(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 June 2022 (23.06.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/133239 A1

(51) International Patent Classification:
C07K 16/42 (2006.01) *A61K 31/165* (2006.01)
A61P 9/12 (2006.01)

(21) International Application Number:
PCT/US2021/064073

(22) International Filing Date:
17 December 2021 (17.12.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/127,959 18 December 2020 (18.12.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: IMMUNOGLOBULIN PROTEINS THAT BIND TO NPR1 AGONISTS

(57) Abstract: The present disclosure provides novel immunoglobulin proteins that bind to a human natriuretic peptide receptor 1 (NPR1) agonist, preferably an anti-NPR1 antibody. In certain embodiments, the proteins of the disclosure comprise at least one immunoglobulin variable domain that binds to an anti-NPR1 antibody. In certain embodiments, the proteins of the disclosure are useful in blocking and/or reversing the effect of an administered anti-NPR1 antibody. In certain embodiments, the antigen-binding proteins are useful for effective management of blood pressure and hemodynamics in humans.

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IMMUNOGLOBULIN PROTEINS THAT BIND TO NPR1 AGONISTS

FIELD OF THE INVENTION

[001] The present disclosure is related to immunoglobulin proteins that specifically bind to natriuretic peptide receptor 1 (NPR1) agonists, and therapeutic methods of using those proteins.

CROSS-REFERENCE TO RELATED APPLICATIONS

[002] This application is being filed on December 17, 2021 as a PCT Patent International Application. This application claims priority to U.S. Provisional Patent Application No. 63/127,959, filed December 18, 2020, the entire content of which is incorporated by reference herein.

SEQUENCE STATEMENT

[003] The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 13, 2021, is named 40848-0104WOU1-SeqListing.txt and is 71 kilobytes in size.

BACKGROUND

[004] Natriuretic peptide receptor 1 (NPR1; also known as NPR-A) belongs to the cell-surface family of the guanylyl cyclase receptors, enzymes that catalyze the conversion of GTP into cyclic GMP. NPR1 is highly expressed in kidney, lungs, adrenal, vasculature, brain, liver, endothelial and adipose tissues and at lower levels in the heart. It is activated by binding to atrial natriuretic peptide (ANP) or brain natriuretic peptide (BNP). NPR1 activation and signaling stimulate many physiologic responses involving many tissues. The ANP-NPR1 system has been well studied for its role in vasorelaxation, natriuresis, diuresis, endothelial permeability and in non-cardiovascular functions like lipolysis and immune cell functions (Potter 2011, *Pharmacol. Ther*. 130: 71-82). Activation of NPR1 leads to natriuresis (excretion of salt by kidneys) and lowers blood pressure.

[005] Currently approved therapeutics intended for agonism of NPR1 present with multiple clinical challenges.

[006] Monoclonal antibodies to NPR1 were first described by Kitano, *et al.,* (1995 *Immunol Lett* 47: 215-22). Activating or agonist anti-NPR1 antibodies are disclosed in, for example, US Patent/Publication Nos. 9090695, and 20160168251, and in WO2010065293. Fully human agonist antibodies that specifically bind to NPR1 protein with high affinity and activate it have been described in US Publication No.

20200123263. R5381, is an agonist of NPR1 that has shown long duration of effect in reduction of systemic blood pressure as compared to current standard-of-care therapies.

[007] *In vivo* studies have shown that R5381 induced significant and persistent reductions of systemic blood pressure, with no evidence of adverse hypotension (*i.e.*, syncope, altered locomotion, death). Because the primary mode of action of certain anti-NPR1 antibodies has been found to be hemodynamic, there is a need for a reversal agent to preempt their hemodynamic effects.

SUMMARY

[008] In an effort to address possible concerns regarding the use of NPR1 agonists (*e.g.*, an activating or agonist anti-NPR1 antibody), reversal agents that bind specifically to such NPR1 agonists were developed, as disclosed herein.

[009] Anti-NPR1 antibodies have been described for the treatment and/or prevention of a disease, disorder, or condition associated with NPR1 and/or for ameliorating at least one symptom associated with such disease, disorder, or condition (*see*, for example, WO2020/086406). The primary mode of action of the anti-NPR1 antibody is hemodynamic. Potential adverse events associated with the lowered blood pressure may include persistent, symptomatic hypotension, reflex tachycardia from compensatory sympathetic nervous system responses (possibly increasing the risk for myocardial infarction, stroke, arrhythmias, heart failure), and decreased cardiac output and end-organ perfusion in subjects with normal (low) venous pressures. Thus, there is a need for a reversal agent (or a rescue agent) that can target and stabilize or reduce or reverse the hemodynamic effects of the anti-NPR1 antibody.

[010] Accordingly, the present disclosure provides an agent that reverses the hemodynamic effects of a natriuretic peptide receptor 1 (NPR1) agonist. The agent is also referred to as a reversal or rescue agent herein.

[011] In another aspect, the present disclosure provides an agent that reverses a reduction in blood pressure associated with the administration of a NPR1 agonist in a subject.

[012] In one embodiment, the agent is selected from the group consisting of an immunoglobulin protein, a vasopressor, an alpha-adrenoreceptor agonist, a steroid, an antidiuretic hormone, an angiogenesis inhibitor, and a small molecule agent that increases blood pressure.

[013] In one embodiment, the agent is an immunoglobulin protein. In one embodiment, the agent specifically binds to the NPR1 agonist. In one embodiment, the NPR1 agonist is an antibody or antigen-binding fragment thereof that binds

specifically to NPR1. In one embodiment, the anti-NPR1 antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) comprising SEQ ID NO:48; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising SEQ ID NO:52. In one embodiment, the anti-NPR1 antibody comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) comprising SEQ ID NOs:49, 50, and 51, respectively; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) comprising SEQ ID NO:53, 54, and 55, respectively. In one embodiment, the anti-NPR1 antibody or antigen-binding fragment thereof comprises a HCVR of SEQ ID NO: 48 and a LCVR of SEQ ID NO: 52. In one embodiment, the anti-NPR1 antibody is a monoclonal antibody. In one embodiment, the anti-NPR1 antibody is an IgG1 or IgG4 antibody. In one embodiment, the anti-NPR1 antibody or antigen-binding fragment thereof comprises a heavy chain comprising SEQ ID NO:56 and a light chain comprising SEQ ID NO:57. In one embodiment, the anti-NPR1 antibody is R5381.

[014] In one embodiment, the rescue agent is an immunoglobulin protein. In one embodiment, the immunoglobulin protein comprises a monoclonal antibody or antigen-binding fragment thereof. In another embodiment, the immunoglobulin protein comprises a bivalent antibody. In another embodiment, the immunoglobulin protein comprises a monovalent or 'one-armed' antibody. In another embodiment, the immunoglobulin protein comprises a recombinant monoclonal antibody. In another embodiment, the immunoglobulin protein comprises a fully human monoclonal antibody that is bivalent or monovalent. In another embodiment, the immunoglobulin protein is a fully human monoclonal antibody that is of IgG1 or IgG4 isotype. In one embodiment, the immunoglobulin protein comprises a Fab fragment. In some embodiments, the immunoglobulin protein comprises a monoclonal antibody or antigen-binding fragment thereof, a bivalent monoclonal antibody, a monovalent monoclonal antibody, a Fab fragment, F(ab)2 fragment, Fv fragment, Fd fragment, scFv, or dAb. In one embodiment, the immunoglobulin protein comprises at least one immunoglobulin variable domain comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR). In one embodiment, the immunoglobulin protein comprises one immunoglobulin variable domain comprising three heavy chain CDRs contained in a HCVR and three light chain CDRs contained in a LCVR. In one embodiment, the immunoglobulin protein further

comprises a multimerizing component, wherein the multimerizing component comprises at least one Fc fragment. In one embodiment, the multimerizing component comprises a first Fc fragment and a second Fc fragment wherein the first Fc fragment or the second Fc fragment, but not both, comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification. In one embodiment, the modification comprises a H315R substitution and a Y316F substitution (EU numbering).

[015] In one embodiment, the immunoglobulin protein comprises a monovalent antibody, wherein the monovalent antibody comprises a heavy chain comprising a heavy chain constant region and a HCVR, and a light chain comprising a light chain constant region and a LCVR wherein the heavy chain is of human IgG1 or IgG4 isotype. In one embodiment, the heavy chain constant region comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification. In one embodiment, the modification comprises a H315R substitution and a Y316F substitution (EU numbering). In one embodiment, the immunoglobulin protein further comprises a multimerizing component, wherein the multimerizing component comprises a Fc fragment. In one embodiment, the Fc fragment is of human IgG1 or IgG4 isotype.

