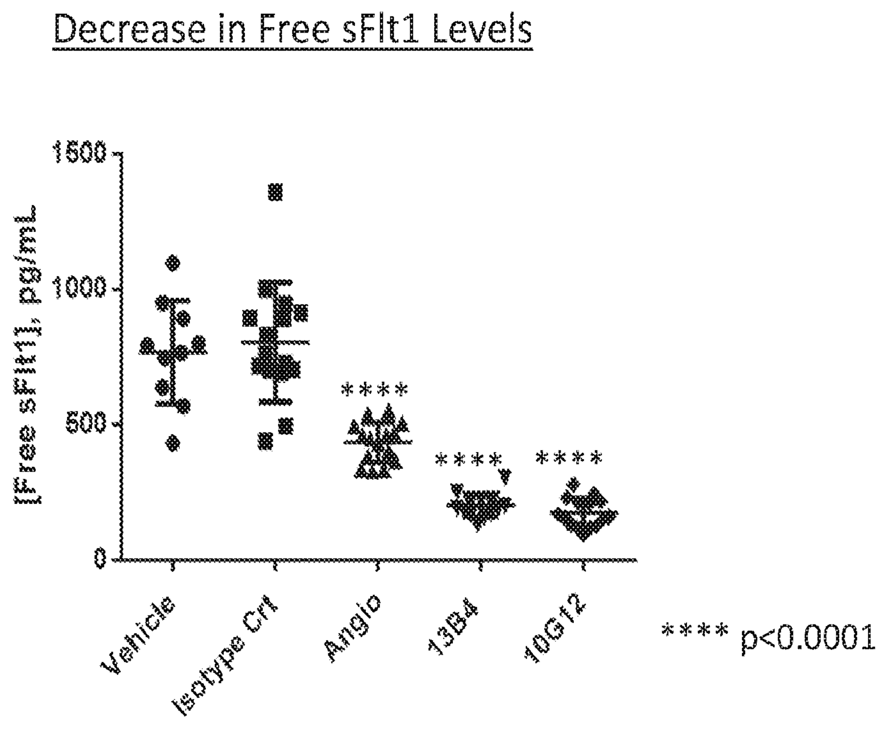
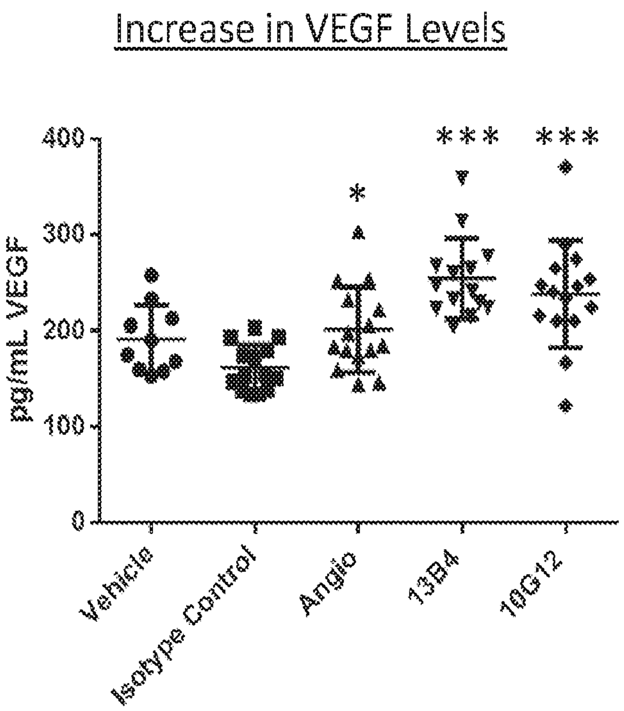

Decrease in Free sFlt1 Levels
[Free sFlt1], pg/mL
1500
1000
500
0
****
****
****
Vehicle
Isotype Crt
Angio
13B4
10Gf2
**** p<0.0001

Figure 3



* Angio vs. isotype p<0.05
*** 13B4/10G12 vs. isotype p<0.001

Figure 4

Isotype Control

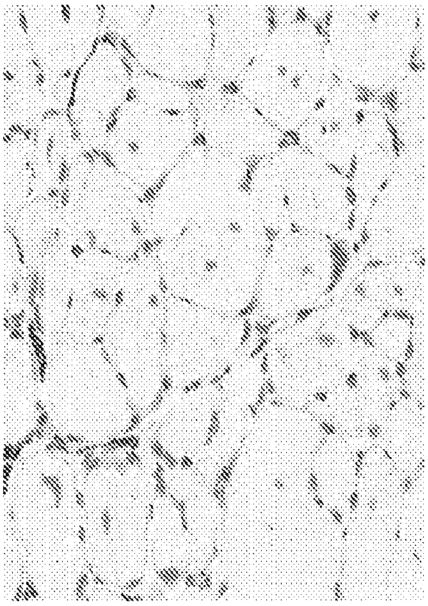


Figure 5A

Commercial control Ab

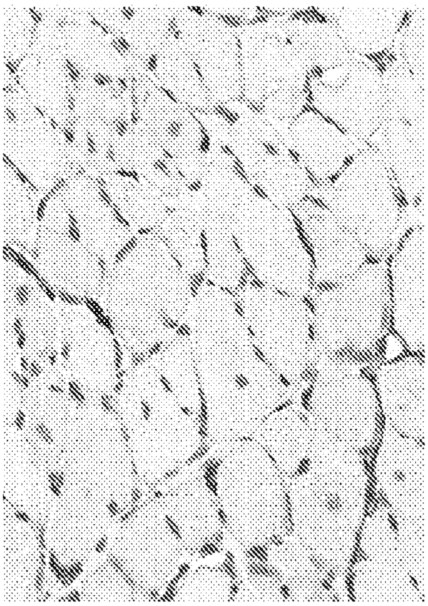


Figure 5B

13B4

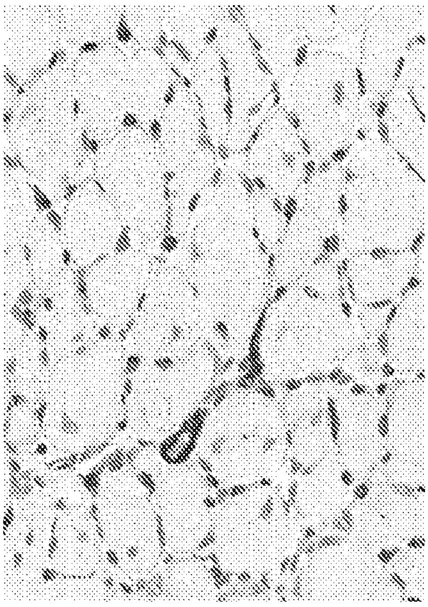


Figure 5C

10G12

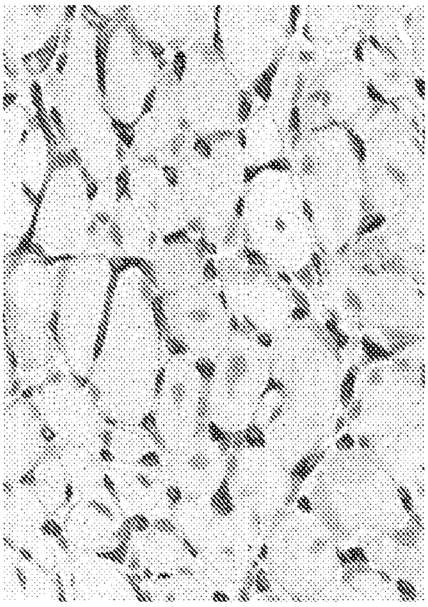


Figure 5D

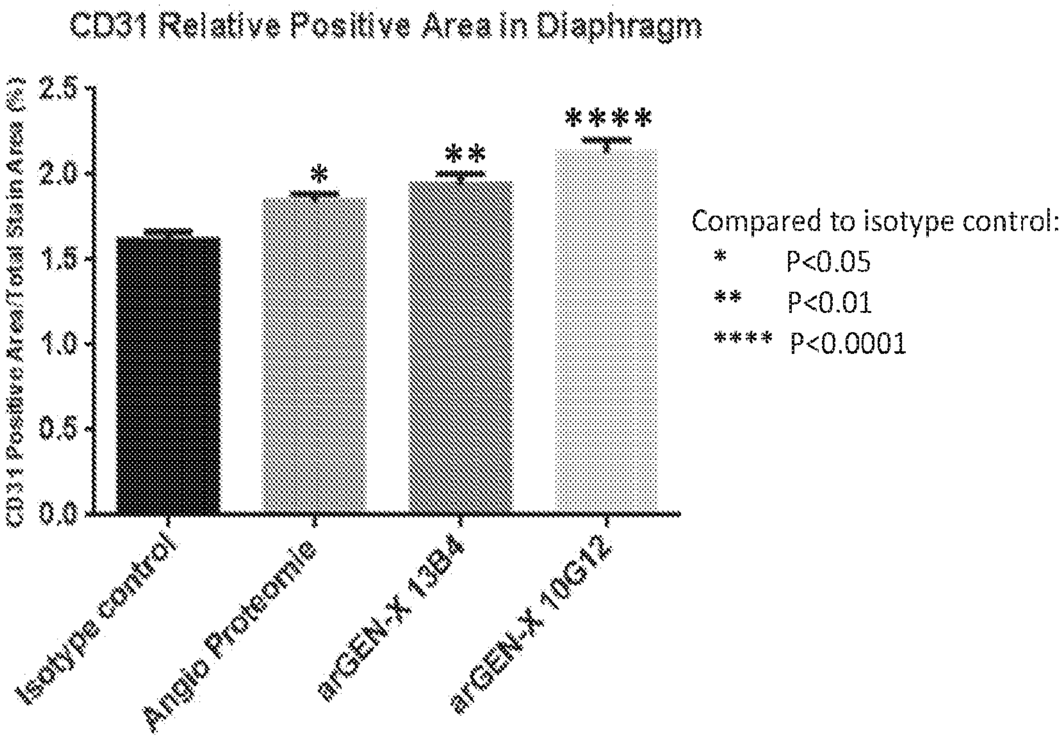


Figure 6A

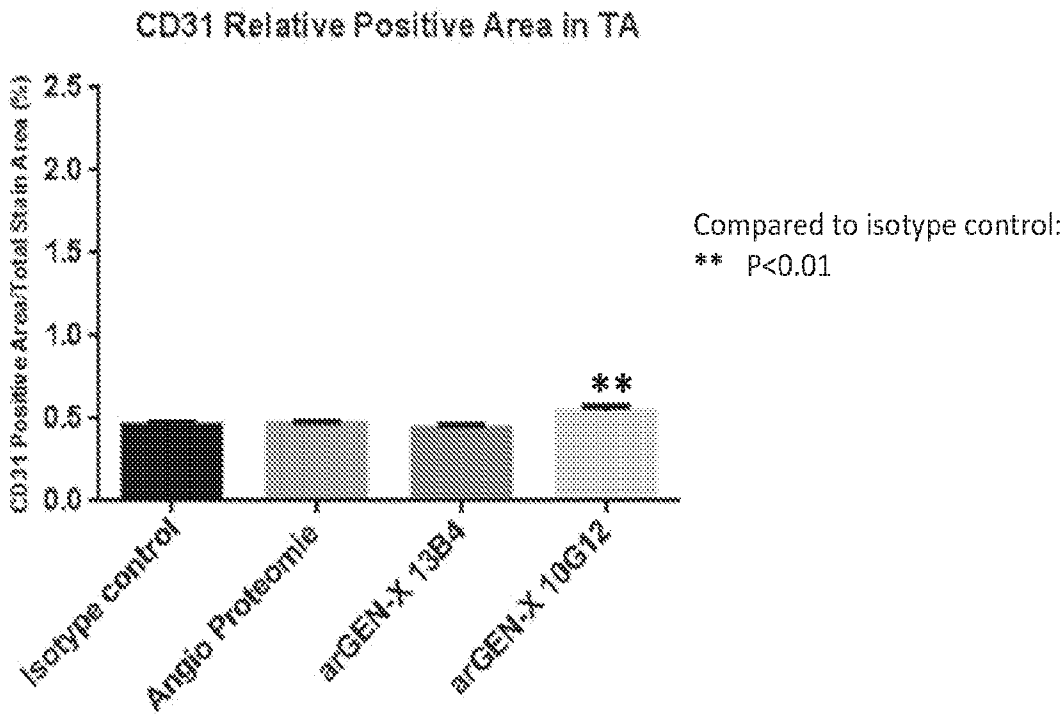


Figure 6B

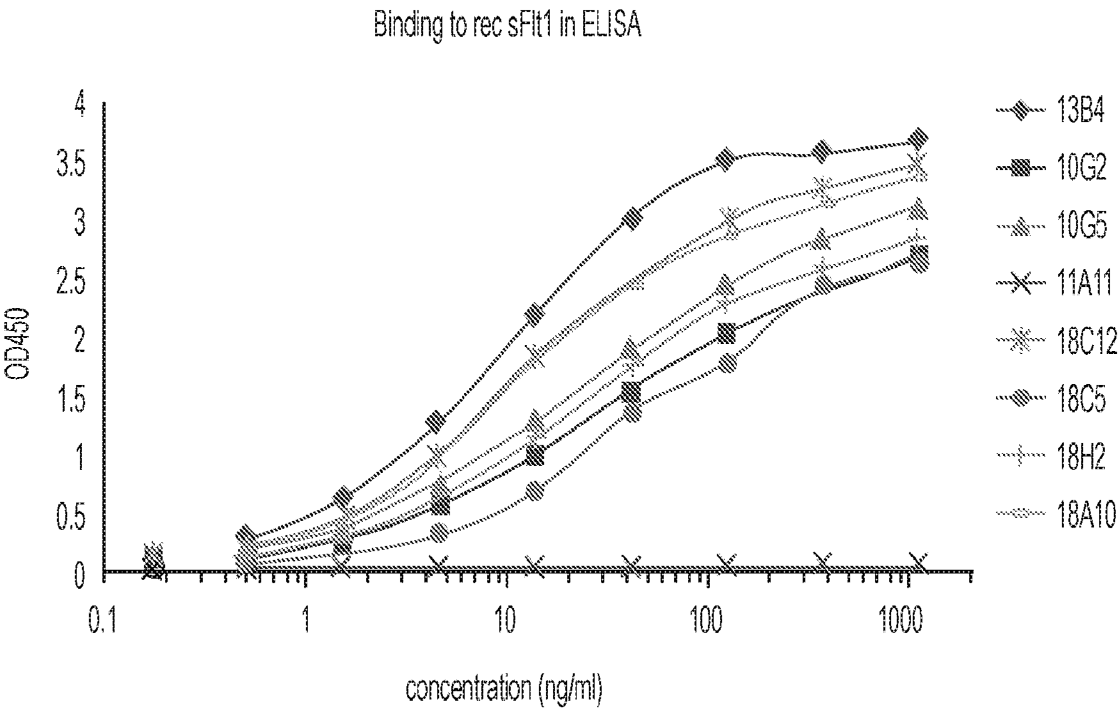

Binding to rec sFlt1 in ELISA
OD450
4
3.5
3
2.5
2
1.5
1
0.5
0
0.1
1
10
100
1000
concentration (ng/ml)
13B4
10G2
10G5
11A11
18C12
18C5
18H2
18A10

Figure 7

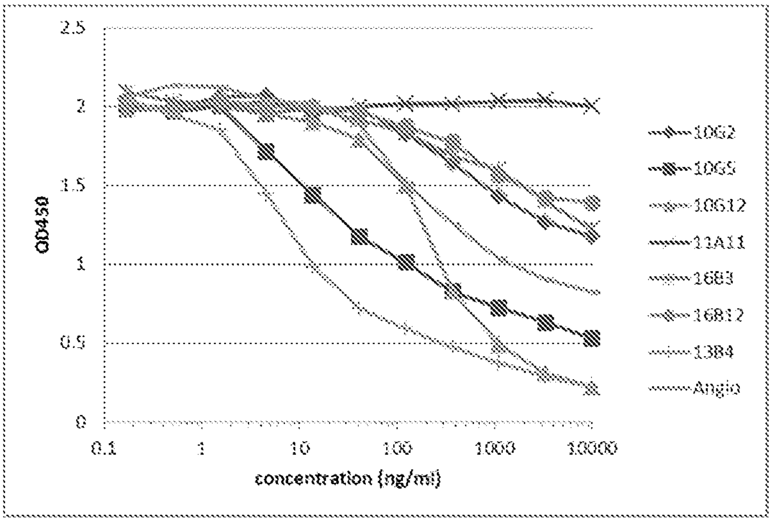

2.5
2
1.5
1
0.5
0
OD450
0.1
1
10
100
1000
10000
concentration (ng/ml)
10G2
10G5
10G12
11A11
16B3
16B12
13B4
Angio

Figure 8

939

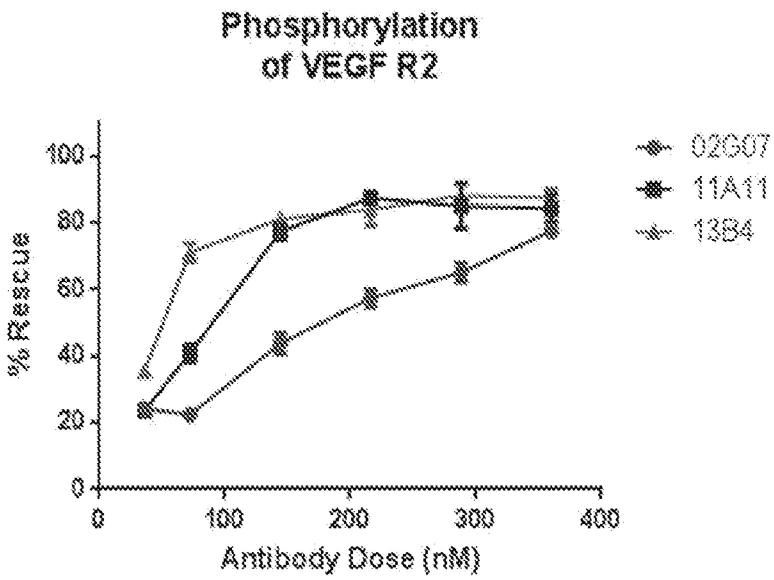

Phosphorylation
of VEGF R2
% Rescue
100
80
60
40
20
0
0
100
200
300
400
Antibody Dose (nM)
02G07
11A11
13B4