[016] In one embodiment, the immunoglobulin protein comprises a Fab fragment comprising one immunoglobulin variable domain comprising three heavy chain CDRs contained in a HCVR and three light chain CDRs contained in a LCVR. In one embodiment, the immunoglobulin protein further comprises a multimerizing component. In one embodiment, the multimerizing component comprises at least one Fc fragment. In one embodiment, the Fc fragment is of isotype IgG1, IgG4 or a variant thereof. In one embodiment, the multimerizing component comprises a first Fc fragment and a second Fc fragment wherein the first Fc fragment or the second Fc fragment, but not both, comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification. In one embodiment, the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in a Fc fragment of IgG1 or IgG4 isotype.

[017] Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDRs) (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDRs) (LCDR1, LCDR2 and LCDR3) of exemplary immunoglobulin proteins. Table 2 sets forth the nucleic acid

sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary immunoglobulin proteins.

[018] Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani, *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin, *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[019] In certain embodiments, the immunoglobulin proteins of the disclosure are antibodies, or antigen-binding fragments thereof, comprising a HCVR and a LCVR, said HCVR comprising an amino acid sequence listed in Table 1 having no more than twelve amino acid substitutions, and/or said LCVR comprising an amino acid sequence listed in Table 1 having no more than ten amino acid substitutions. For example, the present disclosure provides antibodies or antigen-binding fragments thereof comprising a HCVR and a LCVR, said HCVR comprising an amino acid sequence listed in Table 1, said amino acid sequence having one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve amino acid substitutions. In another example, the present disclosure provides antibodies or antigen-binding fragments thereof comprising a HCVR and a LCVR, said LCVR comprising an amino acid sequence listed in Table 1, said amino acid sequence having one, two, three, four, five, six, seven, eight, nine or ten amino acid substitutions. In one embodiment, the present disclosure provides immunoglobulin proteins or antigen-binding fragments thereof comprising a HCVR and a LCVR, said HCVR comprising an amino acid sequence listed in Table 1, said amino acid sequence having at least one amino acid substitution, and/or said LCVR comprising an amino acid sequence listed in Table 1, said amino acid sequence having at least one amino acid substitution.

[020] In certain embodiments, the immunoglobulin proteins of the disclosure are antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[021] In certain embodiments, the immunoglobulin proteins of the disclosure are antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in

Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[022] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[023] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[024] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[025] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[026] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[027] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[028] In certain embodiments, the immunoglobulin proteins of the disclosure are antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the present disclosure

provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary immunoglobulin proteins listed in Table 1. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 8/16 and 28/36.

[029] In certain embodiments, the immunoglobulin proteins of the disclosure are antibodies, or antigen-binding fragments thereof, encoded by nucleic acid molecules disclosed herein. For example, the present disclosure provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[030] The present disclosure also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[031] The present disclosure also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[032] The present disclosure also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[033] The present disclosure also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[034] The present disclosure also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[035] The present disclosure also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[036] The present disclosure also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[037] In certain embodiments, the immunoglobulin proteins of the disclosure are antibodies, or antigen-binding fragments thereof having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield, *et al.*, (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[038] In one aspect, the present disclosure provides an immunoglobulin protein comprising: (i) one immunoglobulin variable domain comprising three heavy chain CDRs (HCDR1, HCDR2, and HCDR3) contained within a HCVR, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within a LCVR. In one embodiment, the HCVR of the immunoglobulin protein comprises an amino acid sequence selected from any of the HCVR sequences in Table 1. In one embodiment, the LCVR of the immunoglobulin protein comprises an amino acid sequence selected from any of the LCVR sequences in Table 1. In one embodiment, the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 22; and the LCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 30. In one embodiment, the antibody or antigen-binding fragment thereof

comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) and three light chain CDRs (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 24; HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 and 26; HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 28; LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12 and 32; LCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14 and 34; and LCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 36. In one embodiment, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprise amino acid sequences selected from (i) SEQ ID NOs: 4, 6, 8, 12, 14 and 16; or (ii) SEQ ID NOs: 24, 26, 28, 32, 34, and 36. In one embodiment, the immunoglobulin protein further comprises a multimerizing component, wherein the multimerizing component comprises at least one Fc fragment. In one embodiment, the Fc fragment is of IgG1 or IgG4 isotype. In one embodiment, the multimerizing component comprises a first Fc fragment and a second Fc fragment, wherein the first Fc fragment or the second Fc fragment, but not both, comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification. In one embodiment, the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in a Fc fragment of IgG1 or IgG4 isotype. In one embodiment, the multimerizing component comprises a Fc fragment comprising the amino acid sequence of SEQ ID NO: 46 and a Fc fragment comprising the amino acid sequence of SEQ ID NO: 58.

SEQ ID NO:58:

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS
SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNRFTQKSLSL
SPGK

[039] In one embodiment, the immunoglobulin protein comprises a bivalent antibody or antigen-binding fragment thereof. In one embodiment, the immunoglobulin protein comprises a monovalent ('one-armed') antibody or antigen-binding fragment thereof. In one embodiment, the immunoglobulin protein comprises a heavy chain comprising the HCVR and a light chain comprising the LCVR, wherein the heavy chain is of human IgG1 or IgG4 isotype. In one embodiment, the heavy chain comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to

Protein A as compared to an immunoglobulin protein lacking the modification. In one embodiment, the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in a heavy chain of IgG1 or IgG4 isotype. In one embodiment, the heavy chain has an amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 38; and the light chain has an amino acid sequence selected from the group consisting of SEQ ID NOs: 20 and 40. In one embodiment, the immunoglobulin protein further comprises a Fc fragment. In one embodiment, the Fc fragment is of IgG1 or IgG4 isotype. In one embodiment, the Fc fragment comprises an amino acid sequence comprising SEQ ID NO: 46.

[040] In one embodiment, the immunoglobulin protein binds specifically to an anti-NPR1 antibody. In one embodiment, the anti-NPR1 antibody is R5381.

[041] In one embodiment, the immunoglobulin protein is REGN9035. In one embodiment, the immunoglobulin protein is REGN9037.

[042] In one aspect, the disclosure provides an isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a heavy chain variable region (HCVR) of an immunoglobulin protein disclosed herein. In another aspect, the disclosure provides an isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a light chain variable region (LCVR) of an immunoglobulin protein disclosed herein. In one aspect, the disclosure provides a vector comprising a polynucleotide molecule disclosed herein. In certain embodiments, the vector is a recombinant expression vector capable of expressing a polypeptide comprising a heavy and/or light chain variable region of an immunoglobulin protein. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules disclosed herein, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 2. In another aspect, the disclosure provides a host cell expressing a vector disclosed herein. For example, the present disclosure provides a host cell comprising a first recombinant expression vector capable of expressing a polypeptide comprising a heavy chain variable region of an immunoglobulin protein; and a second expression vector capable of expressing a polypeptide comprising a light chain variable region of an immunoglobulin protein, as disclosed herein. In one embodiment, the present disclosure provides a host cell comprising a first isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a heavy chain variable region (HCVR) of an immunoglobulin protein disclosed herein and a second isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a light chain variable region (LCVR) of an immunoglobulin protein disclosed herein. In certain embodiments, the host cell

comprises a mammalian cell or a prokaryotic cell. In certain embodiments, the host cell is a Chinese Hamster Ovary (CHO) cell or an *Escherichia coli* (*E. coli*) cell.

[043] In one aspect, the disclosure provides a method of producing an immunoglobulin protein or fragment thereof that specifically binds to an anti-NPR1 antibody or antigen-binding fragment thereof, comprising growing a host cell disclosed herein under conditions permitting production of the antibody or fragment, and recovering the immunoglobulin protein or fragment so produced. In certain embodiments, the present disclosure provides methods of producing an immunoglobulin protein or fragment thereof of the disclosure, comprising introducing into a host cell an expression vector comprising a nucleic acid sequence encoding a HCVR and/or LCVR of an immunoglobulin protein or fragment thereof of the disclosure operably linked to a promoter; culturing the host cell under conditions favorable for expression of the nucleic acid sequence; and isolating the immunoglobulin protein or fragment thereof from the culture medium and/or host cell. The isolated immunoglobulin protein or fragment thereof may be purified using any of the methods known in prior art. In one embodiment, the immunoglobulin proteins of the present disclosure may be purified using reagents and methods employing differential binding to Protein A, as disclosed elsewhere herein.

[044] In one embodiment, the rescue agent is a vasopressor. In another embodiment, the vasopressor is Midodrine.

[045] In one aspect, the disclosure provides a pharmaceutical composition comprising a rescue agent disclosed herein and a pharmaceutically acceptable carrier or diluent. In one embodiment, the composition comprises a combination of a rescue agent and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with the rescue agent. Additional combination therapies and co-formulations involving the rescue agents of the present disclosure are disclosed elsewhere herein.

[046] In one aspect, the disclosure provides a method of reversing the hemodynamic effects of an agonist antibody or antigen-binding fragment that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of a rescue agent disclosed herein to a subject in need thereof.

[047] In another aspect, the disclosure provides a method of reversing a reduction in blood pressure associated with the administration of an agonist antibody or antigen-binding fragment that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, the method comprising administering a pharmaceutical composition

comprising a therapeutically effective amount of a rescue agent disclosed herein to a subject in need thereof.

[048] In certain embodiments, the pharmaceutical composition is administered subcutaneously, intravenously, intradermally, intraperitoneally, intramuscularly, or orally to the subject.

[049] In another aspect, the disclosure provides the use of a rescue agent disclosed herein in the manufacture of a medicament for reversing the hemodynamic effects associated with the administration of an anti-NPR1 antibody in a subject need thereof.

[050] In one embodiment, the subject has a NPR1-associated disease or disorder. In one embodiment, the disease or disorder is hypertension, heart failure and/or chronic kidney disease.

[051] In one aspect, the present disclosure provides a composition comprising: (i) an immunoglobulin protein as disclosed herein; and (ii) and an NPR1 agonist. In one embodiment, the NPR1 agonist is an anti-NPR1 antibody (*e.g.,* R5381). In one embodiment, the composition is used in a method for effective regulation of blood pressure in a subject in need thereof. In one embodiment, the subject has a NPR1-associated disease or disorder. In one embodiment, the disease or disorder is hypertension, heart failure and/or chronic kidney failure.

[052] In one aspect, the disclosure provides an antibody or antigen-binding fragment thereof that competes for binding with an immunoglobulin protein disclosed herein.

[053] In another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that binds to the same epitope as an immunoglobulin protein disclosed herein.

[054] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[055] Figure 1 provides, in bar graph form, a comparison of the dissociative half-life (t½) of anti-R5381 antibodies REGN9035 and REGN9037 in pH 7.4, pH 6.5, pH 6.0, and pH 5.0 buffers.

[056] Figure 2 provides, in line graph form, the pharmacokinetic profiles of anti-R5381 antibodies REGN9035, REGN9037, REGN6580, and REGN6581 in $NPR1^{hu/hu}$ mice.

[057] Figure 3 shows, in line graph form, the effects of bivalent anti-R5381 mAbs on reversing R5381-induced systolic blood pressure-lowering in normotensive $NPR1^{hu/hu}$ mice. Telemetered normotensive $NPR1^{hu/hu}$ mice were randomized into groups based off of systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of R5381 or PBS as described in Table 18. Animals were then

given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent mAb or PBS as described in Table 18. All values are mean pressures over 24 hours for days -2-20 ± SEM, n=4-5 per group. Statistics –two-way ANOVA with Dunnett's; *p<0.05 vs. isotype control mAb.

[058] Figure 4 shows, in line graph form, the effects of bivalent anti-R5381 mAbs on reversing R5381-induced diastolic blood pressure-lowering in normotensive NPR1$^{hu/hu}$ mice. Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based off of systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of R5381 or PBS as described in Table 18. Animals were then given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent mAb or PBS as described in Table 18. All values are mean pressures over 24 hours for days -2-20 ± SEM, n=4-5 per group. Statistics –two-way ANOVA with Dunnett's; *p<0.05 vs. isotype control mAb.

[059] Figure 5 shows, in line graph form, the effects of bivalent anti-R5381 mAbs on reversing R5381 mAb-induced heart rate effects in normotensive NPR1$^{hu/hu}$ mice. Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based off of systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of R5381 or PBS as described in Table 18. Animals were then given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent mAb or PBS as described in Table 18. All values are mean heart rates over 24 hours for days -2-20 ± SEM, n=4-5 per group. Statistics –two-way ANOVA with Dunnett's.

[060] Figure 6 shows, in line graph form, the effects of bivalent anti-R5381 mAbs on reversing R5381 mAb-induced mean arterial blood pressure-lowering in normotensive NPR1$^{hu/hu}$ mice. Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based off of systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of R5381 or PBS as described in Table 18. Animals were then given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent mAb or PBS as described in Table 18. All values are mean pressures over 24 hours for days -2-20 ± SEM, n=4-5 per group. Statistics –two-way ANOVA with Dunnett's; *p<0.05 vs. isotype control mAb.

[061] Figure 7 shows, in line graph form, the effects of bivalent anti-R5381 mAbs on reversing R5381-induced systolic blood pressure-lowering in normotensive NPR1$^{hu/hu}$ mice. Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based off of systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of R5381 or PBS as described in Table 23. Animals were then given a single 50 mg/kg subcutaneous injection of an anti-R5381 bivalent mAb or isotype control mAb as described in Table 23. All values are mean pressures over 24

hours for days -3-21 ± SEM, n=4-5 per group. Statistics –two way ANOVA with Dunnett's; *p<0.05 PBS vs. isotype control mAb; **p<0.01 PBS vs. isotype control mAb; !p<.05 REGN6580 vs. isotype control mAb; #p<0.05 REGN6581 vs. isotype control.

[062] Figure 8 shows, in line graph form, the effects of monovalent anti-R5381 mAbs on reversing R5381-induced systolic blood pressure-lowering in normotensive $NPR1^{hu/hu}$ mice. Telemetered normotensive $NPR1^{hu/hu}$ mice were randomized into groups based off of body weight. Animals were given a single 5 mg/kg subcutaneous injection of an NPR1 agonist mAb or isotype control mAb as described in Table 28. Animals were then given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent (REGN6580) or monovalent (REGN9035 or REGN9037) mAb or PBS as described in Table 28. All values are mean ± SEM, n=4-5 per group. Statistics –two way ANOVA with Dunnett's; *p<0.05 REGN6580 s.c. vs. isotype control mAb; $^{\#}$p<0.05 REGN9035 s.c. vs. isotype control mAb; $^{!}$p<0.05 REGN9037 s.c. vs. isotype control mAb; $^{@}$p<0.05 REGN6580 i.v. vs. isotype control mAb; $^{\&}$p<0.05 REGN9035 i.v. vs. isotype control mAb; $^{>}$p<0.05 REGN9037 i.v. vs. isotype control mAb.

[063] Figure 9 shows, in line graph form, the acute effects of monovalent and bivalent anti-R5381 mAbs on reversing R5381-induced blood pressure-lowering in normotensive $NPR1^{hu/hu}$ mice. Telemetered normotensive $NPR1^{hu/hu}$ mice were randomized into groups based off of body weight. Animals were given a single 5 mg/kg subcutaneous injection of R5381 or PBS control as described in Table 28. Animals were then given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent (REGN6580) or monovalent (REGN9035 or REGN9037) mAb or isotype control mAb as described in Table 28. All values are mean ± SEM, n=4-5 per group.

[064] Figure 10 shows, in line graph form, the effects of monovalent anti-R5381 mAbs on reversing R5381-induced cGMP generation in normotensive $NPR1^{hu/hu}$ mice. Telemetered normotensive $NPR1^{hu/hu}$ mice were randomized into groups based off of body weight. Animals were given a single 5 mg/kg subcutaneous injection of an NPR1 agonist mAb or isotype control mAb as described in Table 28. Animals were then given a single 50 mg/kg intravenous injection of an anti-R5381 bivalent (REGN6580) or monovalent (REGN9035 or REGN9037) mAb or PBS as described in Table 28. Urine was collected overnight from study day 21 to 22. All values are mean ± SEM, n=5-6 per group. Statistics –ANOVA with Dunnett's; ****p<0.0001 vs. PBS + PBS; #### p<0.0001 vs. R5381 + isotype control mAb.

[065] Figure 11 shows that three doses of 2.5 mg/kg Midodrine administered three days after a single dose of 25 mg/kg R5381 reverse the blood pressure-lowering effects of R5381. Male cynomolgus monkeys weighing 3 to 5 kg were surgically

implanted with a radio telemetry transmitter. On Day 0, animals each received a single IV bolus of saline (PBS; n=10) or 25 mg/kg R5381 (n=13). On Day 3, animals each received 3 doses of 2.5 mg/kg/dose midodrine (n=6 for saline group; n=7 for R5381 group) or water/vehicle (n=4 for saline group; n=6 for R5381 group) administered by oral gavage, with each dose spaced 3 to 4 hours apart, as indicated by dotted lines on the x-axis. Blood pressure measurements were collected for each animal pre-dose (for baseline measurements) and during a 4-day post-dose monitoring period. Mean changes from baseline systolic blood pressure for each treatment group between 35 and 72 hours post-R5381-dose are shown. Data are expressed as the group mean ± standard error of the mean.