Figure 9

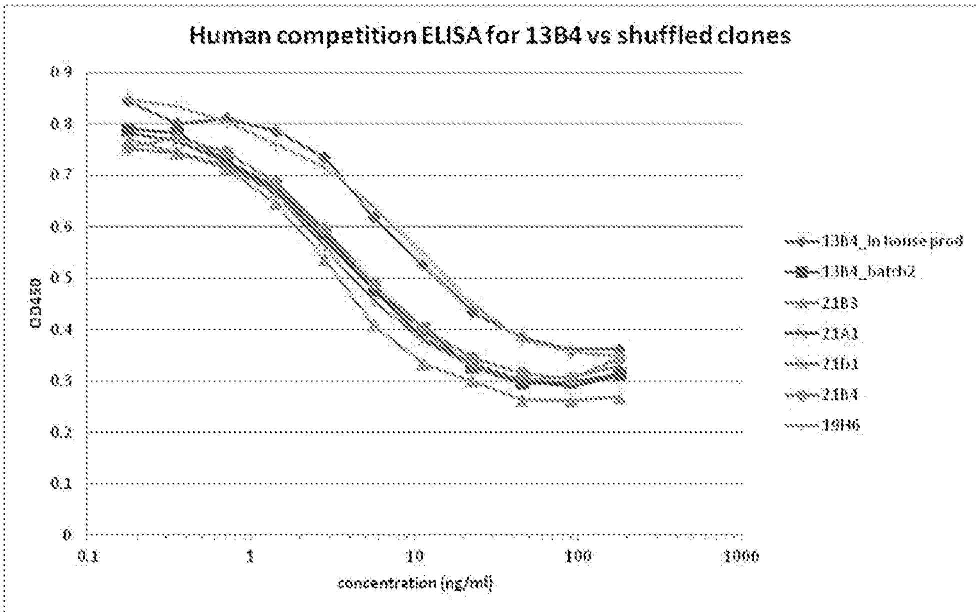

Human competition ELISA for 13B4 vs shuffled clones
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD450
0.1
1
10
100
1000
concentration (ng/ml)
13B4_In house prod
13B4_batch2
21B3
21A1
21D1
21B4
19H6

Figure 10

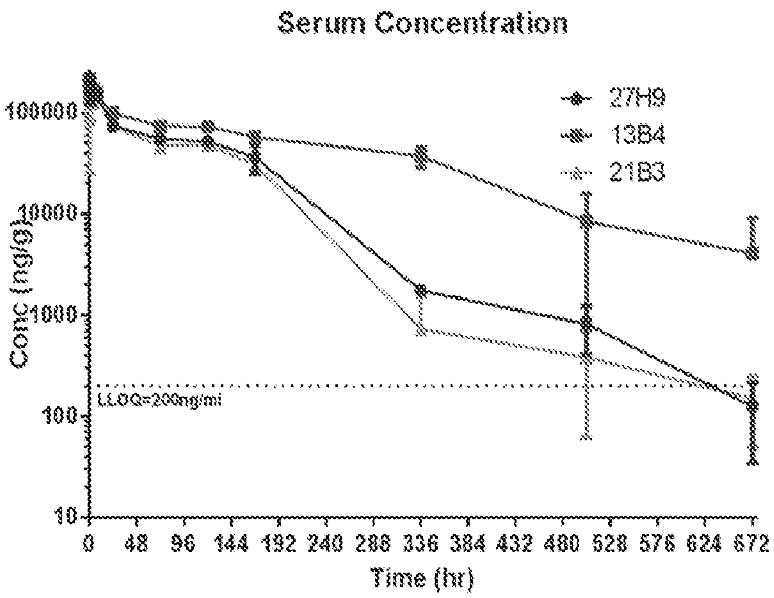

Serum Concentration
27H9
13B4
21B3
100000
10000
1000
100
10
Conc (ng/g)
LLOQ=200ng/ml
0 48 96 144 192 240 288 336 384 432 480 528 576 624 672
Time (hr)

Figure 11

Isotype **13B4** **21B3**

Diaphragm

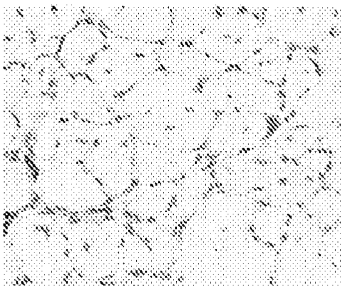


Figure 12A

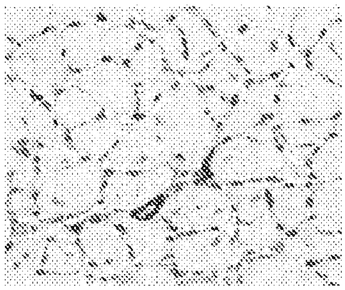


Figure 12B

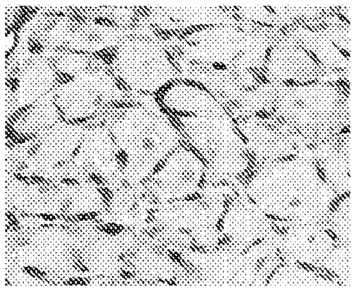


Figure 12C

Tibialis Anterior

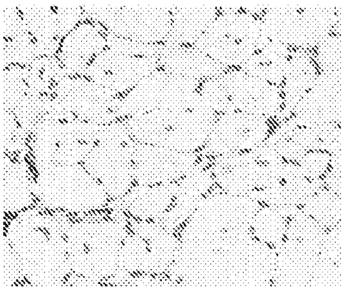


Figure 12D

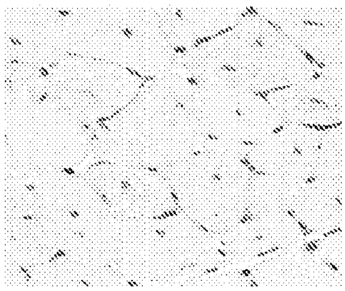


Figure 12E

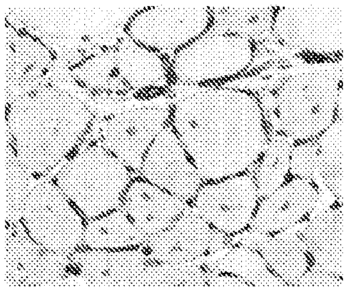


Figure 12F

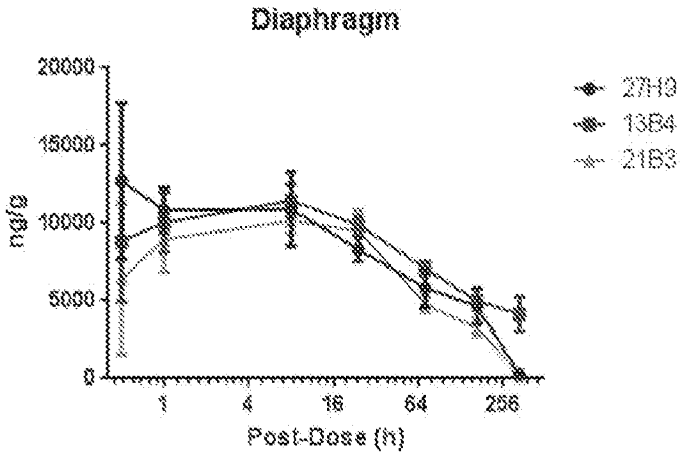


Figure 13A

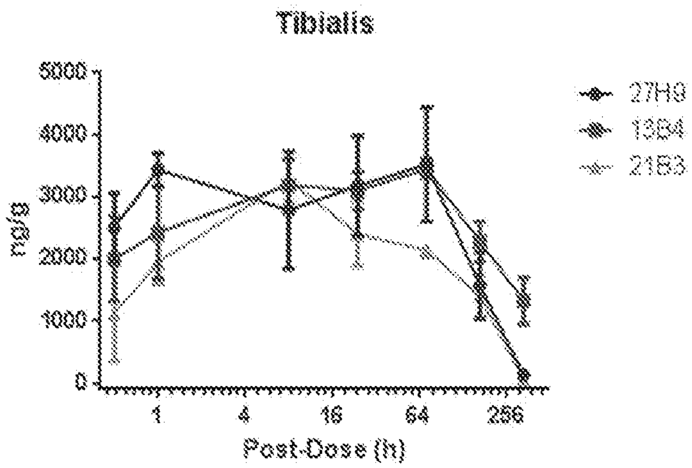


Figure 13B

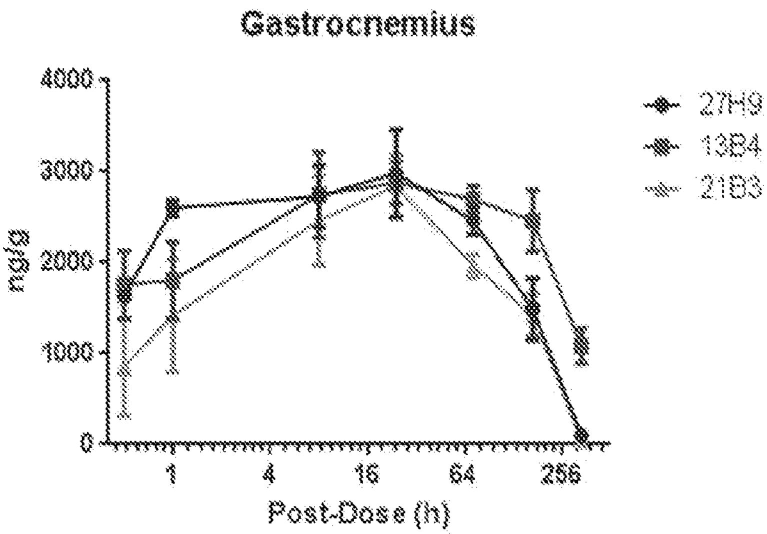


Figure 13C

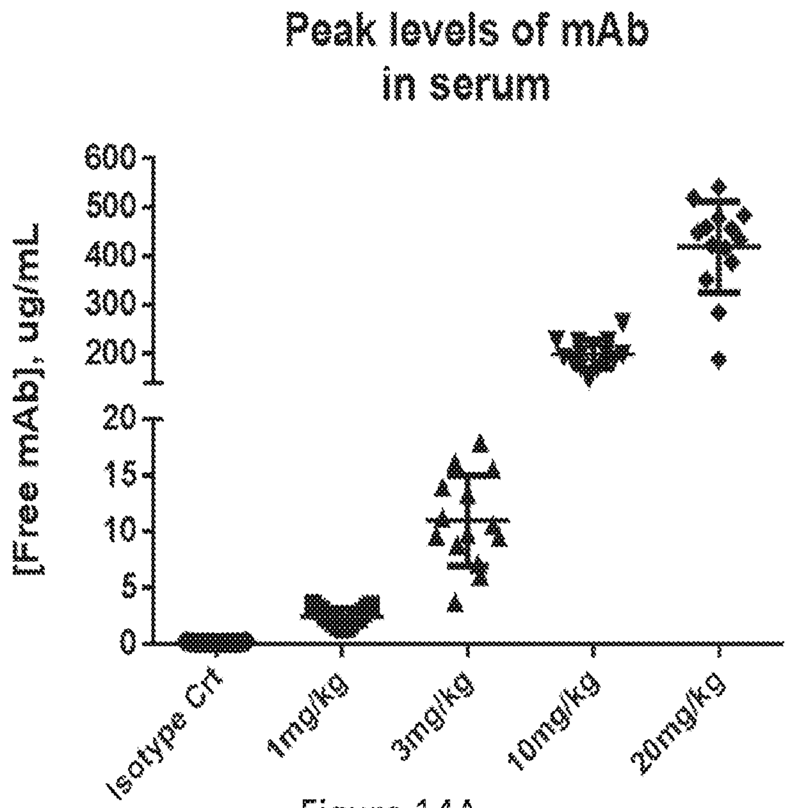


Figure 14A

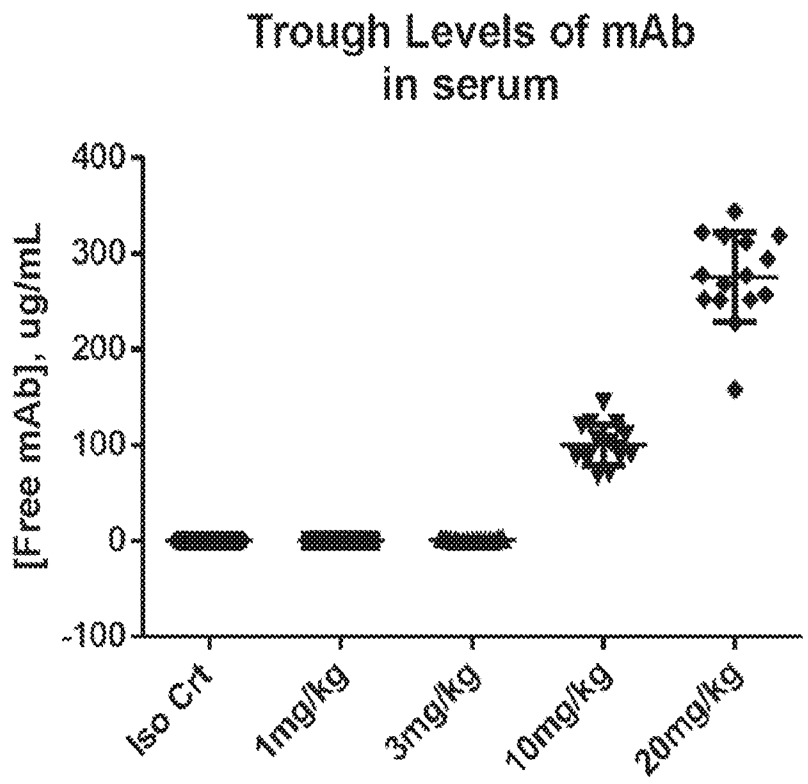


Figure 14B

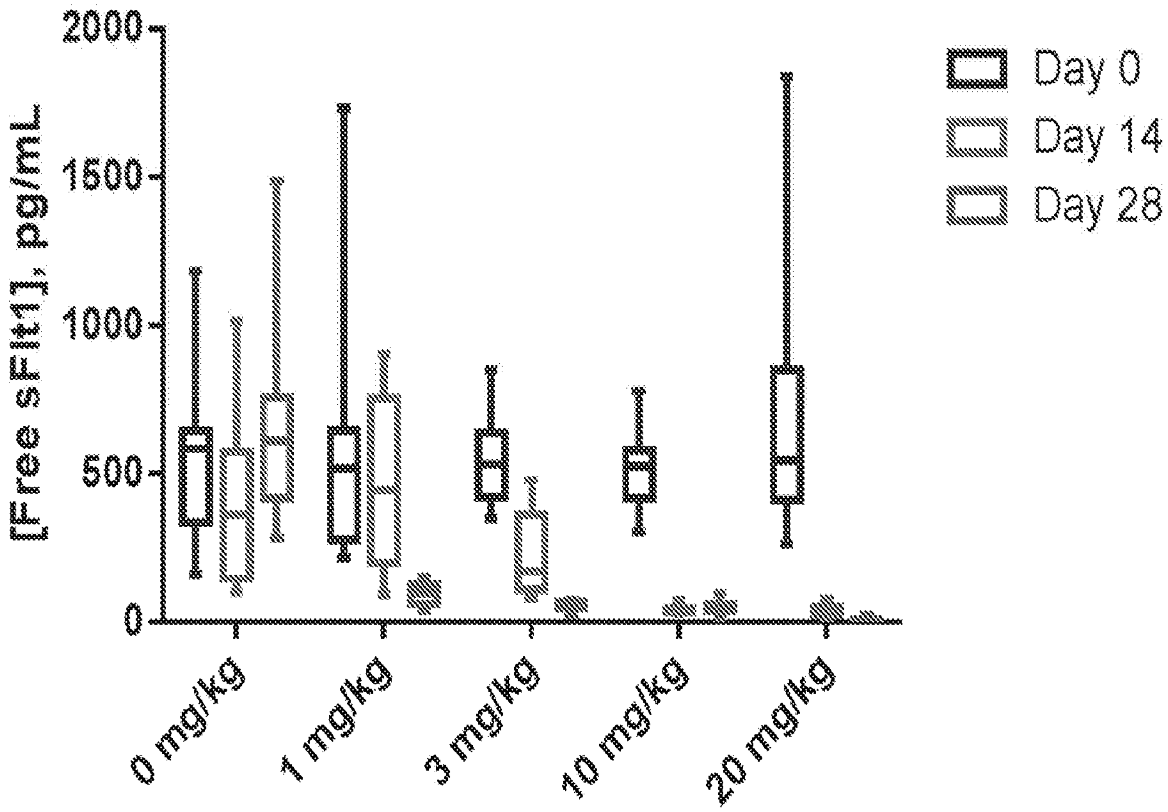

[Free sFlt1], pg/mL
2000
1500
1000
500
0
0 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
20 mg/kg
Day 0
Day 14
Day 28

Figure 15

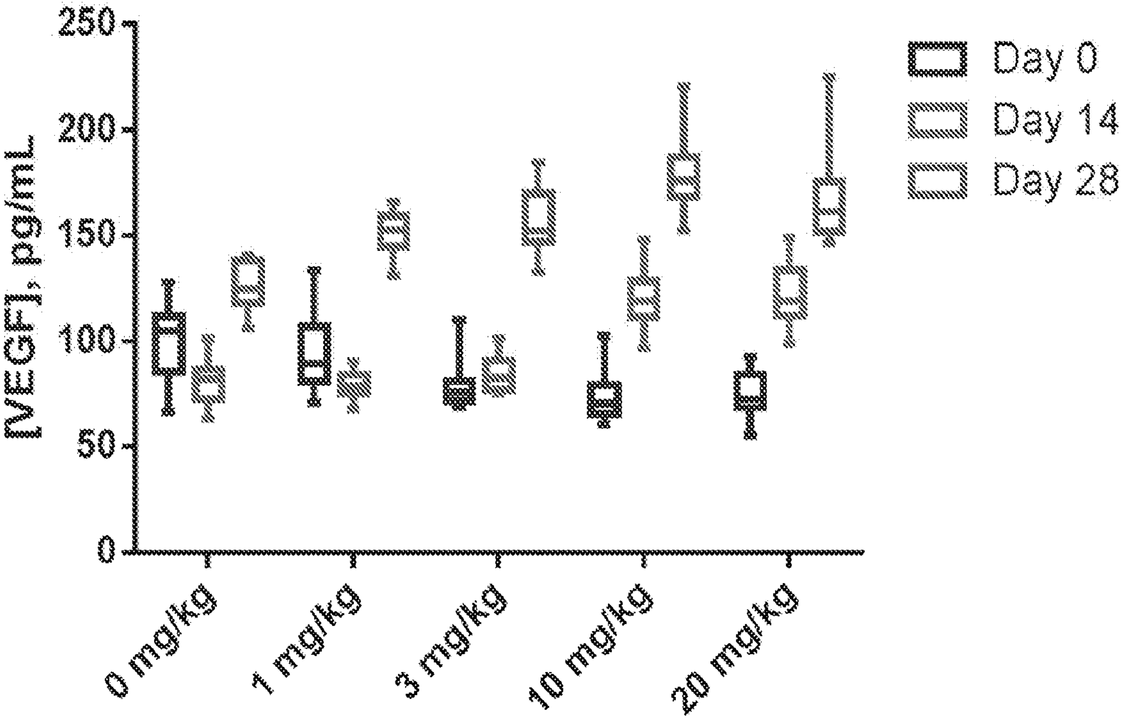

250
200
150
100
50
0
[VEGF], pg/mL
Day 0
Day 14
Day 28
0 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
20 mg/kg

Figure 16

Isotype Control

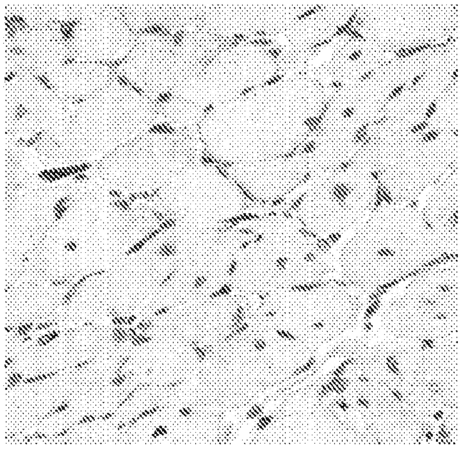


Figure 17A

21B3 1mg/kg

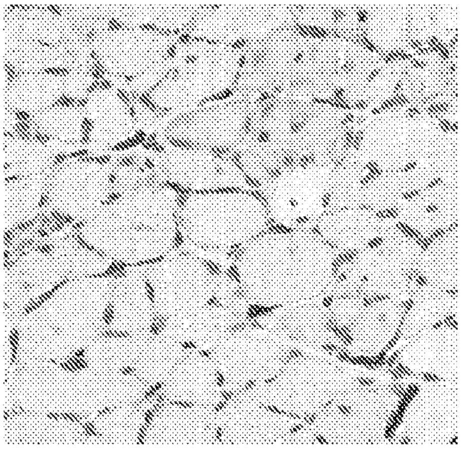


Figure 17B

21B3 3mg/kg

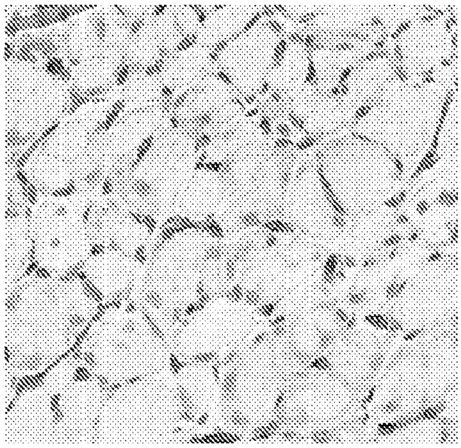


Figure 17C

21B3 10mg/kg

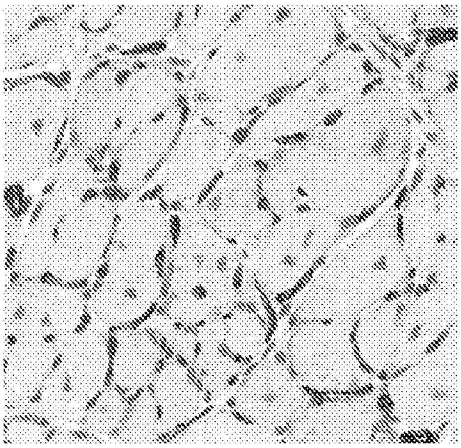


Figure 17D

21B3 20mg/kg

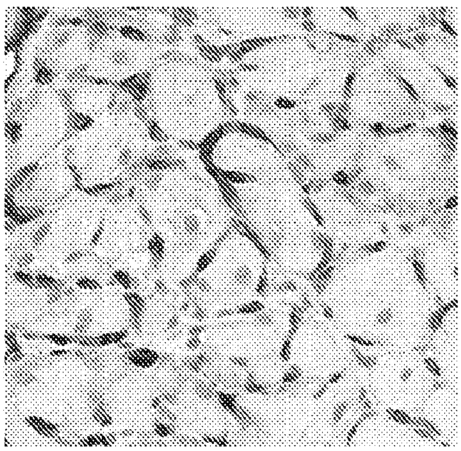
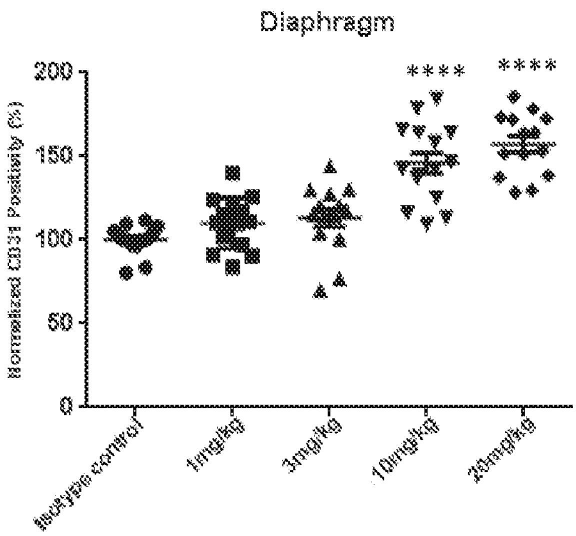


Figure 17E



**** p <0.0001 vs. isotype

Figure 18

Isotype Control

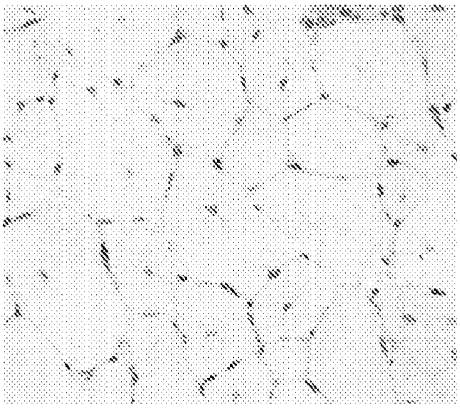


Figure 19A

21B3 1mg/kg

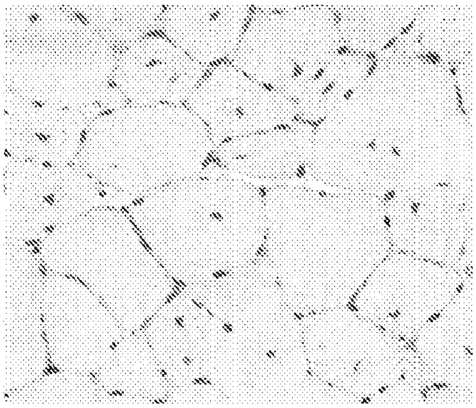


Figure 19B

21B3 3mg/kg

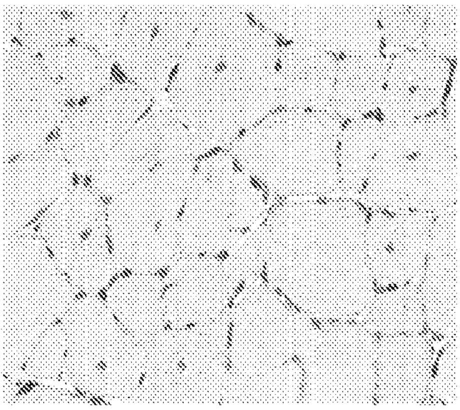


Figure 19C

21B3 10mg/kg

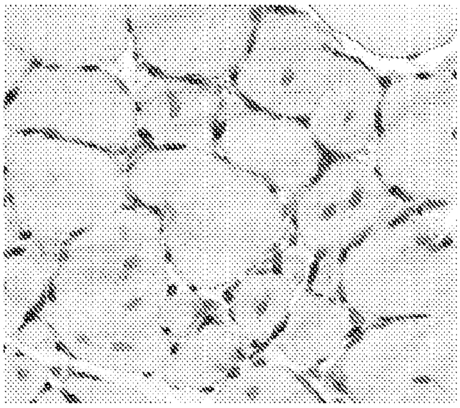


Figure 19D

21B3 20mg/kg

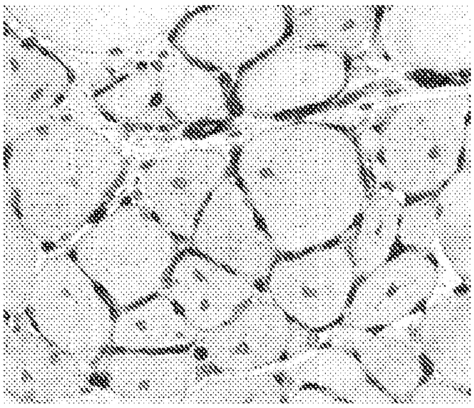
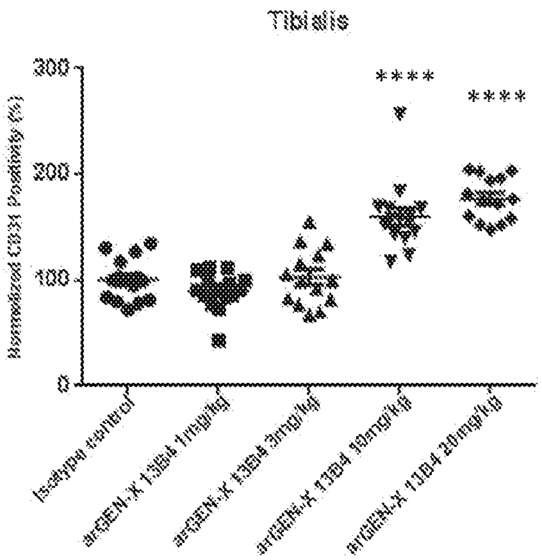


Figure 19E



**** p <0.0001 vs. isotype

Figure 20

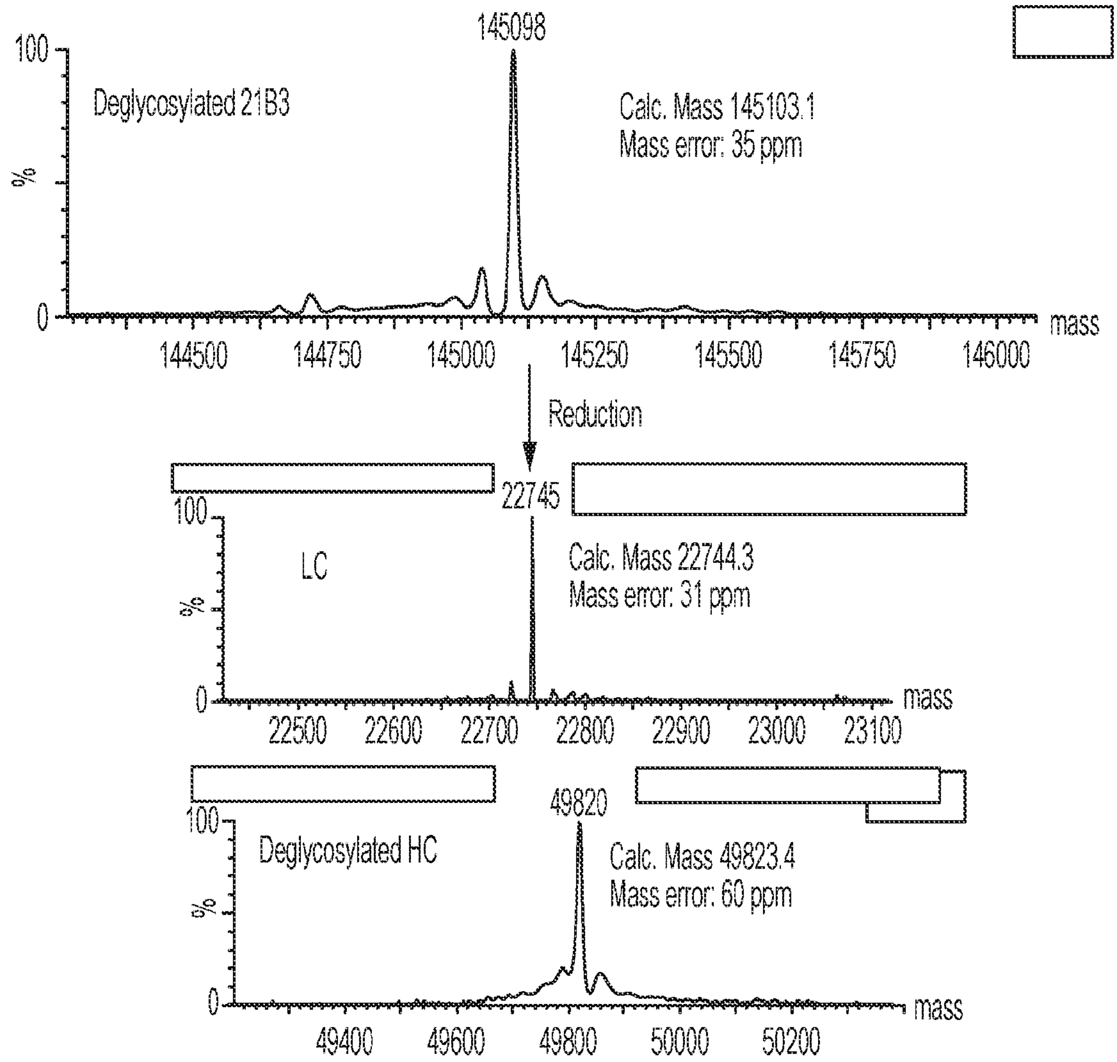


Figure 21A

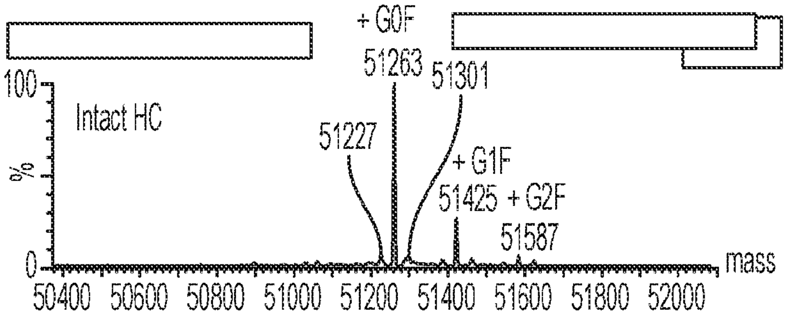


Figure 21B

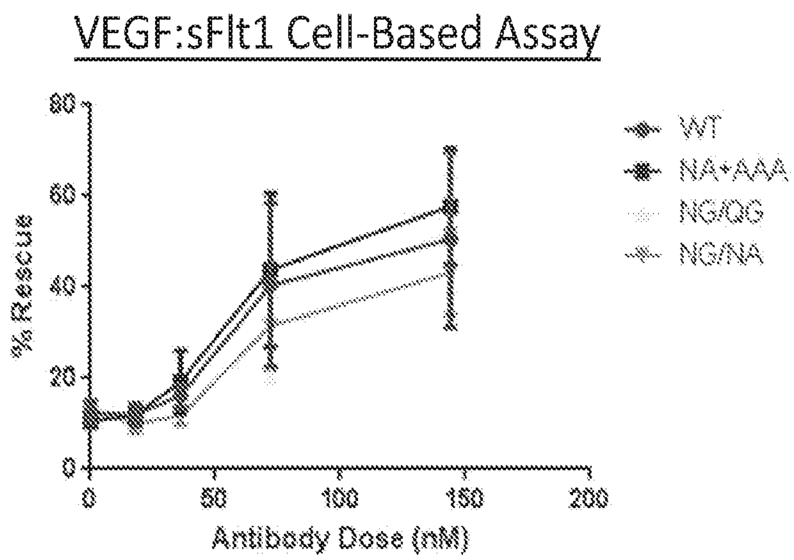


Figure 22A

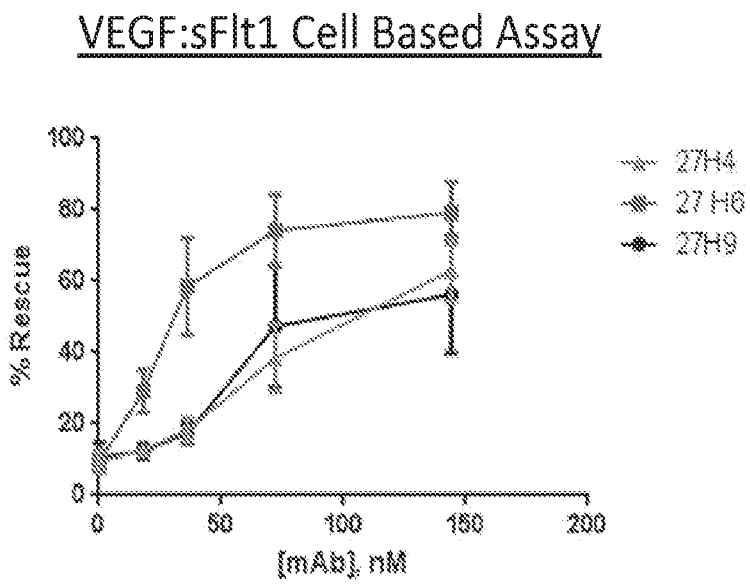


Figure 22B

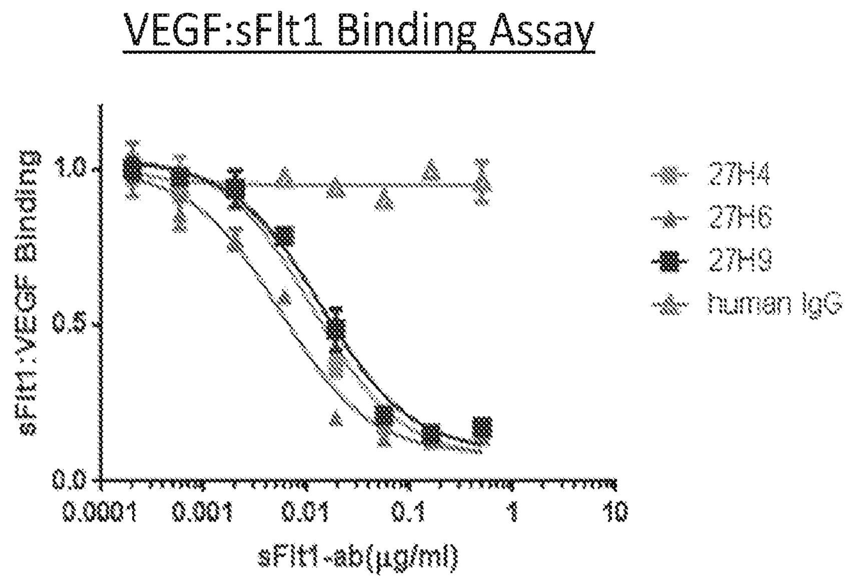

VEGF:sFlt1 Binding Assay
sFlt1:VEGF Binding
1.0
0.5
0.0
0.0001
0.001
0.01
0.1
1
10
sFlt1-ab(µg/ml)
27H4
27H6
27H9
human IgG