[066] Figure 12 shows that three doses of 2.5 mg/kg Midodrine administered 3 days after a single dose of 25 mg/kg reverse the R5381-induced heart rate effects. Male cynomolgus monkeys weighing 3 to 5 kg old were surgically implanted with a radio telemetry transmitter. On Day 0, animals each received a single IV bolus of saline (PBS; n=10) or 25 mg/kg R5381 (n=13). On Day 3, animals each received 3 doses of 2.5 mg/kg/dose midodrine (n=6 for saline group; n=7 for R5381 group) or water/vehicle (n=4 for saline group; n=6 for R5381 group) administered by oral gavage, with each dose spaced 3 to 4 hours apart, as indicated by dotted lines on the x-axis. Heart rate measurements were collected for each animal pre-dose (for baseline measurements) and during a 4-day post-dose monitoring period. Mean changes from baseline heart rate for each treatment group between 35 and 72 hours post-R5381-dose are shown. Data are expressed as the group mean ± standard error of the mean.

DETAILED DESCRIPTION

[067] Before the present methods are described, it is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

[068] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[069] The term “NPR1”, also called “NPRA,” refers to natriuretic peptide receptor 1 (also known as natriuretic peptide receptor A). NPR1 is a homodimeric transmembrane guanylate cyclase, an enzyme that catalyzes cGMP synthesis. The protein has 4 distinct regions comprising an extracellular ligand-binding domain, a single transmembrane-spanning region, an intracellular protein kinase-like homology domain, and a guanylyl cyclase catalytic domain. The amino acid sequence of full-length NPR1 protein is exemplified by the amino acid sequence provided in UniProtKB/Swiss-Prot as accession number P16066.1 (SEQ ID NO:59):

```
        1 mpgprrpags rlrlllllll ppllllllrgs hagnltvavv lplantsypw
swarvgpave
       61 lalaqvkarp dllpgwtvrt vlgssenalg vcsdtaapla avdlkwehnp
avflgpgcvy
      121 aaapvgrfta hwrvplltag apalgfgvkd eyalttragp syaklgdfva
alhrrlgwer
      181 qalmlyayrp gdeehcfflv eglfmrvrdr lnitvdhlef aeddlshytr
llrtmprkgr
      241 viyicsspda frtlmllale aglcgedyvf fhldifgqsl qgggqgpaprr
pwergdgqdv
      301 sarqafqaak iitykdpdnp eyleflkqlk hlayeqfnft medglvntip
asfhdgllly
      361 iqavtetlah ggtvtdgeni tqrmwnrsfq gvtgylkids sgdretdfsl
wdmdpengaf
      421 rvvlnyngts qelvavsgrk lnwplgyppp dipkcgfdne dpacnqdhls
tlevlalvgs
      481 lsllgilivs ffiyrkmqle kelaselwrv rwedvepssl erhlrsagsr
ltlsgrgsny
      541 gsllttegqf qvfaktayyk gnlvavkrvn rkrieltrkv lfelkhmrdv
qnehltrfvg
      601 actdppnici lteycprgsl qdilenesit ldwmfryslt ndivkgmlfl
hngaicshgn
      661 lkssncvvdg rfvlkitdyg lesfrdldpe qghtvyakkl wtapellrma
sppvrgsqag
      721 dvysfgiilq eialrsgvfh vegldlspke iiervtrgeq ppfrpslalq
shleelgllm
      781 qrcwaedpqe rppfqqirlt lrkfnrenss nildnllsrm eqyannleel
veertqayle
      841 ekrkaeally qilphsvaeq lkrgetvqae afdsvtiyfs divgftalsa
estpmqvvtl
      901 lndlytcfda vidnfdvykv etigdaymvv sglpvrngrl hacevarmal
alldavrsfr
      961 irhrpqeqlr lrigihtgpv cagvvglkmp ryclfgdtvn tasrmesnge
alkihlsset
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1021 kavleefggf elelrgdvem kgkgkvrtyw llgergsstr g

[070] The term "NPR1" includes recombinant NPR1 protein or a fragment thereof. The term also encompasses NPR1 protein or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence.

[071] The term "NPR1 agonist," as used herein, refers to a molecule that activates, increases or potentiates NPR1 activity or that stabilizes the activated conformation of NPR1. In preferred embodiments, the term "NPR1 agonist" refers to an antibody or antigen-binding fragment thereof that binds specifically to NPR1 and activates or increases at least one biological activity of NPR1. Such an agonist anti-NPR1 antibody may bind to NPR1 either in the presence or absence of a ligand (*e.g.,* ANP or BNP). In certain embodiments, the biological activity includes, but is not limited to, decrease or reduction of blood pressure in a subject upon administration of the agonist anti-NPR1 antibody. In certain embodiments, the biological activity includes hemodynamic changes (*e.g.,* reduction of blood pressure) in a subject having a disease or disorder such as hypertension, heart failure, or chronic kidney disease. The term includes agonist anti-NPR1 antibodies disclosed in, for example, US Publication No: 20200123263. In a specific embodiment, the term refers to an anti-NPR1 antibody comprising a HCVR of SEQ ID NO: 48 and a LCVR of SEQ ID NO: 52. In another specific embodiment, the term refers to R5381 (also known as REGN5381). R5381 is a fully human anti-NPR1 monoclonal antibody comprising a heavy chain that comprises the amino acid sequence of SEQ ID NO: 56 and a light chain that comprises the amino acid sequence of SEQ ID NO: 57.

[072] As used herein, a "reversal agent" or "rescue agent" is an agent that reverses the hemodynamic effects of an NPR1 agonist. The terms "reversal agent" and "rescue agent" are used interchangeably herein. In certain embodiments, the reversal agent, as referred to herein, reverses hemodynamic changes associated with the administration (to a subject) of an agonist antibody or antigen-binding fragment that binds specifically to NPR1. The term "reverses" includes increasing the blood pressure of the subject, whose blood pressure has been reduced as a result of the administration of an NPR1 agonist. The increase in blood pressure can be measured using any standard blood pressure assessment means (*e.g.,* sphygmomanometer) known in the art. The increase may be to its pre-agonist antibody treatment level, or to a level that results in adequate hemodynamic stability. Hemodynamic effects may include indirect effects, effects associated with a drop in blood pressure. These effects can likewise be reversed using the agents disclosed herein. Hemodynamic effects, as referred to herein, may include physiological parameters such as blood pressure and

heart rate, or clinical signs such as dizziness, lightheadedness, blurred vision, nausea, fatigue. In certain embodiments, the reversal agent binds specifically to the NPR1 agonist and reverses hemodynamic effects caused by the NPR1 agonist. In specific embodiments, the reversal agent comprises an immunoglobulin protein as disclosed herein.

[073] The term "immunoglobulin protein," as used herein refers to antigen-binding molecules that comprise at least one immunoglobulin variable domain. The at least one variable domain is the antigen-binding domain and comprises a heavy chain variable region and a light chain variable region. In certain embodiments, the variable domain is comprised in a Fab, Fv, single chain Fv, or any other antigen-binding fragment of an antibody, as disclosed elsewhere herein. In some embodiments, the variable domain is comprised in a monovalent or bivalent antibody. The term includes, but is not limited to, antibodies and antigen-binding fragments thereof, monovalent antibodies, bivalent antibodies and antigen-binding fragments thereof. The immunoglobulin protein may also comprise a multimerizing component linked to the variable domain. As disclosed elsewhere herein, the multimerizing component may comprise a Fc fragment of an antibody or a truncated heavy chain of an antibody. For example, an immunoglobulin protein of the present disclosure may comprise a single variable domain within a Fab wherein the Fab is linked to at least one Fc fragment. In certain embodiments, the immunoglobulin protein comprises: (i) a heavy chain comprising a heavy chain constant region and a heavy chain variable region, (ii) a light chain comprising a light chain constant region and a light chain variable region, and (iii) a polypeptide comprising a Fc fragment or a truncated heavy chain. In certain embodiments, the Fc domain polypeptide is a "dummy Fc," which refers to an Fc domain polypeptide that is not linked to an antigen binding domain. The immunoglobulin proteins comprising a single variable domain may be referred to as "one-armed" or "single arm" or "monovalent" antibodies. In the context of the disclosure, the term refers to an antibody or antigen-binding fragment thereof that binds to the variable region of another antibody ("anti-idiotype antibody"). In specific embodiments, the term refers to an antibody (or antigen-binding fragment thereof) that comprises a single variable domain that binds specifically to an anti-NPR1 antibody or antigen-binding fragment thereof. In one embodiment, the antibody or antigen-binding fragment thereof that specifically binds an anti-NPR1 antibody is a competitive binder. The one-armed antibodies of the present disclosure may comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein. Exemplary one-armed anti-R5381 antibodies disclosed herein include REGN9035 and REGN9037.

[074] The term "antibody", as used herein, unless specified otherwise, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.*, IgM) or antigen-binding fragments thereof. In the context of the present disclosure, the term “bivalent antibodies” refers to “full antibody molecules,” *i.e.*, comprising 2 heavy chains and 2 light chain. Each heavy chain is comprised of a heavy chain variable region (“HCVR” or “V_H”) and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region (“LCVR or “V_L”) and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, 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. In certain embodiments of the disclosure, the FRs of the antibody (or antigen-binding fragment thereof) may be identical to the human germline sequences or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[075] In certain embodiments, the term “antibody” or “antigen-binding molecule” includes monovalent antigen binding molecules. A monovalent antigen binding molecule comprises a single antigen binding domain that is formed by a single heavy chain and a single light chain. The monovalent antigen binding molecule further includes a polypeptide comprising at least an Fc domain of a heavy chain. In certain embodiments, the Fc domain polypeptide is a “dummy Fc,” which refers to an Fc domain polypeptide that is not linked to an antigen binding domain. In certain embodiments, the monovalent antibody has a complete heavy chain, a complete light chain, and a truncated heavy chain.

[076] “Fc fragment” or “Fc region,” as used herein, refers to the fragment crystallizable region of an immunoglobulin, which is the tail region of an antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system. This property allows antibodies to activate the immune system. In IgG, IgA, and IgD antibody isotypes, the Fc region or Fc domain is derived from the second and third constant domains (CH2 and CH3)of the antibody's heavy chain; IgM and IgE Fc regions contain three heavy chain constant domains (C_H domains 2–4) in the polypeptide chain. In the context of the present disclosure, the term refers to Fc region derived from human Fc domain, unless specified otherwise. In certain embodiments, the Fc fragment is derived from human IgG1, IgG2, IgG3, or IgG4 isotypes.

[077] As used herein, a "multimerizing component" refers to any macromolecule that has the ability to associate with a second macromolecule of the same or similar structure or constitution. For example, a multimerizing component may be a polypeptide comprising an immunoglobulin C_H3 domain. A non-limiting example of a multimerizing component is an Fc portion of an antibody (comprising a C_H2-C_H3 domain), *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group. In certain embodiments, the immunoglobulin proteins of the present disclosure comprise a multimerizing component that comprises at least one Fc fragment. In certain embodiments, the immunoglobulin proteins comprise two Fc fragments. The first and second Fc fragments may be of the same IgG isotype such as, *e.g.*, IgG1/IgG1, IgG2/IgG2, IgG4/IgG4. Alternatively, the first and second Fc fragments may be of different IgG isotypes such as, *e.g.*, IgG1/IgG2, IgG1/IgG4, IgG2/IgG4, etc. In certain embodiments, the 2 Fc fragments have identical sequence. In certain embodiments, the 2 Fc fragments differ from each other by one or more amino acids. In certain embodiments, the first Fc fragment or the second Fc fragment, but not both Fc fragments, comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification. In certain embodiments, the multimerizing component is an Fc fragment or an amino acid sequence of 1 to about 200 amino acids in length containing at least one cysteine residues. In other embodiments, the multimerizing component is a cysteine residue, or a short cysteine-containing peptide. Other multimerizing components include peptides or polypeptides comprising or consisting of a leucine zipper, a helix-loop motif, or a coiled-coil motif. The multimerizing component comprising, *e.g.*, Fc domains, may comprise one or more amino acid changes (*e.g.*, insertions, deletions or substitutions) as compared to the wild-type, naturally occurring version of the Fc domain, as disclosed elsewhere herein.

[078] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan, *et al.,* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos, *et al.,* 2002 *J Mol Biol* 320:415-428).

[079] CDR residues not contacting antigen can be identified based on previous studies (for example, residues H60-H65 in CDRH2 are often not required), from regions of

Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[080] The fully human immunoglobulin proteins (that specifically bind to anti-NPR1 antibodies) disclosed herein may comprise one or more amino acid substitutions, insertions, and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present disclosure includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations").

[081] A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments that comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present disclosure may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated

to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic biological properties, reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present disclosure.

[082] The present disclosure also includes fully human immunoglobulin proteins (that specifically bind to anti-NPR1 antibodies) comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present disclosure includes fully human immunoglobulin proteins (that specifically bind to anti-NPR1 antibodies) having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[083] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an anti-NPR1 antibody. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. In certain embodiments, the term "antigen-binding fragment" refers to a polypeptide fragment of a multi-specific antigen-binding molecule. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[084] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an

isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.*, monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[085] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H - V_H, V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[086] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present disclosure include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present disclosure may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[087] The term "human antibody", or “fully human antibody”, or “fully human immunoglobulin protein”, as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced

by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", or "fully human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse), have been grafted onto human FR sequences. The term includes antibodies that are recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[088] The term "recombinant", as used herein, refers to antibodies or antigen-binding fragments thereof of the disclosure created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, *e.g.*, DNA splicing and transgenic expression. The term refers to antibodies expressed in a non-human mammal (including transgenic non-human mammals, *e.g.*, transgenic mice), or a cell (*e.g.*, CHO cells) expression system or isolated from a recombinant combinatorial human antibody library.

[089] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with, for example, an anti-NPR1 antibody that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-8}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, that bind specifically to an anti-NPR1 antibody (*e.g.*, R5381). Moreover, multi-specific antibodies that bind to one domain in an anti-NPR1 antibody and one or more additional antigens, or a bi-specific that binds to two different regions of an anti-NPR1 antibody, are nonetheless considered antibodies that "specifically bind", as used herein.

[090] The term "high affinity" antibody refers to those mAbs having a binding affinity to an anti-NPR1 antibody, expressed as K_D, of at least 10^{-8} M; preferably 10^{-9} M; more preferably 10^{-10} M, even more preferably 10^{-11} M, as measured by surface plasmon resonance, *e.g.*, BIACORE™ or solution-affinity ELISA.

[091] By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from an anti-NPR1 antibody, with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[092] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring,

enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The “antigen” of the immunoglobulin proteins described herein is an anti-NPR1 antibody (for example, R5381) or antigen-binding fragment thereof (*i.e.*, fragment of the antibody that binds NPR1). The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to bind to an anti-NPR1 antibody.

[093] In specific embodiments, antibody or antibody fragments of the disclosure may be conjugated to a moiety such as a ligand or a therapeutic moiety (“immunoconjugate”), a second rescue agent, or any other therapeutic moiety useful for reversing the hemodynamic effects of an anti-NPR1 antibody.

[094] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds an anti-NPR1 antibody, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than anti-NPR1 antibodies).

[095] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[096] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction. In the context of the present disclosure, the “antigen” of the immunoglobulin proteins is an anti-NPR1 antibody (for example, R5381) or antigen-binding fragment thereof (*i.e.*, fragment of the antibody that binds NPR1).

[097] The term “epitope” refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. The “antigen” of the immunoglobulin proteins is an anti-NPR1 antibody (for example, R5381) or antigen-binding fragment thereof (*i.e.*, fragment of the antibody that binds NPR1). A single antigen may have more than one epitope. Thus, different immunoglobulin proteins (antibodies) may bind to different areas on an antigen and may have different biological effects. The term “epitope” also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be

conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[098] The term “cross-competes”, as used herein, means an antibody or antigen-binding fragment thereof binds to an antigen and inhibits or blocks the binding of another antibody or antigen-binding fragment thereof. The “antigen” of the immunoglobulin proteins is an anti-NPR1 antibody (for example, R5381) or antigen-binding fragment thereof (*i.e.*, fragment of the antibody that binds NPR1). The term also includes competition between two antibodies in both orientations, *i.e.*, a first antibody that binds and blocks binding of second antibody and vice-versa. In certain embodiments, the first antibody and second antibody may bind to the same epitope. Alternatively, the first and second antibodies may bind to different, but overlapping epitopes such that binding of one inhibits or blocks the binding of the second antibody, *e.g.*, *via* steric hindrance. Cross-competition between antibodies may be measured by methods known in the art, for example, by a real-time, label-free bio-layer interferometry assay. Cross-competition between two antibodies may be expressed as the binding of the second antibody that is less than the background signal due to self-self binding (wherein first and second antibodies is the same antibody). Cross-competition between 2 antibodies may be expressed, for example, as % binding of the second antibody that is less than the baseline self-self background binding (wherein first and second antibodies is the same antibody).

[099] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0100] As applied to polypeptides, the term "substantial similarity" or “substantially similar” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity.

Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) *Methods Mol. Biol.* 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet, *et al.,* (1992) *Science* 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0101] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the disclosure to a database containing a large number of sequences from different organisms is the

computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul, *et al.*, (1990) *J. Mol. Biol.* 215: 403-410 and (1997) *Nucleic Acids Res.* 25:3389-3402, each of which is herein incorporated by reference.