Figure 23

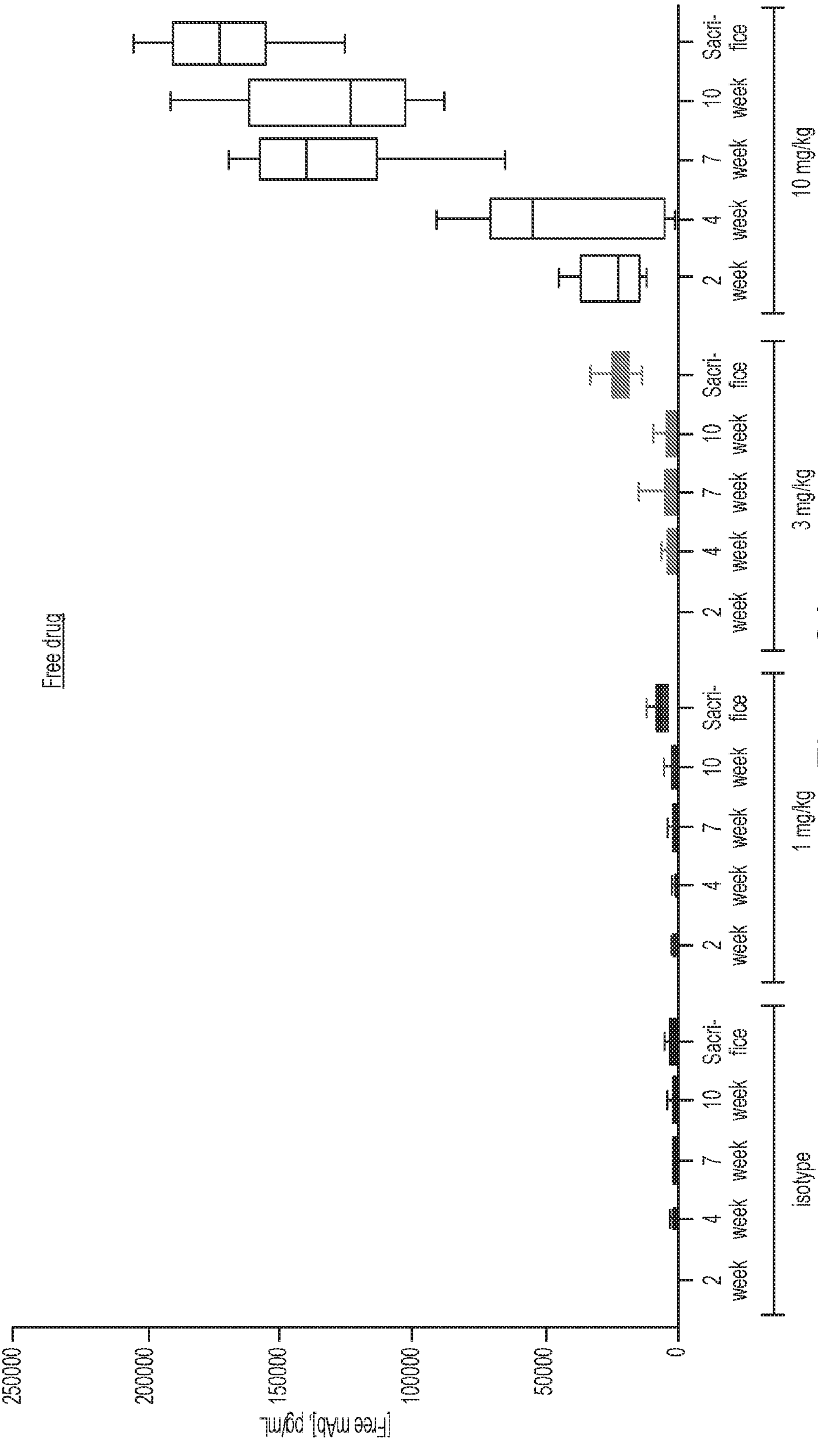

Free drug
[Free mAb], pg/mL
250000
200000
150000
100000
50000
0
2 week
4 week
7 week
10 week
Sacri-fice
isotype
1 mg/kg
3 mg/kg
10 mg/kg

Figure 24

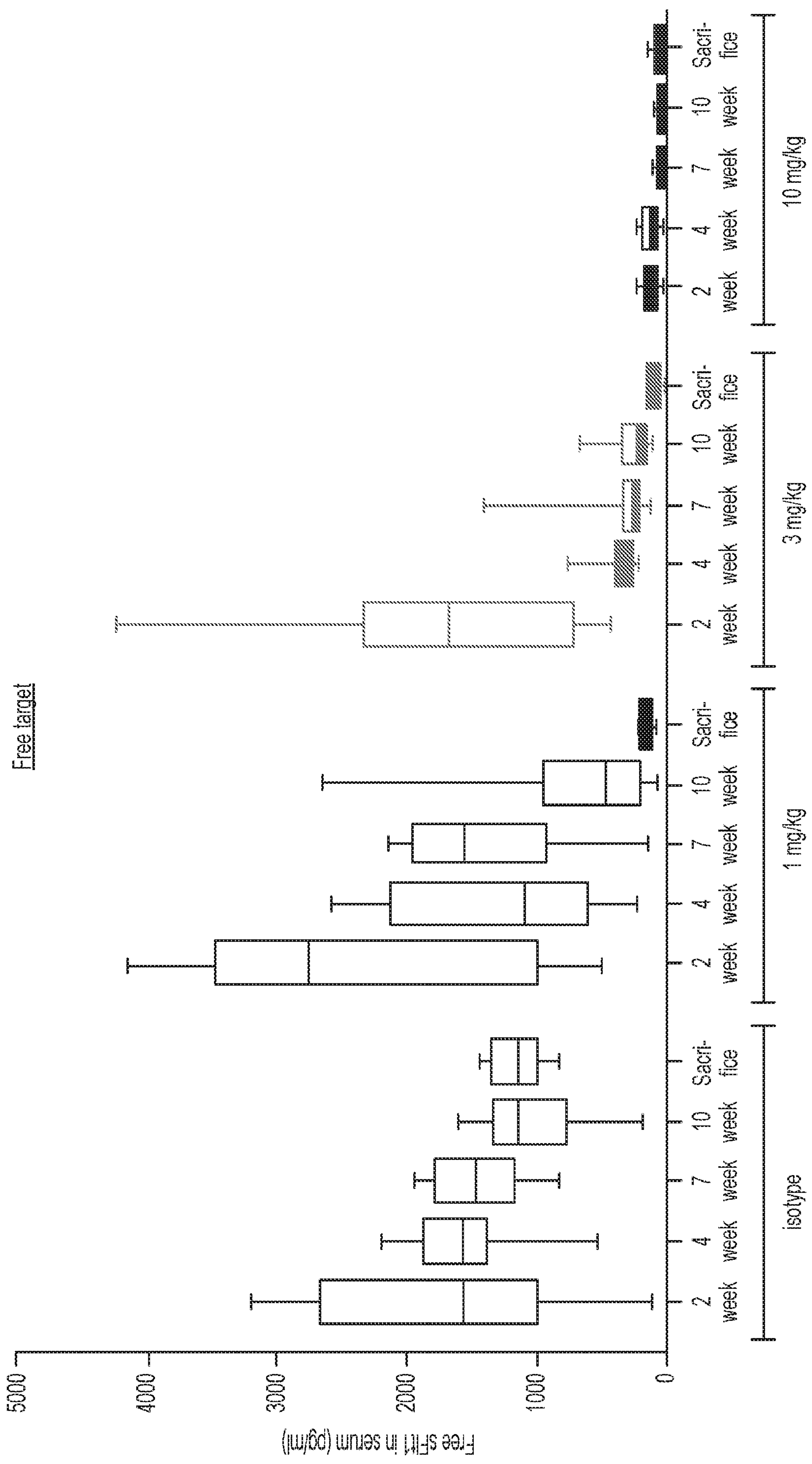

Free target
Free sFlt1 in serum (pg/ml)
0
1000
2000
3000
4000
5000
2 week
4 week
7 week
10 week
Sacri-fice
isotype
1 mg/kg
3 mg/kg
10 mg/kg

Figure 25

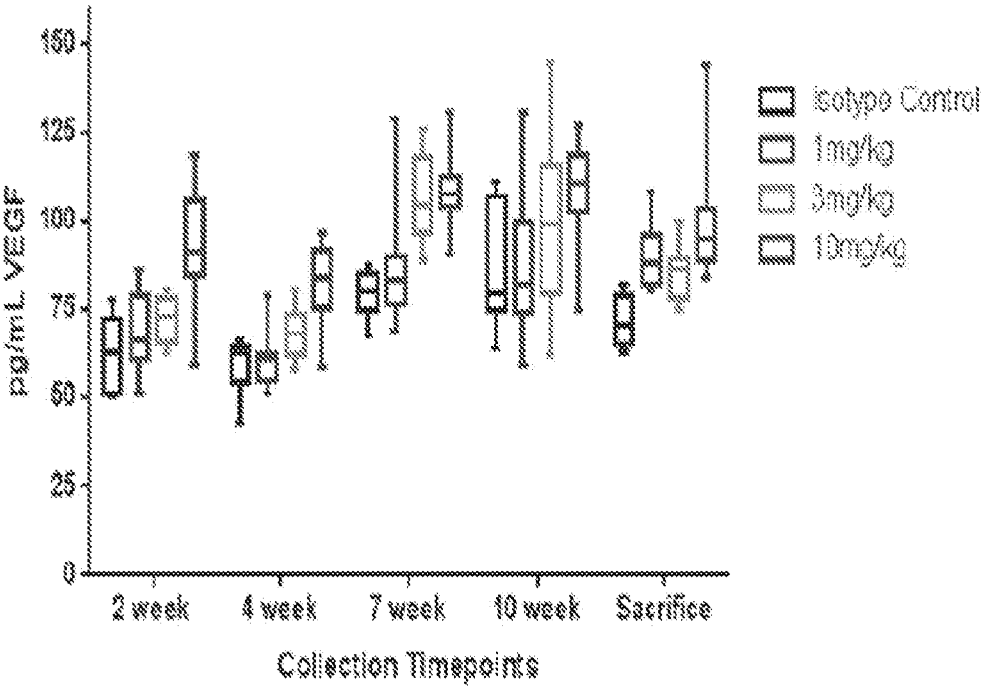

150
125
100
75
50
25
0
pg/mL VEGF
2 week
4 week
7 week
10 week
Sacrifice
Collection Timepoints
Isotype Control
1mg/kg
3mg/kg
10mg/kg

Figure 26

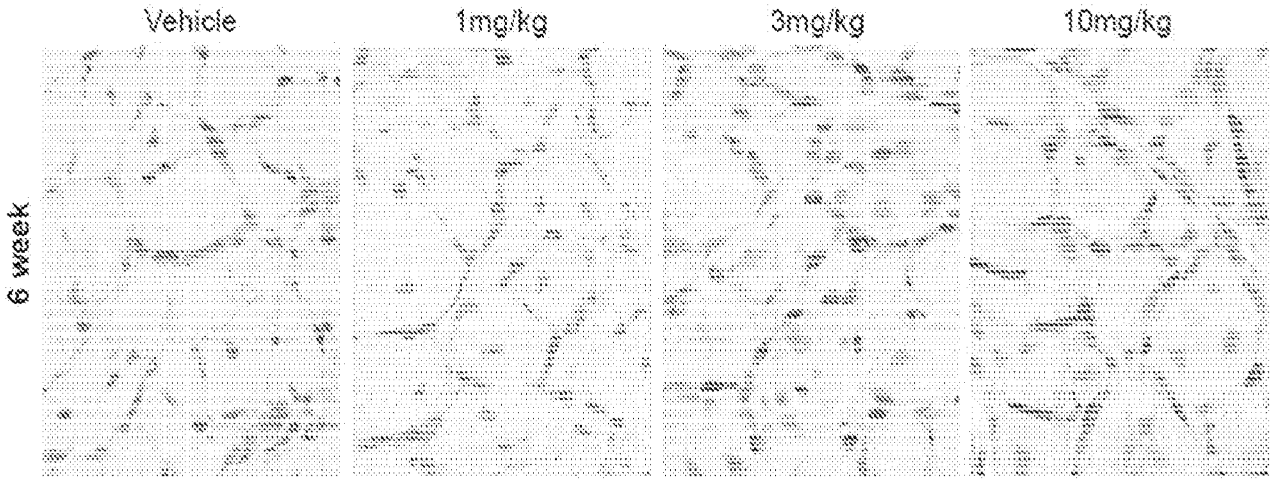


Figure 27A Figure 27B Figure 27C Figure 27D

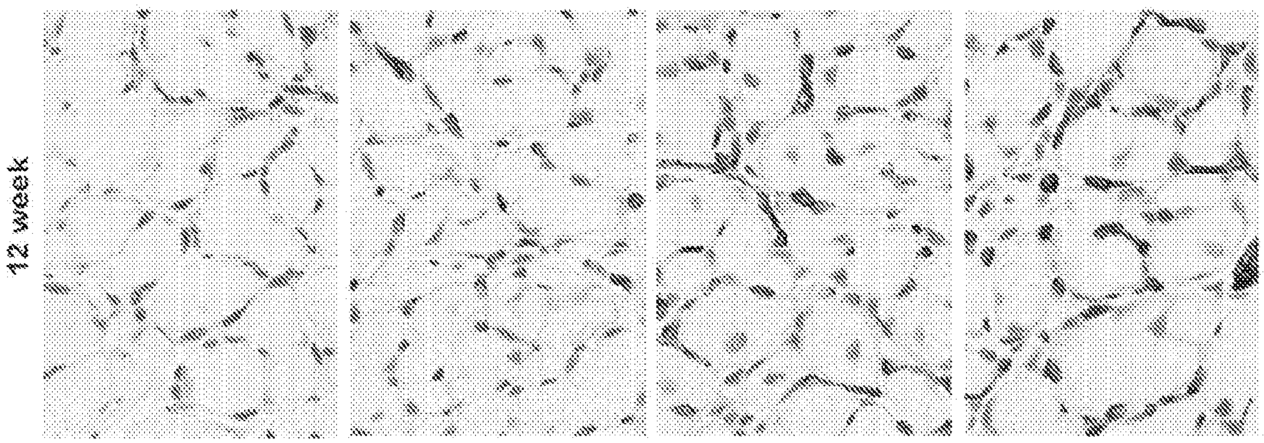


Figure 27E Figure 27F Figure 27G Figure 27H

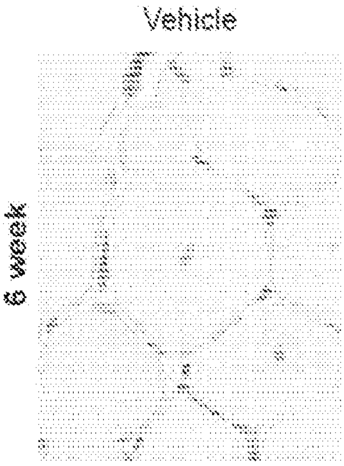


Figure 28A

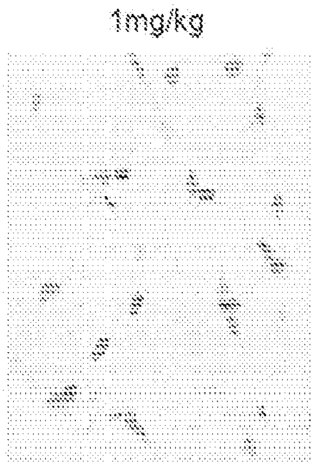


Figure 28B

Figure 28C

Figure 28D

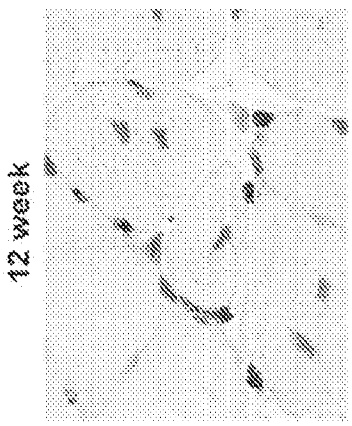


Figure 28E

Figure 28F

Figure 28G

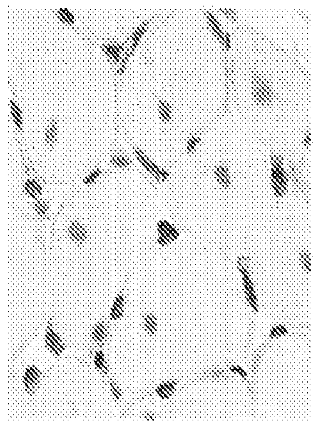


Figure 28H

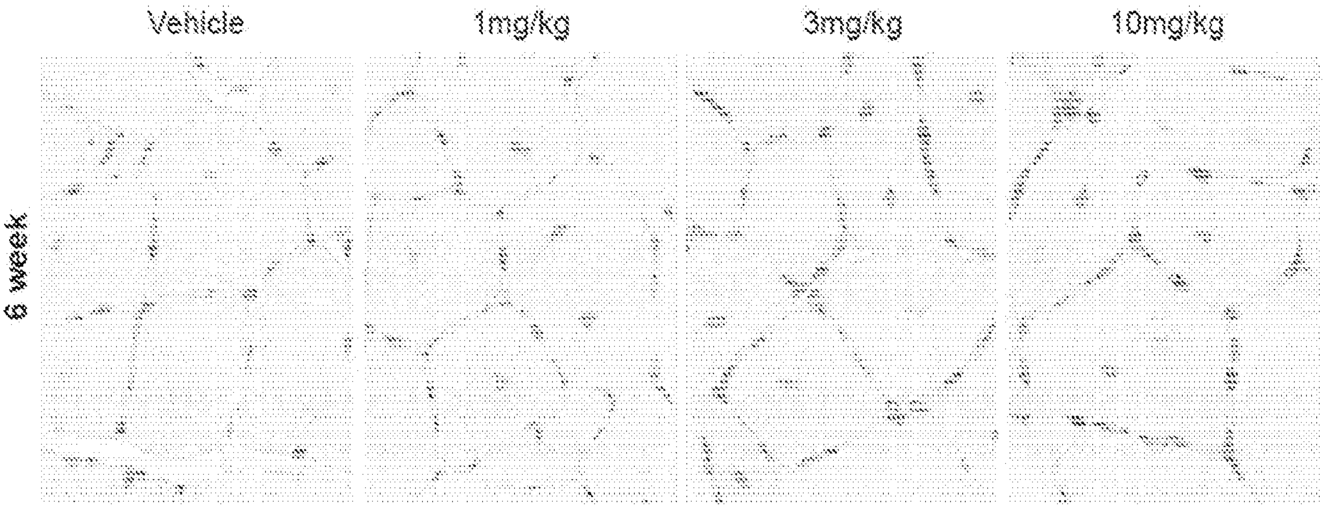


Figure 29A Figure 29B Figure 29C Figure 29D

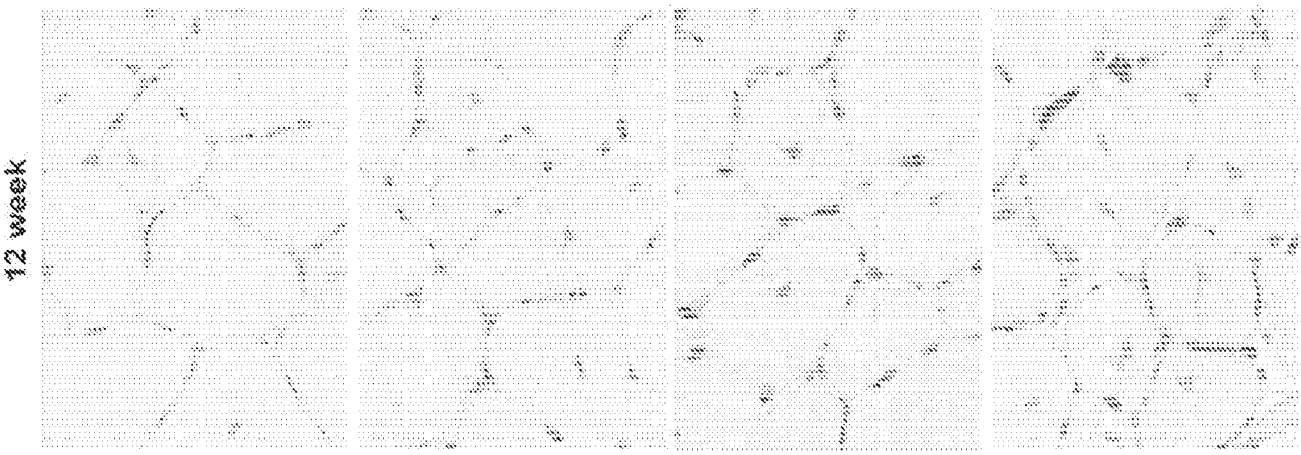


Figure 29E Figure 29F Figure 29G Figure 29H

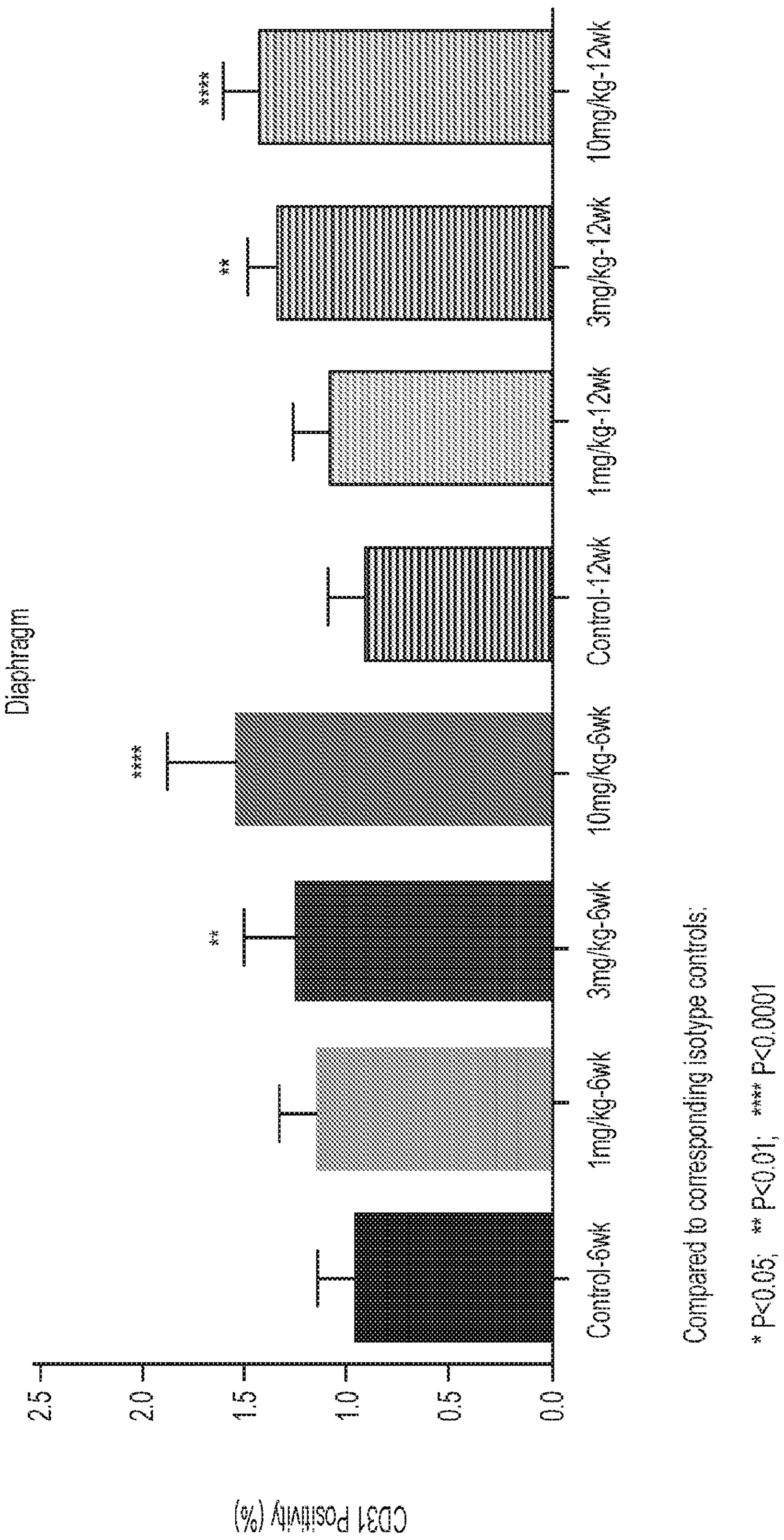

Diaphragm
CD31 Positivity (%)
0.0
0.5
1.0
1.5
2.0
2.5
Control-6wk
1mg/kg-6wk
3mg/kg-6wk
10mg/kg-6wk
Control-12wk
1mg/kg-12wk
3mg/kg-12wk
10mg/kg-12wk
**
****
**
****
Compared to corresponding isotype controls:
* P<0.05; ** P<0.01; **** P<0.0001

Figure 30A

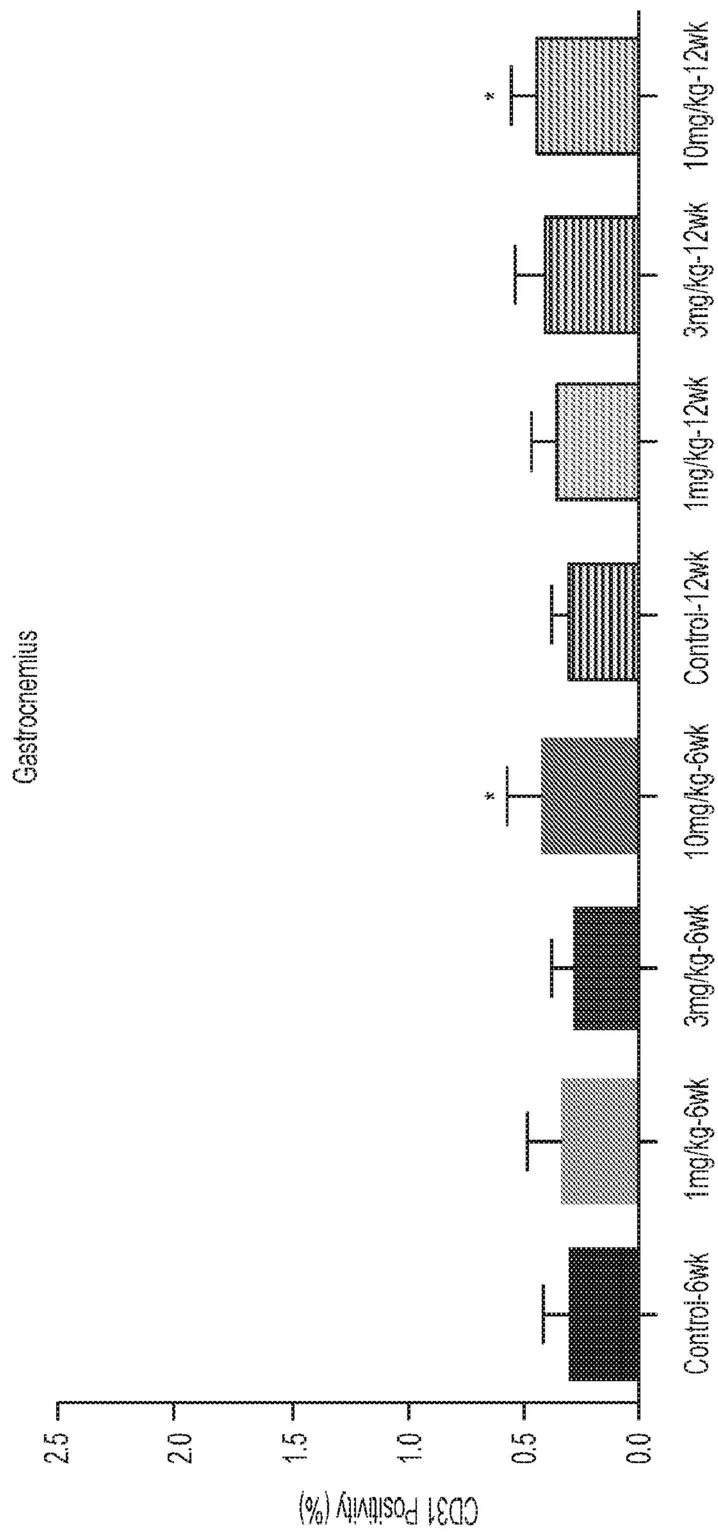

Gastrocnemius
CD31 Positivity (%)
2.5
2.0
1.5
1.0
0.5
0.0
Control-6wk
1mg/kg-6wk
3mg/kg-6wk
10mg/kg-6wk
Control-12wk
1mg/kg-12wk
3mg/kg-12wk
10mg/kg-12wk
*
*

Figure 30B

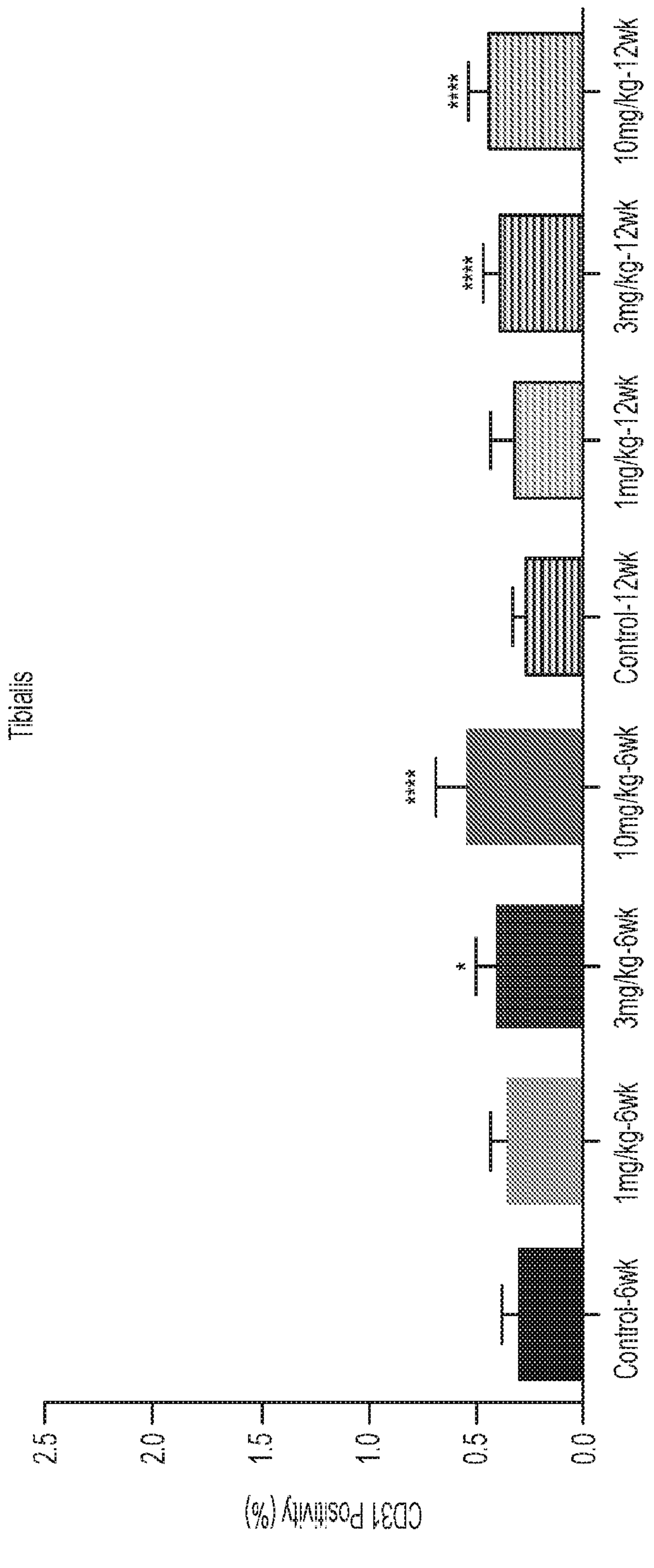

Tibialis
CD31 Positivity (%)
2.5
2.0
1.5
1.0
0.5
0.0
*
****
****
****
Control-6wk
1mg/kg-6wk
3mg/kg-6wk
10mg/kg-6wk
Control-12wk
1mg/kg-12wk
3mg/kg-12wk
10mg/kg-12wk