[0102] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding). For example, a therapeutically effective amount of a rescue agent according to the invention is, in one embodiment, the amount that results in some degree of reversal of the hemodynamic effects of a natriuretic peptide receptor 1 (NPR1) agonist.

[0103] As used herein, the term "subject" refers to an animal, preferably a mammal, more preferably a human. In specific embodiments of the disclosure, the subject has experienced, is experiencing, or may experience a drop in blood pressure or alteration in other hemodynamic parameters associated with the administration of an agonist antibody or antigen-binding fragment thereof that specifically binds NPR1.

[0104] As used herein, the terms "treat", "treating", or "treatment" refer to the reduction or amelioration of the severity of at least one symptom or indication of a disease or disorder due to the administration of a therapeutic agent such as an immunoglobulin protein described herein to a subject in need thereof. The terms include inhibition of progression of disease or of worsening of a symptom/indication. The terms also include positive prognosis of disease, *i.e.*, the subject may be free of disease or may have reduced disease upon administration of a therapeutic agent such as an antibody of the present disclosure. The therapeutic agent may be administered at a therapeutic dose to the subject.

[0105] The terms "prevent", "preventing" or "prevention" refer to inhibition of manifestation of a disease or disorder or any symptoms or indications of such a disease or disorder upon administration of a therapeutic agent.

Preparation of Human Immunoglobulin Proteins

[0106] Immunoglobulin variable (antigen-binding) domains specific for particular antigens can be prepared by any antibody-generating technology known in the art. Once obtained, they can be appropriately arranged to produce an immunoglobulin protein molecule of the present disclosure using routine methods. (A discussion of exemplary immunoglobulin protein components and formats that can be used to construct the immunoglobulin protein molecules of the present disclosure is provided elsewhere herein.) In certain embodiments, one or more of the individual components

(*e.g.*, heavy and light variable regions) of the proteins of the disclosure are derived from chimeric, humanized, or fully human antibodies. Methods for making such antibodies are well known in the art. For example, human antibodies can be generated in transgenic mice.

[0107] Any such known methods can be used in the context of the present disclosure to make human antibodies that specifically bind to an anti-NPR1 antibody. In one embodiment, the anti-NPR1 antibody is R5381.

[0108] An immunogen comprising any one of the following can be used to generate antibodies to an anti-NPR1 antibody. In certain embodiments, the immunoglobulin proteins (antibodies) of the disclosure are obtained from mice immunized with a full length anti-NPR1 antibody (*e.g.,* R5381) or with DNA encoding the protein or fragment thereof. Alternatively, the protein or a fragment thereof may be produced using standard biochemical techniques and modified and used as immunogen.

[0109] In some embodiments, the immunogen may be a recombinant anti-NPR1 antibody or antigen-binding fragment thereof expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells.

[0110] One or more of the heavy and/or light chains of the immunoglobulin proteins of the present disclosure can be prepared using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies. Using VELOCIMMUNE® technology, high affinity chimeric antibodies to an anti-NPR1 antibody (*e.g.,* R5381) are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0111] As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the disclosure, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen binding and target specificity characteristics reside in the variable region.

Bioequivalents

[0112] The immunoglobulin proteins of the present disclosure encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind an anti-NPR1 antibody, for example, R5381. Such variant proteins comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence but exhibit biological activity that is essentially equivalent to that of the described proteins (*e.g.*, antibodies). Likewise, the immunoglobulin proteins' (*e.g.*, antibody-encoding) DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an immunoglobulin protein (*e.g.*, antibody or antibody fragment) of the disclosure.

[0113] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives, if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0114] In one embodiment, two immunoglobulin proteins are bioequivalent, if there are no clinically meaningful differences in their safety, purity, or potency.

[0115] In one embodiment, two immunoglobulin proteins are bioequivalent, if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0116] In one embodiment, two immunoglobulin proteins are bioequivalent, if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0117] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the immunoglobulin protein or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b)

an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the immunoglobulin protein (*e.g.*, antibody (or its target)) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an immunoglobulin protein.

[0118] Bioequivalent variants of the immunoglobulin proteins (*e.g.*, antibodies) of the disclosure may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. The "biological activity" of the immunoglobulin proteins of the disclosure (for example, antibody or antibody fragment that specifically binds R5381) includes, but is not limited to, specifically binding the antigen (*e.g.*, the anti-NPR1 antibody R5381), reversing the hemodynamic effects of the anti-NPR1 antibody, and increasing the blood pressure, which has dropped as a result of the administration of an anti-NPR1 antibody. Blood flow, cardiac loading, and/or heart rate are also positively affected in certain embodiments. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent immunoglobulin proteins may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Immunoglobulin Proteins Comprising Fc Variants

[0119] According to certain embodiments of the present disclosure, immunoglobulin proteins are provided comprising an Fc domain comprising one or more mutations that enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present disclosure includes immunoglobulin proteins that bind anti-NPR1 antibodies and that comprise a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the immunoglobulin protein when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* H/L/R/S/P/Q or K) and/or 434 (*e.g.,* A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,*

308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[0120] For example, the present disclosure includes immunoglobulin proteins that comprise an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present disclosure.

[0121] The present disclosure also includes immunoglobulin proteins comprising a first C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the immunoglobulin protein to Protein A as compared to a protein lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A, and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H315R by EU numbering. The second C_H3 may further comprise a Y316F by EU numbering. In one embodiment, the first Ig C_H3 domain binds Protein A, and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). See, for example, US Patent No. 8,586,713. Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies

[0122] The present disclosure also includes immunoglobulin proteins that bind anti-NPR1 antibodies and that comprise a chimeric heavy chain constant (C_H) region,

wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the immunoglobulin proteins of the disclosure may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the disclosure comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (*See*, *e.g.*, U.S. Patent Application Publication 2014/0243504, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Immunoglobulin Proteins

[0123] In general, the immunoglobulin proteins of the present disclosure function by binding to a NPR1 agonist (such as an anti-NPR1 antibody) and reversing its hemodynamic effects. For example, the present disclosure includes antibodies and antigen-binding fragments of antibodies that bind (the parental hybridoma of) an anti-NPR1 antibody with a K_D of less than about 3 nM at 25°C and less than about 7 nm at 37°C, as measured by surface plasmon resonance, *e.g.,* using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind the parental hybridoma of an anti-NPR1 antibody with a K_D of less than about 25 nM, less than about 10 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 0.75 nM, or less than about 0.5 nM, as measured by surface plasmon resonance, *e.g.,* using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0124] The present disclosure also includes immunoglobulin proteins and fragments thereof that bind to a NPR1 agonist in a pH-sensitive manner. For example, the k_d and dissociative half-life (t½) of REGN9035 and REGN9037 vary in pH 7.4, pH 6.5, pH 6.0,

and pH 5.0 buffers, as shown in Example 5 herein. For REGN9035, the dissociative half-life (t½) decreases significantly with decreasing pH, from about 12 minutes to about 0.6 minutes; for REGN9037, the dissociative half-life (t½) varies slightly with decreasing pH, between about 13 and about 17 minutes. The k_d for each likewise varies, for REGN9035, between about 9.96E-04 and 1.97E-02, and for REGN9037, between about 8.67E-04 and about 6.64E-04.

[0125] The present disclosure also includes immunoglobulin proteins and fragments thereof that inhibit activation of hNPR1 that has been induced by an anti-NPR1 antibody. For example, bivalent and monovalent anti-R5381 antibodies and antigen-binding fragments thereof block R5381-induced activation of hNPR1 in the presence and absence of endogenous ligands (*e.g.*, ANP, BNP). Maximum inhibition (about 97% to about 106%) of R5381-induced activation of NPR1 was measured by cGMP accumulation, *e.g.*, using the assay format as defined in Example 6 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the disclosure inhibit at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 100%, at least about 101%, at least about 102%, at least about 103%, at least about 104%, or at least about 105%, at least about 106% anti-NPR1 antibody-induced activation of hNPR1.