Figure 30C

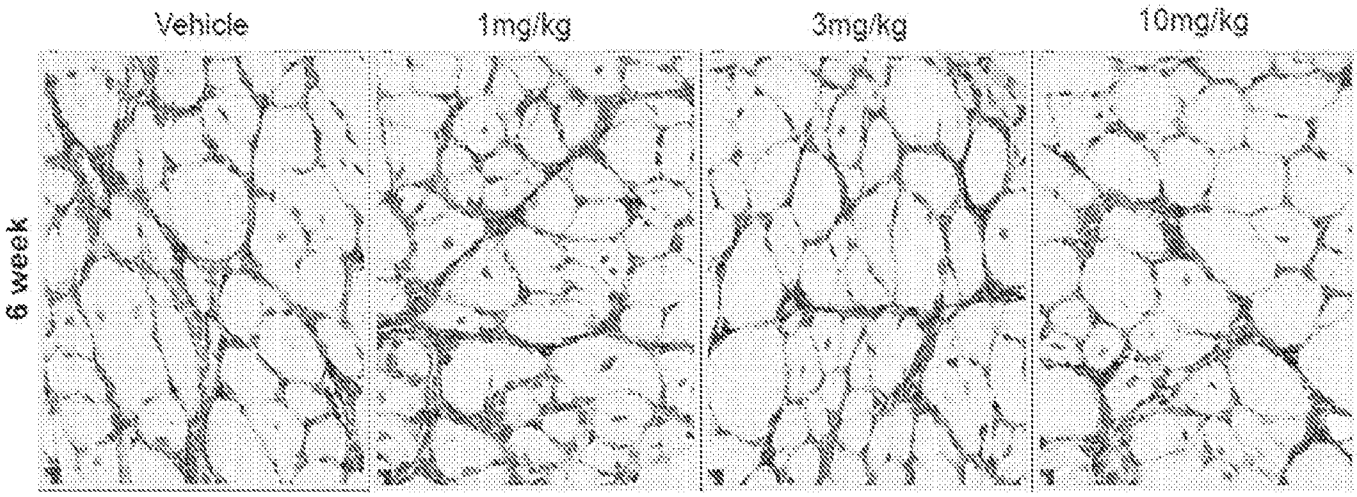


Figure 31A Figure 31B Figure 31C Figure 31D

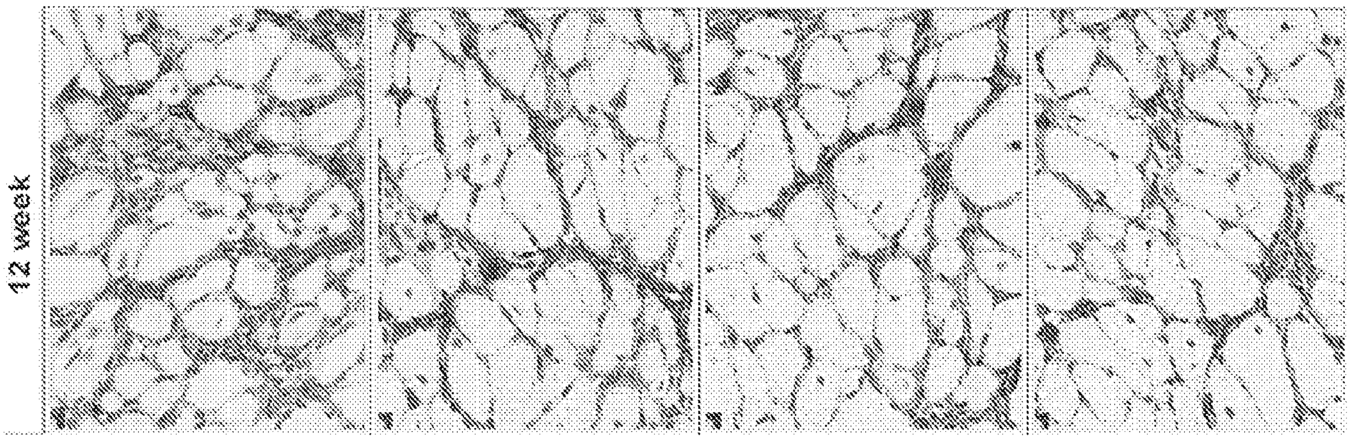


Figure 31E Figure 31F Figure 31G Figure 31H

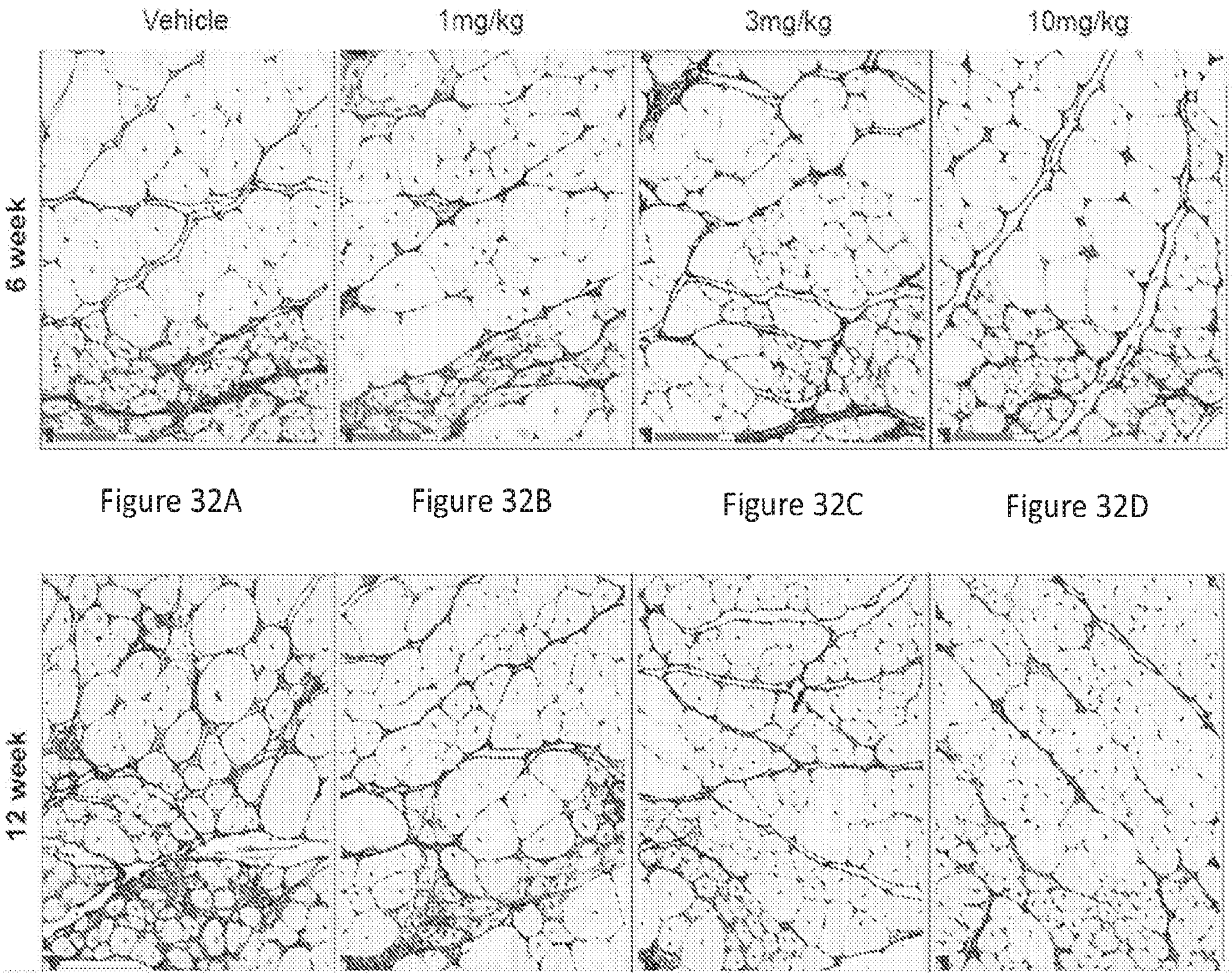


Figure 32A Figure 32B Figure 32C Figure 32D

Figure 32E Figure 32F Figure 32G Figure 32H

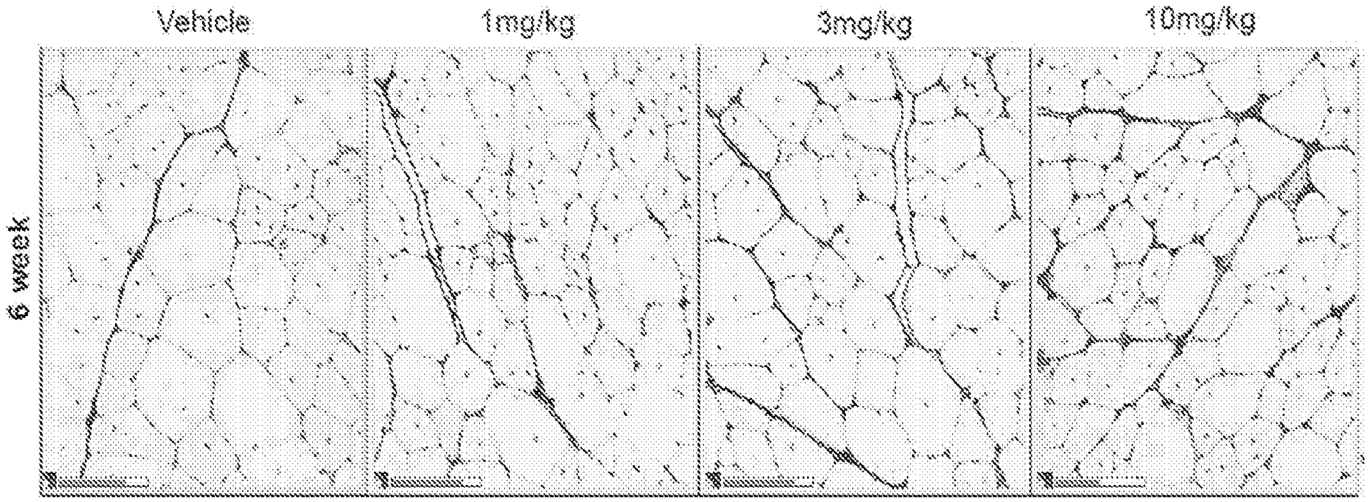


Figure 33A Figure 33B Figure 33C Figure 33D

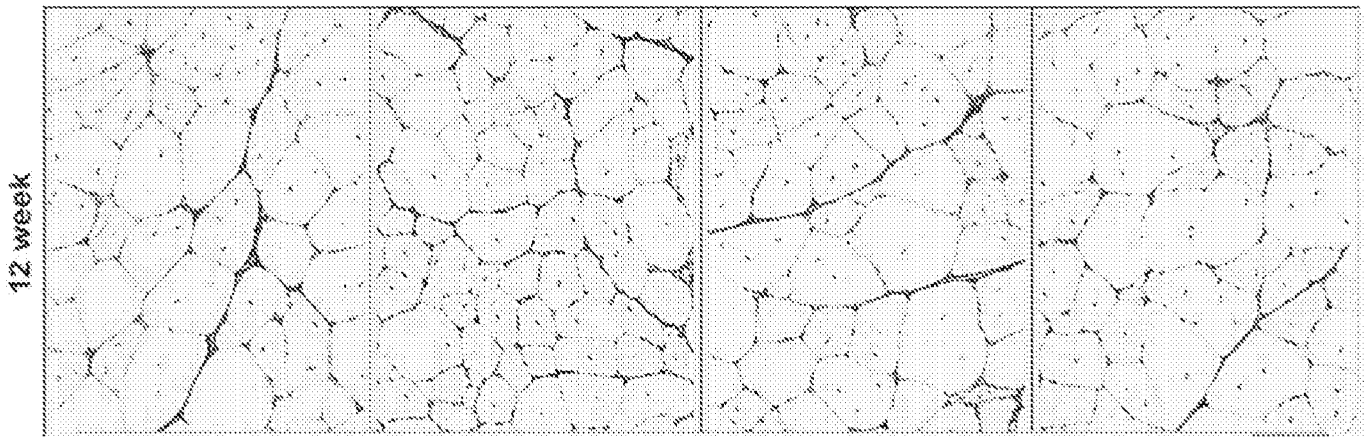


Figure 33E Figure 33F Figure 33G Figure 33H

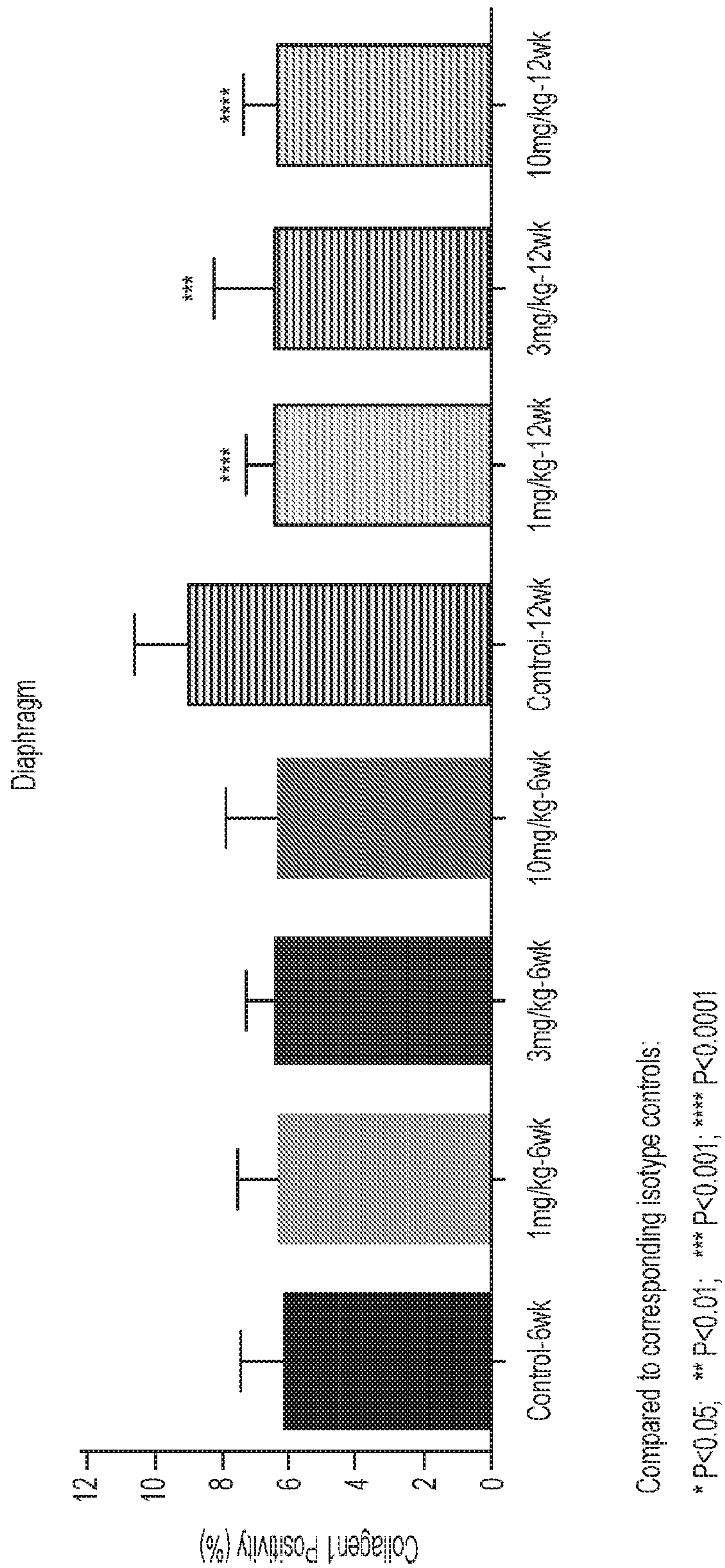

Diaphragm
Collagen1 Positivity (%)
0
2
4
6
8
10
12
Control-6wk
1mg/kg-6wk
3mg/kg-6wk
10mg/kg-6wk
Control-12wk
1mg/kg-12wk
3mg/kg-12wk
10mg/kg-12wk
****
***
****
Compared to corresponding isotype controls:
* P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001

Figure 34A

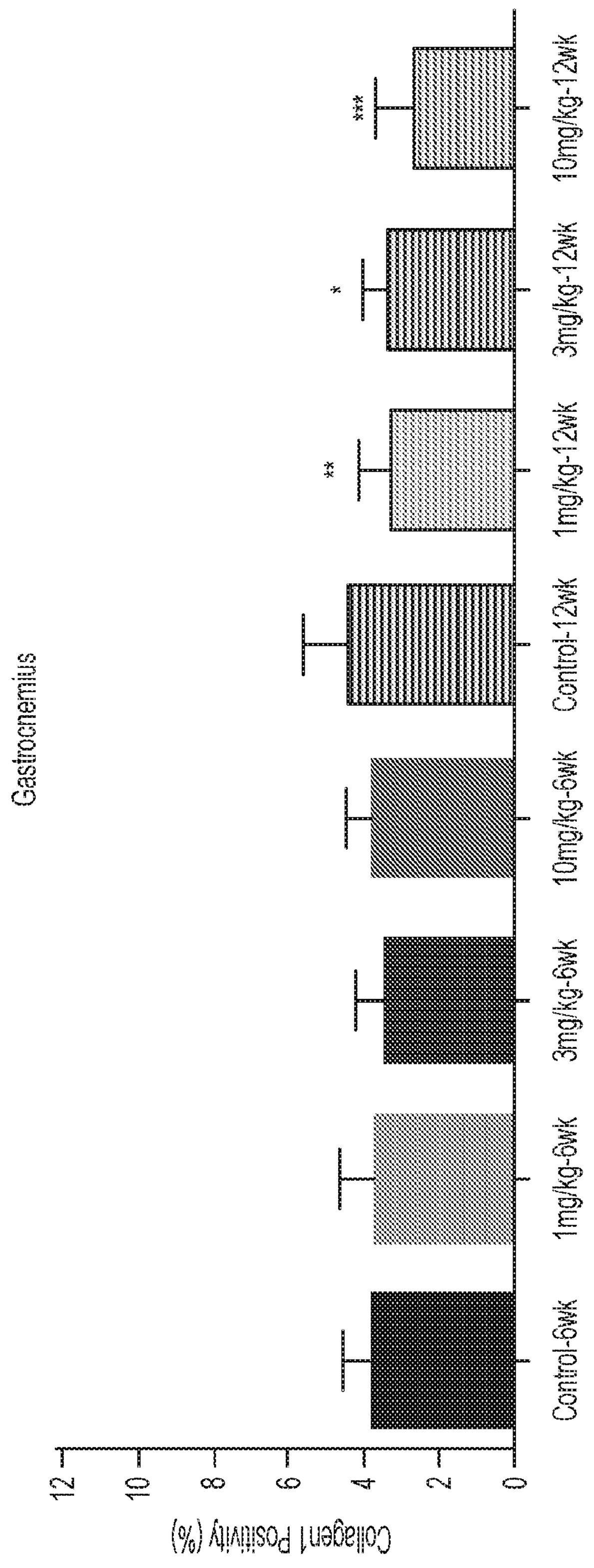

Gastrocnemius
Collagen1 Positivity (%)
12
10
8
6
4
2
0
Control-6wk
1mg/kg-6wk
3mg/kg-6wk
10mg/kg-6wk
Control-12wk
**
1mg/kg-12wk
*
3mg/kg-12wk
***
10mg/kg-12wk

Figure 34B

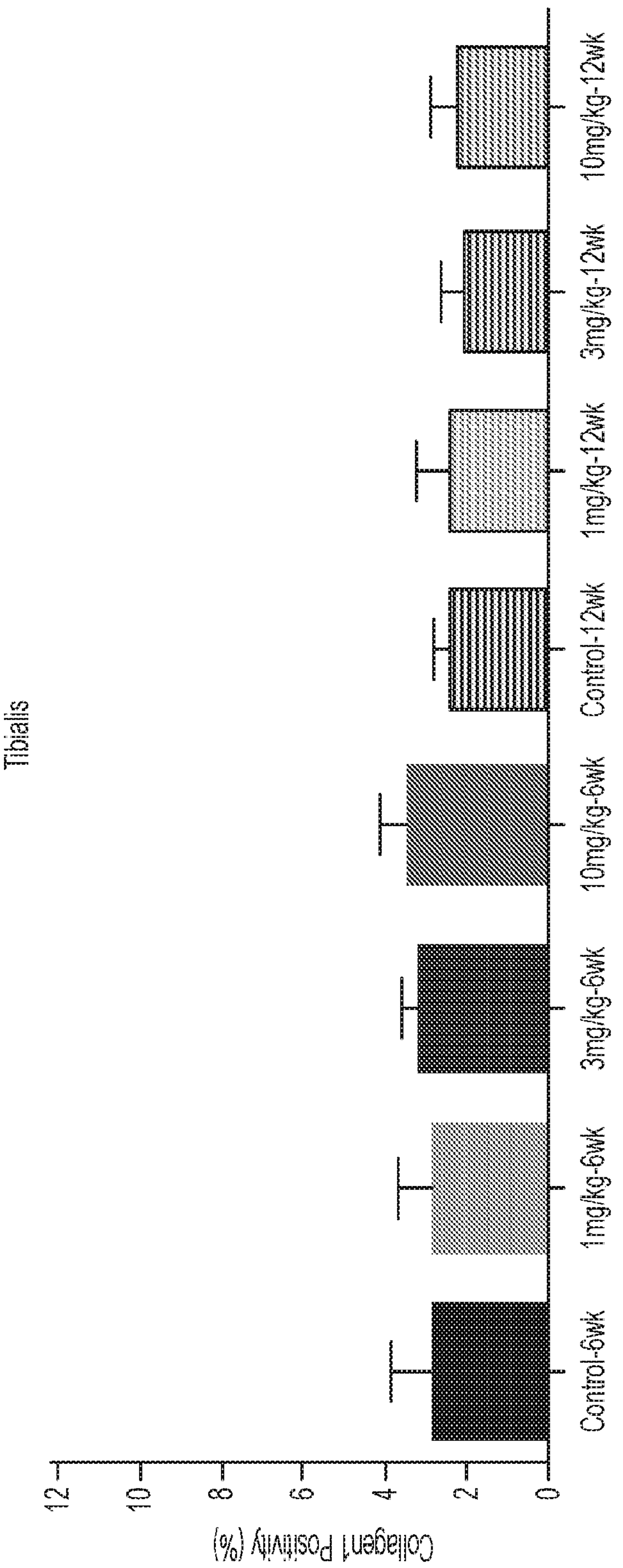

Tibialis
Collagen1 Positivity (%)
0
2
4
6
8
10
12
Control-6wk
1mg/kg-6wk
3mg/kg-6wk
10mg/kg-6wk
Control-12wk
1mg/kg-12wk
3mg/kg-12wk
10mg/kg-12wk

Figure 34C

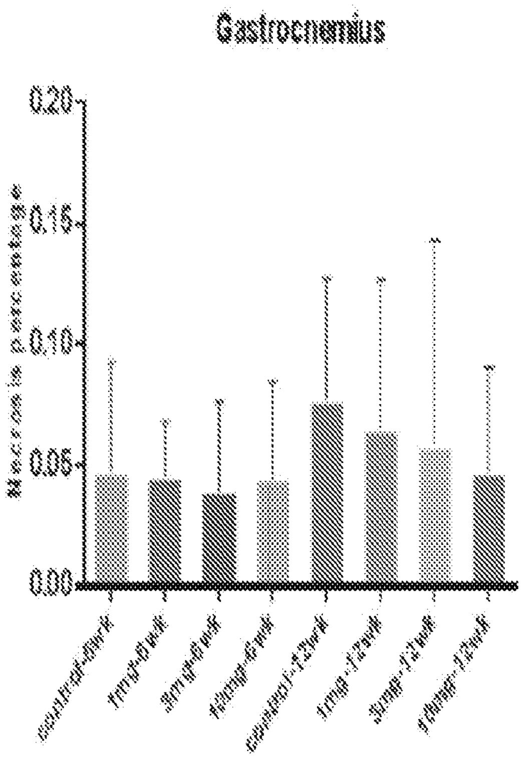


Figure 35A

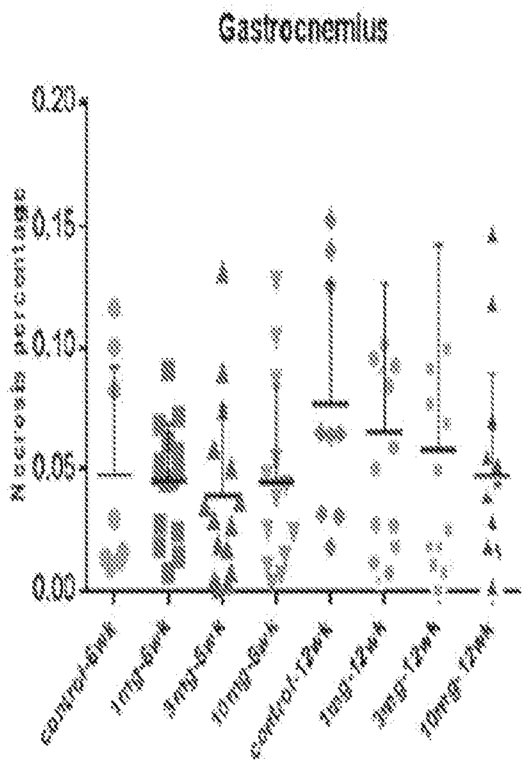


Figure 35B

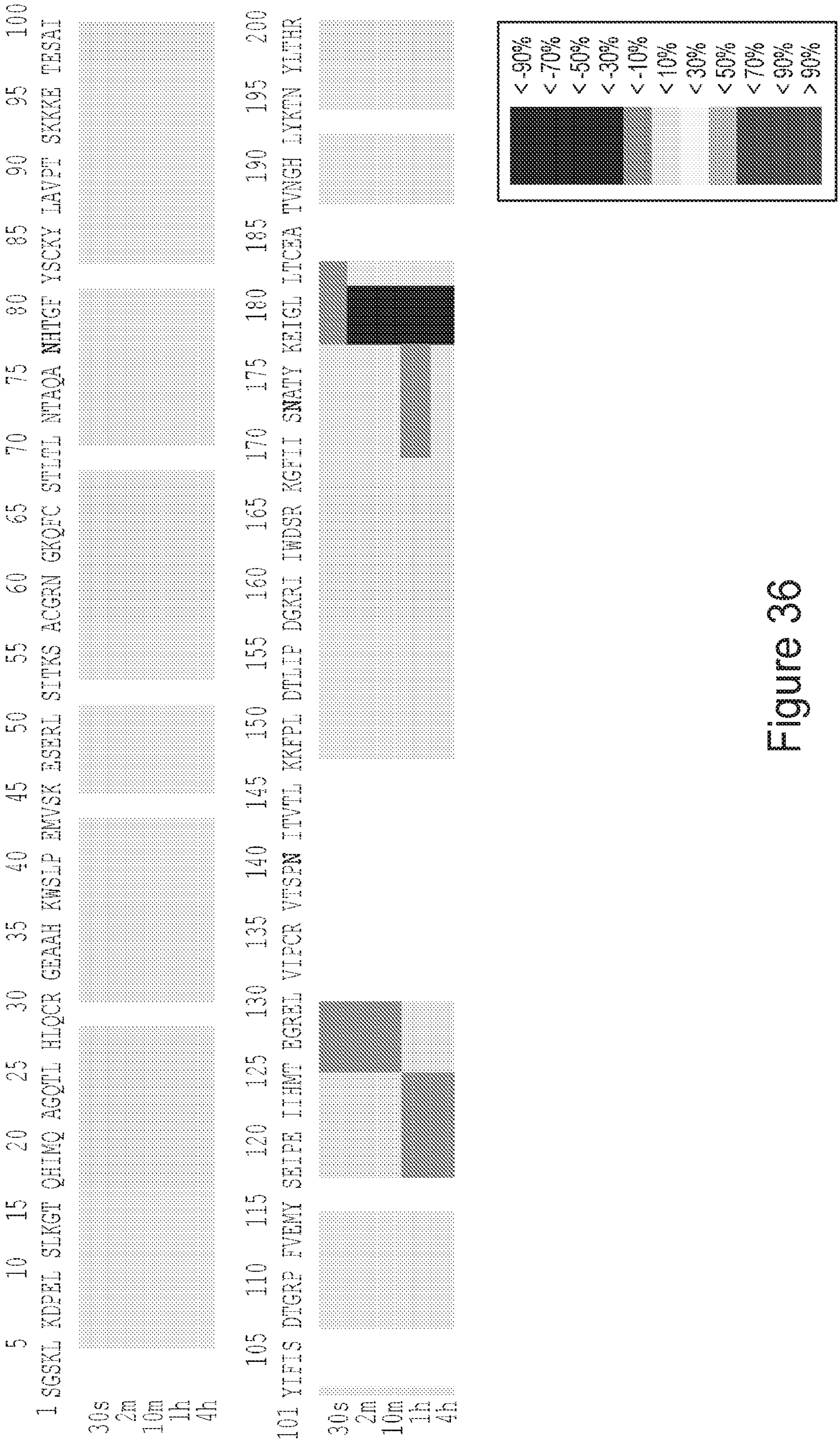

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
1 SGSKL KDPEL SLKGT QHIMQ AGQTL HLQCR GEAAH KWSLP EMVSK ESERL SITKS ACGRN GKQFC STLTL NTAQA NHTGF YSCKY LAVPT SKKKE TESAI
30s
2m
10m
1h
4h
105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200
101 YIFIS DTGRP FVEMY SEIPE IIHMT EGREL VIPCR VTSPN ITVTL KKFPL DTLIP DGKRI IWDSR KGFII SNATY KEIGL LTCEA TVNGH LYKTN YLTHR
30s
2m
10m
1h
4h
< -90%
< -70%
< -50%
< -30%
< -10%
< 10%
< 30%
< 50%
< 70%
< 90%
> 90%

Figure 36

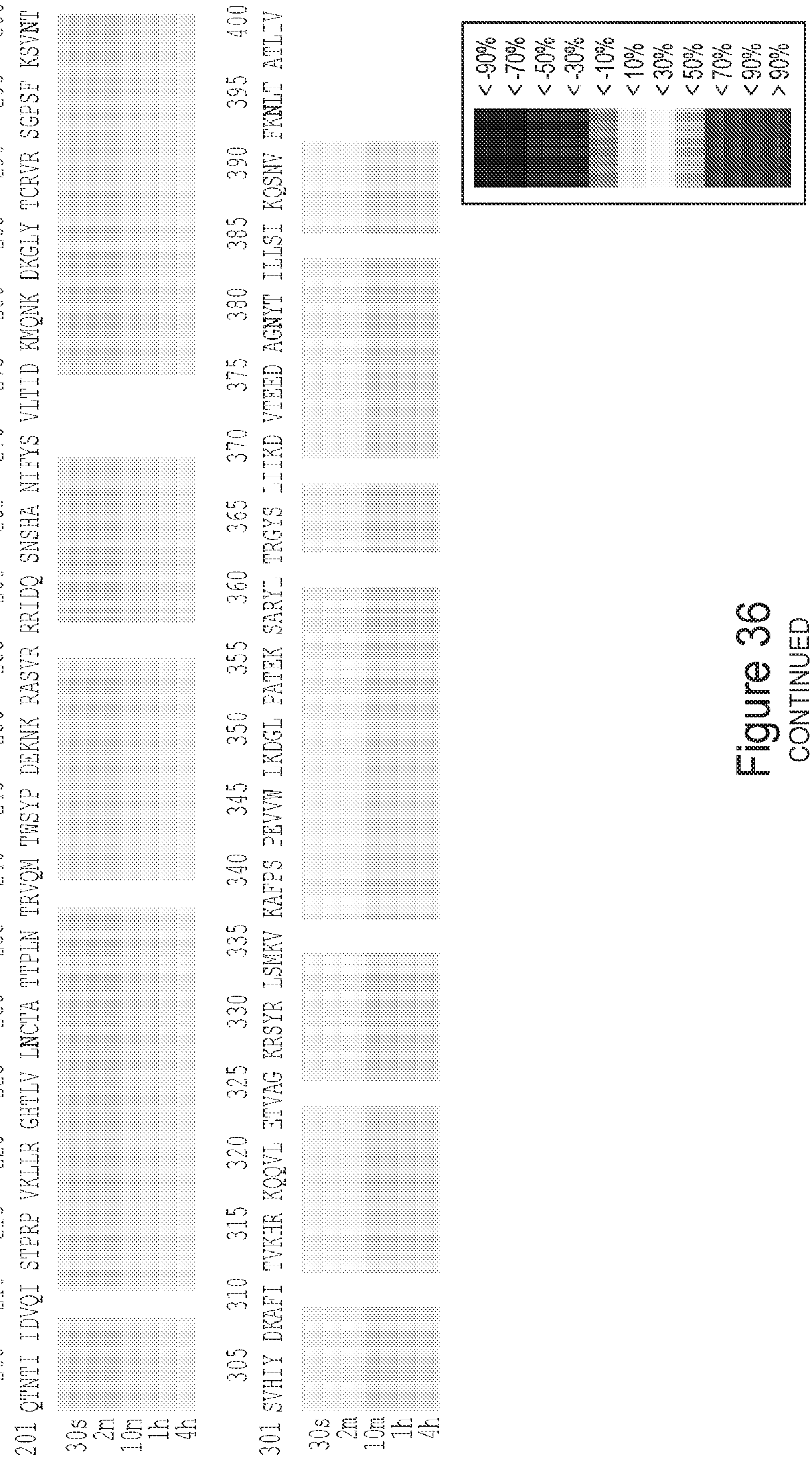


Figure 36
CONTINUED

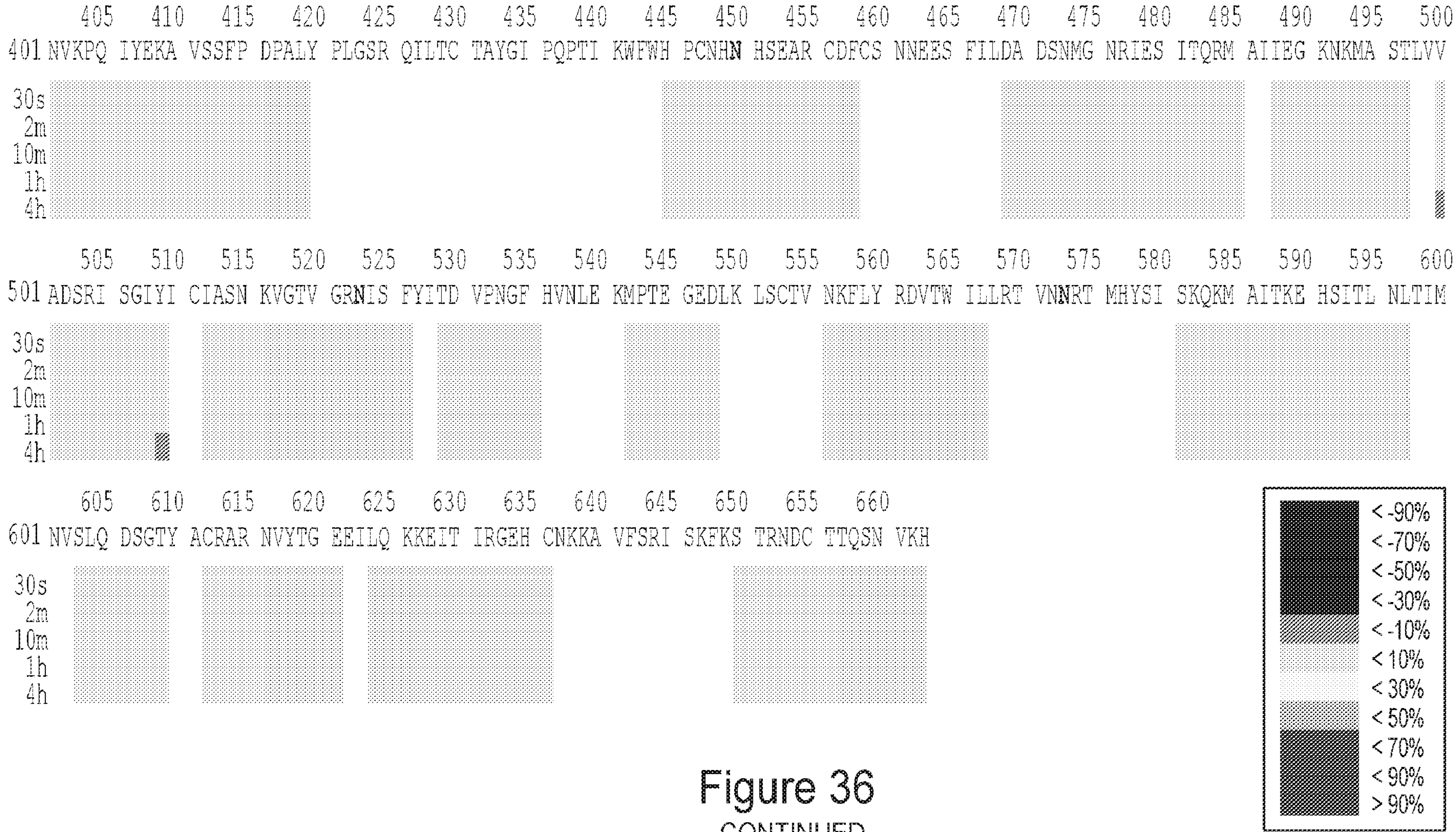


Figure 36
CONTINUED

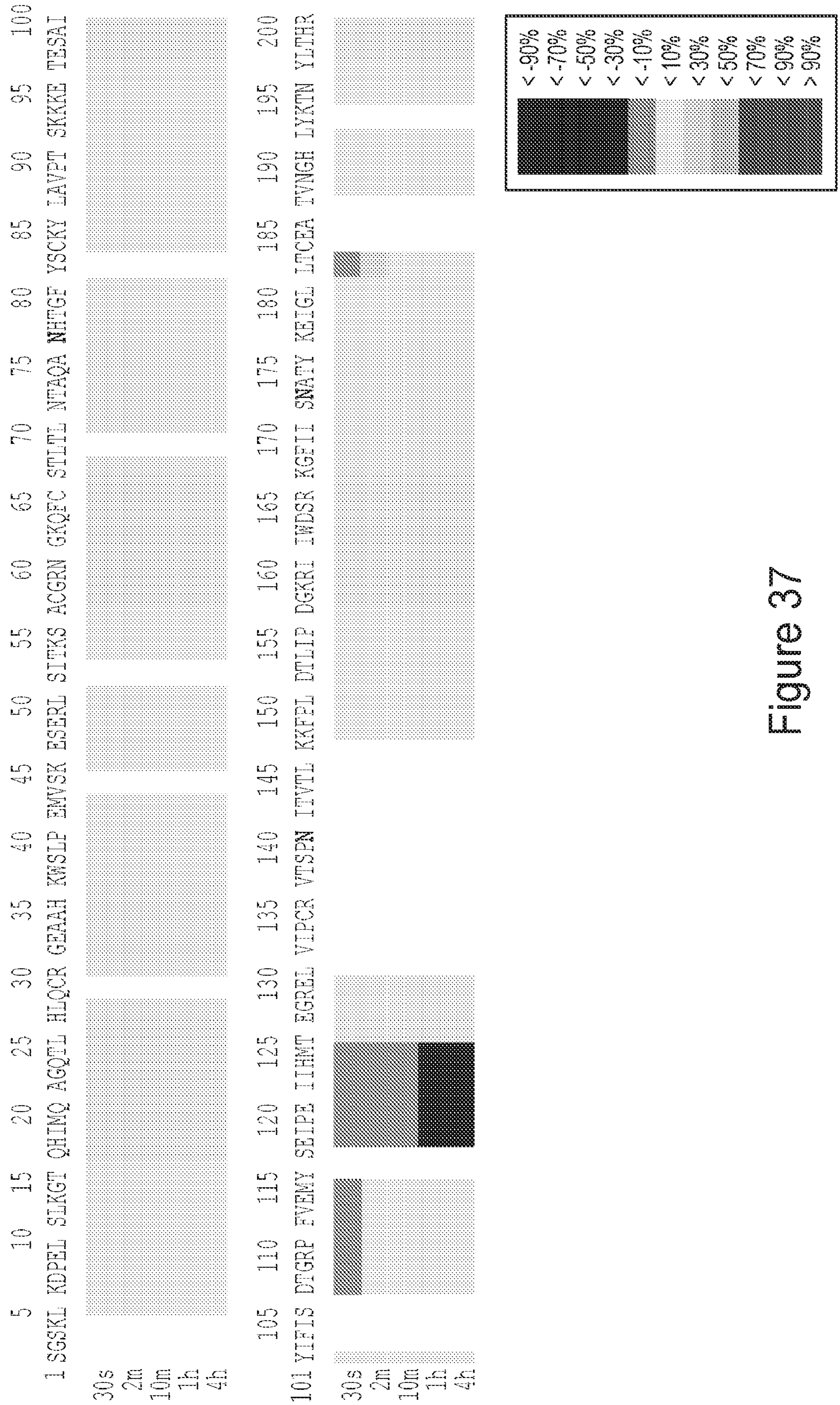

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
1 SGSKL KDPEL SLKGT QHIMQ AGQTL HLQCR GEAAH KWSLP EMVSK ESERL SITKS ACGRN GKQFC STLTL NTAQA NHTGF YSCKY LAVPT SKKKE TESAI
30s
2m
10m
1h
4h
105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200
101 YIFIS DTGRP FVEMY SEIPE IIHMT EGREL VIPCR VTSPN ITVTL KKFPL DTLIP DGKRI IWDSR KGFII SNATY KEIGL LTCEA TVNGH LYKTN YLTHR
30s
2m
10m
1h
4h
< -90%
< -70%
< -50%
< -30%
< -10%
< 10%
< 30%
< 50%
< 70%
< 90%
> 90%