[0126] The present disclosure also includes immunoglobulin proteins and fragments thereof that block anti-NPR1 antibody binding to hNPR1. For example, bivalent and monovalent anti-R5381 antibodies and antigen-binding fragments thereof block biotin-R5381 from binding to human NPR1, as assessed using blocking ELISA, as described in Example 7, or a substantially similar assay. About 99% and about 100% blocking were assessed for the monovalent /one-armed and bivalent anti-R5381 antibodies, respectively. In certain embodiments, the antibodies or antigen-binding fragments of the disclosure block about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% anti-NPR1 antibody binding to hNPR1.

[0127] The present disclosure also includes immunoglobulin proteins and fragments thereof that form minimal to none circulating immune complexes (CIC) with an agonist anti-NPR1 antibody. For example, bivalent and monovalent anti-R5381 antibodies and antigen-binding fragments thereof do not form detectable CICs with R5381, as assessed using Clq-CIC assay, as described in Example 8, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the

disclosure do not form CICs with anti-NPR1 antibodies. The presence of CICs in the circulation statistically correlates with disease activity.

[0128] The present disclosure also includes immunoglobulin proteins and fragments thereof that reverse the hemodynamic effects of an anti-NPR1 antibody. For example, bivalent anti-R5381 antibodies and antigen-binding fragments thereof were able to clear R5381 from mice faster and more effectively than isotype control, as assessed via immunoassay, as described in Example 10, or a substantially similar assay. Serum concentrations of R5381 were significantly lower at day 7 and undetectable at day 22. As another example, bivalent and monovalent anti-R5381 antibodies and antigen-binding fragments thereof rapidly and persistently reversed the blood pressure-lowering effects of R5381, as assessed collecting systolic pressure, diastolic pressure, pulse pressure, and mean arterial pressure, as well as heart rate, as described in Examples 11 and 12, or a substantially similar assay. The bivalent and monovalent anti-R5381 antibodies and antigen-binding fragments thereof also inhibited NPR1-induced cGMP production, as assessed in urine using ELISA, as described in Examples 11 and 12, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the disclosure increase blood pressure back to baseline levels (*i.e.*, back to levels before drop in blood pressure due to administration of an anti-NPR1 antibody).

[0129] In one embodiment, the present disclosure provides an isolated recombinant immunoglobulin protein or antigen-binding fragment thereof that binds specifically to an anti-NPR1 antibody (such as R5381), wherein the immunoglobulin protein exhibits one or more of the following characteristics: (a) comprises a fully human monoclonal antibody; (b) comprises a fully human monovalent or one-armed antibody; (c) comprises a single immunoglobulin domain and a multimerizing component comprising at least one Fc fragment; (d) binds to an anti-NPR1 antibody at 25ºC and at 37ºC with a dissociation constant (K_D) of less than 7nM, as measured in a surface plasmon resonance assay; (e) shows pH-dependent dissociation; (f) inhibits about 97% to about 106% of an anti-NPR1 antibody-induced activation of NPR1; (g) blocks at least about biotin-anti-NPR1-agonist antibody from binding to human NPR1; (h) does not form detectable CICs with an anti-NPR1 antibody; (i) clears an anti-NPR1 antibody from serum faster than isotype control; (j) reverses the blood pressure-lowering effects of an anti-NPR1 antibody; and (k) comprises a HCVR comprising an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1 and a LCVR comprising an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

[0130] The immunoglobulin proteins of the present disclosure may possess one or more of the afore-mentioned biological characteristics, or any combinations thereof. Other biological characteristics of the immunoglobulin proteins of the present disclosure will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Epitope Mapping and Related Technologies

[0131] The present disclosure includes immunoglobulin proteins that interact with one or more amino acids found within one or more regions of the anti-NPR1 antibody molecule. The epitope to which the immunoglobulin proteins bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within any of the aforementioned domains of the anti-NPR1 antibody molecule (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within either or both of the afore-mentioned domains of the antibody molecule (*e.g.*, a conformational epitope).

[0132] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke, (2004) *Methods Mol. Biol*. 248: 443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, (2000) *Prot. Sci*. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled

residues that correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring, (1999) *Analytical Biochemistry* 267: 252-259; Engen and Smith, (2001) *Anal. Chem*. 73: 256A-265A.

[0133] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. In the context of the present disclosure, the antigen is an anti-NPR1 antibody. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0134] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the disclosure into groups of antibodies binding different epitopes.

[0135] In certain embodiments, the present disclosure includes immunoglobulin proteins and fragments thereof that interact with one or more epitopes found within the extracellular domain of an anti-NPR1 antibody. The epitope(s) may consist of one or more contiguous sequences of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within the extracellular domain of an anti-NPR1 antibody. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within an anti-NPR1 antibody.

[0136] The present disclosure includes antibodies that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary immunoglobulin proteins listed in Table 1. Likewise, the present disclosure also includes antibodies that compete for binding to an anti-NPR1 antibody or a fragment thereof with any of the specific exemplary immunoglobulin proteins listed in Table 1. For example, the present

disclosure includes antibodies that cross-compete for binding to an anti-NPR1 antibody or a fragment thereof with one or more antibodies listed in Table 1.

[0137] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference immunoglobulin protein by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference immunoglobulin protein of the disclosure, the reference antibody is allowed to bind to an anti-NPR1 antibody protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the anti-NPR1 antibody protein molecule is assessed. If the test antibody is able to bind to an anti-NPR1 antibody following saturation binding with the reference immunoglobulin protein, it can be concluded that the test antibody binds to a different epitope than the reference immunoglobulin protein. On the other hand, if the test antibody is not able to bind to an anti-NPR1 antibody protein following saturation binding with the reference immunoglobulin protein, then the test antibody may bind to the same epitope as the epitope bound by the reference immunoglobulin protein of the disclosure.

[0138] To determine if an antibody competes for binding with a reference immunoglobulin protein, the above-described binding methodology is performed in two orientations: in a first orientation, the reference antibody is allowed to bind to an anti-NPR1 antibody protein under saturating conditions followed by assessment of binding of the test antibody to the anti-NPR1 antibody molecule. In a second orientation, the test antibody is allowed to bind to an anti-NPR1 antibody molecule under saturating conditions followed by assessment of binding of the reference antibody to the anti-NPR1 antibody molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the anti-NPR1 antibody molecule, then it is concluded that the test antibody and the reference antibody compete for binding to the anti-NPR1 antibody. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0139] Two antibodies bind to the same or overlapping epitope, if each competitively inhibits (blocks) binding of the other to the antigen. The antigen is an anti-NPR1 antibody. That is, a 1-, 5-, 10-, 20-, or 100-fold excess of one antibody inhibits binding of the other by at least 50%, but preferably 75%, 90%, or even 99% as measured in a competitive binding assay (see, *e.g.,* Junghans, *et al.*, *Cancer Res.* 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some

amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0140] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0141] The disclosure further encompasses a human immunoglobulin protein conjugated to a therapeutic moiety ("immunoconjugate"), to treat a NPR1-associated disease or disorder (*e.g.*, hypertension) and/or to ameliorate the hemodynamic effects associated with the therapeutic use of an anti-NPR1 antibody. As used herein, the term "immunoconjugate" refers to an immunoglobulin protein that is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a peptide or protein, or a therapeutic agent. The said protein may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, peptide, or therapeutic agent at any location along the molecule, so long as it is able to bind its target, an anti-NPR1 antibody. Examples of immunoconjugates include antibody-drug conjugates and antibody-toxin fusion proteins. In one embodiment, the agent may be a second different antibody to NPR1 protein. The type of therapeutic moiety that may be conjugated to the rescue agent will take into account the condition to be treated and the desired therapeutic effect to be achieved. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081.

Therapeutic Administration and Formulations

[0142] The disclosure provides therapeutic compositions comprising immunoglobulin proteins of the present disclosure. Therapeutic compositions in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing

vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell, *et al.*, "Compendium of excipients for parenteral formulations", PDA (1998) *J Pharm Sci Technol* 52:238-311.