Figure 37

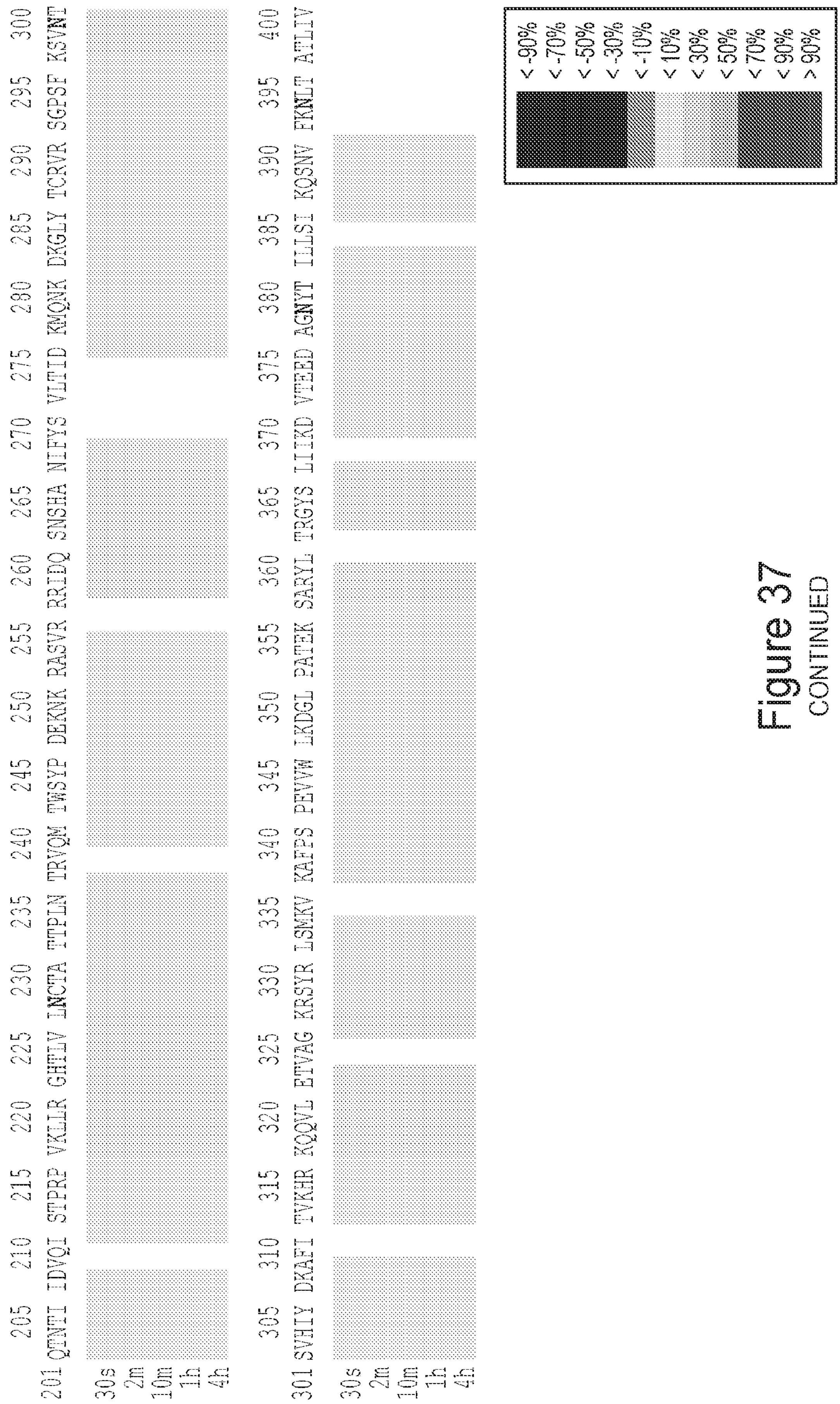


Figure 37
CONTINUED

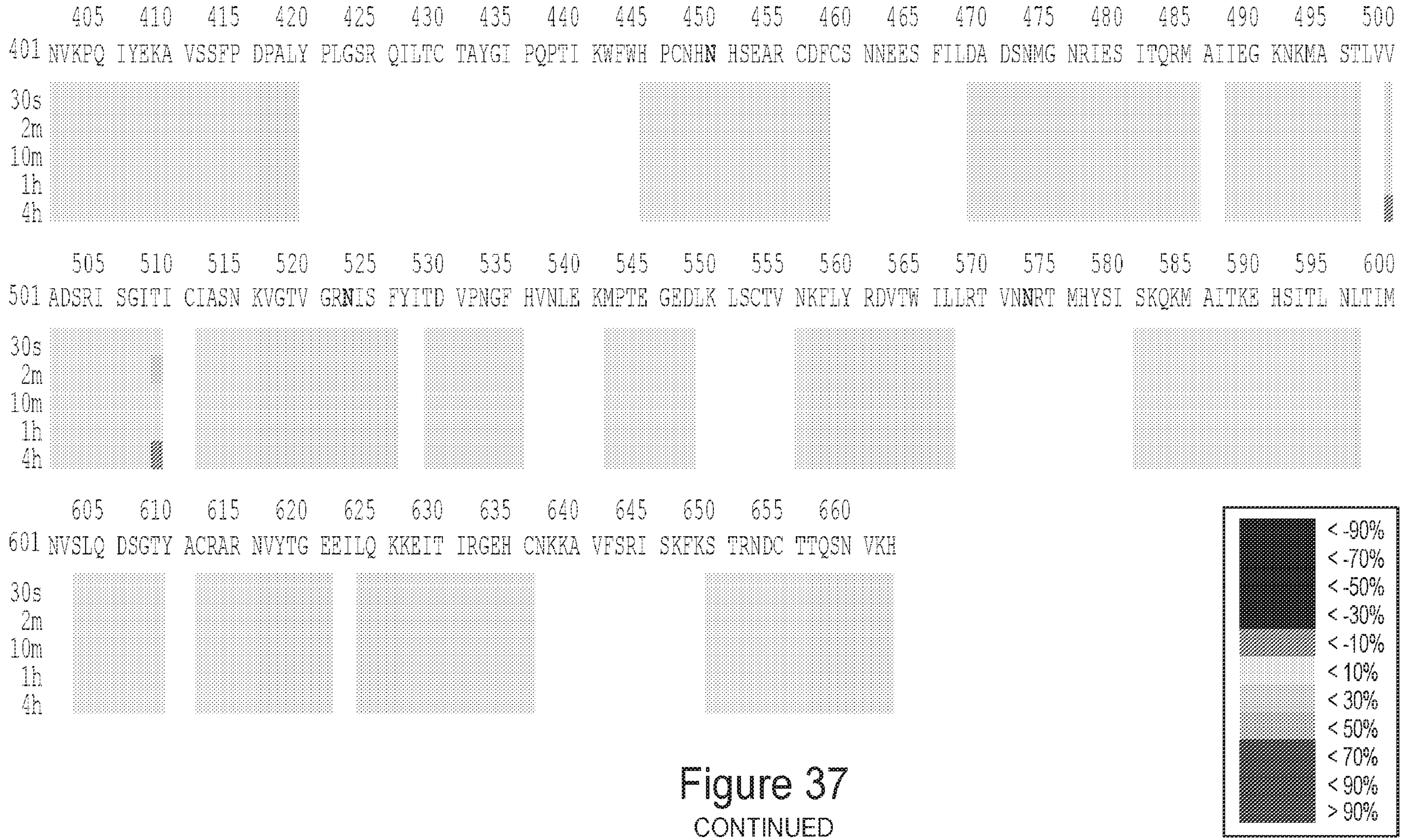


Figure 37
CONTINUED

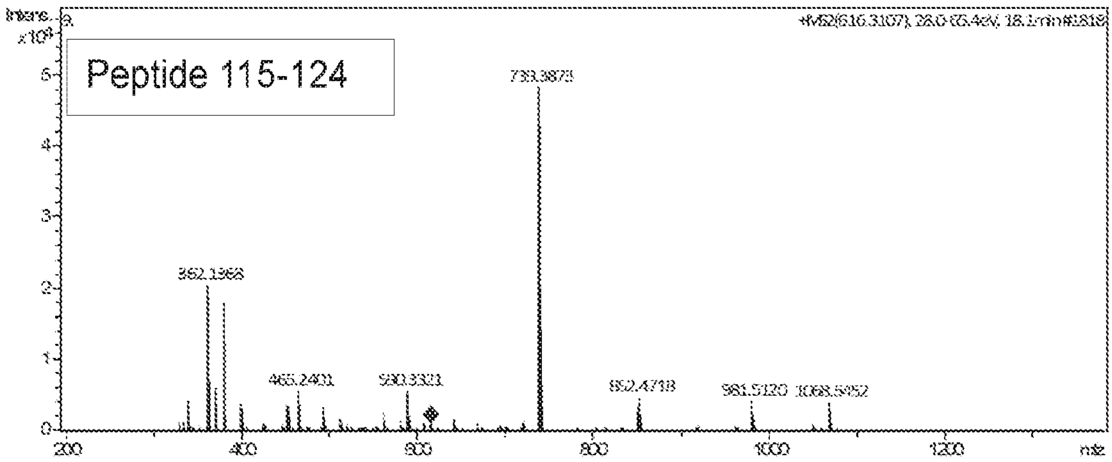


Figure 38A

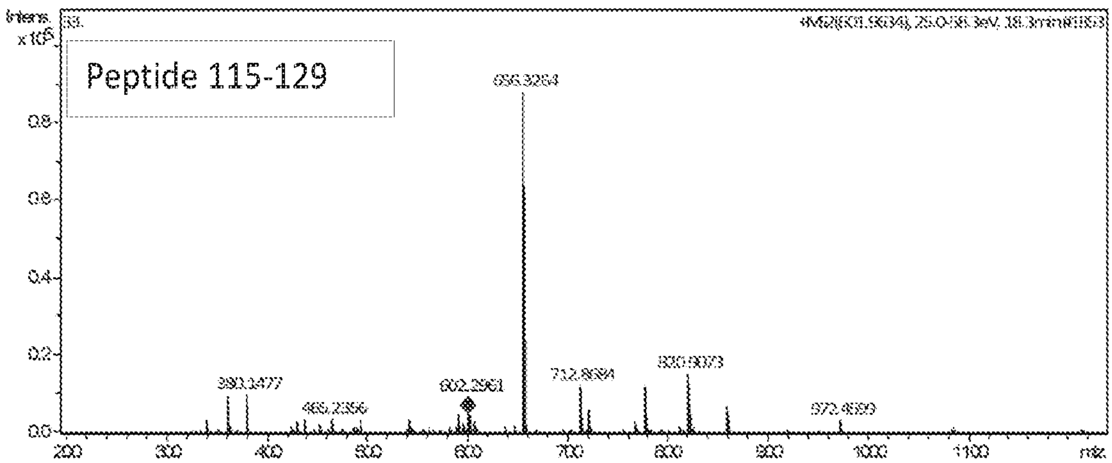


Figure 38B

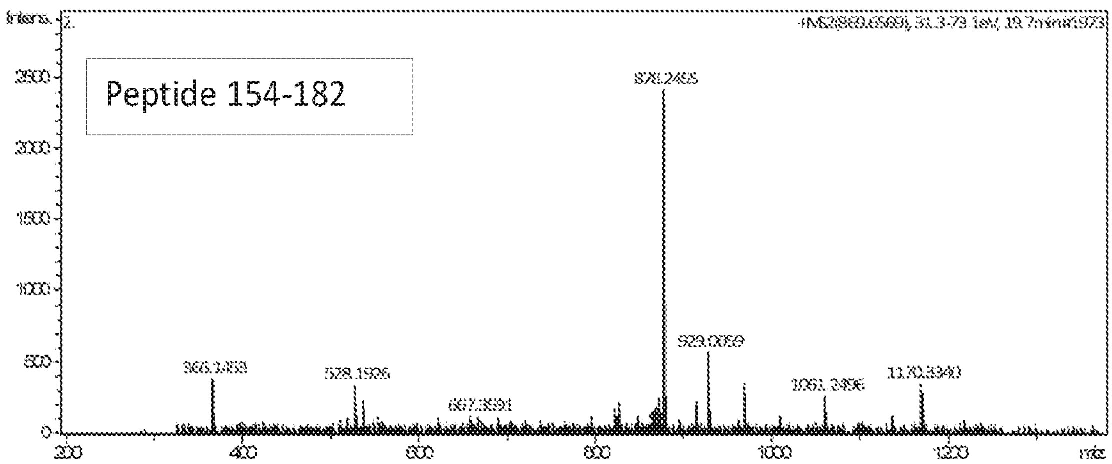


Figure 38C

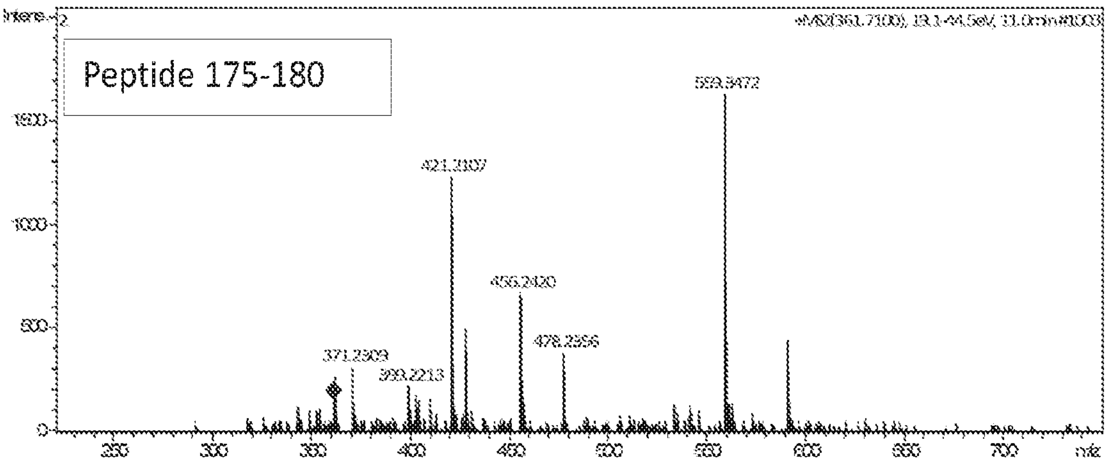


Figure 38D

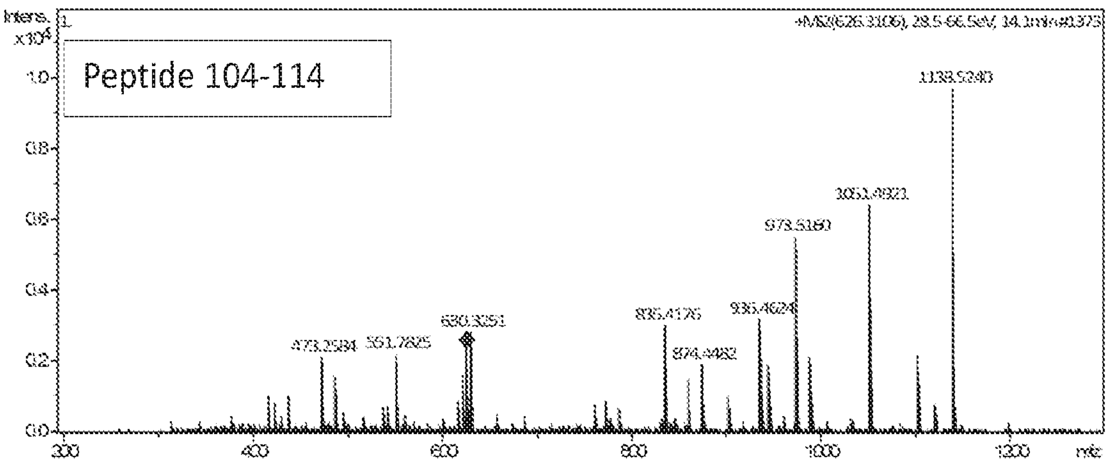


Figure 38E