[0143] The dose of immunoglobulin protein may vary depending upon the age and the size of a subject to be administered, conditions, route of administration, and the like. When a protein of the present disclosure is used for reversing the hemodynamic effects of an anti-NPR1 antibody in an adult patient, or for preventing such hemodynamic effects, it is advantageous to administer the immunoglobulin protein of the present disclosure normally at a single dose of about 0.1 to about 100 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the immunoglobulin proteins of the disclosure can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 600 mg, about 5 to about 500 mg, or about 10 to about 400 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the immunoglobulin protein or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0144] Various delivery systems are known and can be used to administer the pharmaceutical composition of the disclosure, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu, *et al.*, (1987) *J. Biol. Chem.* 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) *Science* 249:1527-1533).

[0145] The use of nanoparticles to deliver the immunoglobulin proteins of the present disclosure is also contemplated herein. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated

nanoparticles and methods of preparation and use are described in detail by Arruebo, M., *et al*., 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in *J. Nanomat.* Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles may be developed and conjugated to antibodies contained in pharmaceutical compositions to target cells. Nanoparticles for drug delivery have also been described in, for example, US 8257740, or US 8246995, each incorporated herein in its entirety.

[0146] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0147] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known.

[0148] A pharmaceutical composition of the present disclosure can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present disclosure. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0149] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the immunoglobulin protein contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the immunoglobulin protein is contained in about 5 to about 300 mg and in about 10 to about 300 mg for the other dosage forms.

Therapeutic Uses of the Immunoglobulin Proteins

[0150] The present disclosure includes methods comprising administering to a subject in need thereof a therapeutic composition comprising an immunoglobulin protein. The therapeutic composition can comprise any of the immunoglobulin proteins as disclosed herein and a pharmaceutically acceptable carrier or diluent. As used herein, the expression "a subject in need thereof" means a human or non-human animal that would benefit from an increase in blood pressure or a reversal of an effect due to an administration of a NPR1 agonist.

[0151] The immunoglobulin proteins of the disclosure (and therapeutic compositions comprising the same) are useful, *inter alia*, for treating any disease or disorder in which an increase in blood pressure would be beneficial. In particular, the immunoglobulin proteins of the present disclosure may be used for the treatment, prevention, and/or amelioration of any disease or disorder associated with or mediated by NPR1 expression or activity. The mechanism of action by which the therapeutic methods of the disclosure are achieved include binding to an agonist anti-NPR1 antibody and removal/clearance of the agonist antibody. Removal of the agonist anti-NPR1 antibody results in an increase in blood pressure.

[0152] The immunoglobulin proteins of the present disclosure may be used to treat, *e.g.*, any NPR1-associated disease or disorder in a subject that has been administered an NPR1 agonist, and wherein a reversal of the hemodynamic effects of the NPR1 agonist is desired. Examples of NPR1-associated disease or disorder include, but are not limited to, hypertension, heart failure, obesity, renal failure, chronic kidney disease, macular edema, glaucoma, stroke, lung disorders, pulmonary fibrosis, inflammation, asthma, skeletal growth disorders, bone fractures, diabetes, and cancer. Administration of a therapeutic composition comprising an immunoglobulin protein of the present disclosure may lead to prevention of one or more adverse effects related to lowered blood pressure. Potential adverse events associated with the lowered blood pressure may include persistent, symptomatic hypotension, reflex tachycardia from compensatory sympathetic nervous system responses (possibly increasing the risk for myocardial infarction, stroke, arrhythmias, heart failure), and decreased cardiac output and end-organ perfusion in subjects with normal (low) venous pressures.

[0153] In one embodiment, the immunoglobulin proteins of the disclosure are used for the preparation of a pharmaceutical composition or medicament for treating patients having hypotension as a result of administration of an anti-NPR1 antibody. In another embodiment, the immunoglobulin proteins are used as adjunct therapy with any other agent, or any other therapy known to those skilled in the art useful for increasing blood pressure and/or addressing symptoms associated with a drop in blood pressure.

[0154] The present disclosure also provides a composition comprising: (i) a therapeutic amount of an immunoglobulin protein as disclosed herein; and (ii) an agonist anti-NPR1 antibody for use in a method for effective regulation of blood pressure and/or hemodynamic changes in a subject wherein the subject suffers from a NPR1-associated disease or disorder.

Non-Immunoglobulin Protein Reversal Agents

[0155] In certain embodiments of the disclosure, the agent for use in reversing the hemodynamic effects of an anti-NPR1 antibody or antigen-binding fragment thereof (*i.e.*, the reversal agent) is selected from the group consisting of a vasopressor, an alpha-adrenoreceptor agonist, a steroid, an antidiuretic hormone, a vascular endothelial growth factor (VEGF) antagonist / an angiogenesis inhibitor, and a small molecule agent that increases blood pressure.

[0156] A vasopressor is an agent that constricts the blood vessels, increasing blood pressure. An alpha-adrenoreceptor agonist (α-agonist) binds to α-receptors on vascular smooth muscle and induces smooth contraction and vasoconstriction, increasing blood pressure. An antidiuretic hormone is a hormone released by the posterior pituitary, which acts on the kidneys to increase the re-absorption of water, causing vasoconstriction in the cardiovascular system. Vasopressors and antidiuretic hormones are known in the art.

[0157] In another embodiment, the reversal agent is a medication for the treatment of anti-NPR1 antibody-induced hemodynamic effects, selected from the group consisting of, but not limited to, midodrine, Levophed, norepinephrine, phenylephrine, fludrocortisone, Orvaten, Northera, ephedrine, Vazculep, droxidopa, Akovaz, Biorphen, Corphedra, and Emerphed.

[0158] Thus, the present disclosure includes reversal agents other than immunoglobulin proteins (including antibodies) that can reverse the hemodynamic effects of anti-NPR1 antibody therapy. For example, the α1-adrenergic receptor agonist, Midodrine can reverse the blood pressure and heart rate effects of R5381, as described herein in Example 14.

Combination Therapies

[0159] In certain embodiments, it is contemplated to use the immunoglobulin proteins and other reversal agents of the present disclosure in combination with one or more other known hypotensive therapies to manage a subject's blood pressure. In specific embodiments, the immunoglobulin proteins of the disclosure are used in combination with NPR1 agonists, preferably with anti-NPR1 antibodies. In one specific

embodiment, the immunoglobulin proteins of the present disclosure are used in combination with R5381 for effective management of blood pressure in a subject in need thereof.

[0160] Combination therapies may include an immunoglobulin protein of the disclosure and any additional therapeutic agent that may be advantageously combined with an immunoglobulin protein of the disclosure, or with a biologically active fragment of an immunoglobulin protein of the disclosure. The immunoglobulin proteins of the present disclosure may be combined synergistically with one or more drugs or therapy used to increase blood pressure or address effects associated with a drop in blood pressure.

[0161] As used herein, the term “in combination with” means that additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the immunoglobulin protein or other reversal agent of the present disclosure. The term “in combination with” also includes sequential or concomitant administration of an immunoglobulin protein or other reversal agent and a second therapeutic agent.

[0162] In specific embodiments, the immunoglobulin proteins of the disclosure are administered to a subject in combination with NPR1 agonists, *e.g.*, R5381. In further embodiments, the immunoglobulin proteins of the disclosure and the NPR1 agonists, *e.g.*, R5381, are administered to a subject concurrently (at the same time), either together in one composition or separately in more than one composition. In still further embodiments, the immunoglobulin proteins of the disclosure and the NPR1 agonists, *e.g.*, R5381, are administered to a subject sequentially, the NPR1 agonists, *e.g.*, R5381, followed by the immunoglobulin proteins of the disclosure.

[0163] The additional therapeutically active component(s) may be administered to a subject prior to administration of an immunoglobulin protein or other reversal agent of the present disclosure. For example, a first component may be deemed to be administered "prior to" a second component, if the first component is administered 1 week before, 72 hours before, 60 hours before, 48 hours before, 36 hours before, 24 hours before, 12 hours before, 6 hours before, 5 hours before, 4 hours before, 3 hours before, 2 hours before, 1 hour before, 30 minutes before, or less than 30 minutes before administration of the second component. In other embodiments, the additional therapeutically active component(s) may be administered to a subject after administration of an immunoglobulin protein or other reversal agent of the present disclosure. For example, a first component may be deemed to be administered "after" a second component, if the first component is administered 30 minutes after, 1 hour after, 2 hours after, 3 hours after, 4 hours after, 5 hours after, 6 hours after, 12 hours

after, 24 hours after, 36 hours after, 48 hours after, 60 hours after, 72 hours after or more after administration of the second component. In yet other embodiments, the additional therapeutically active component(s) may be administered to a subject concurrent with administration of an immunoglobulin protein or other reversal agent of the present disclosure.

[0164] "Concurrent" administration, for purposes of the present disclosure, includes, *e.g.*, administration of an immunoglobulin protein or other reversal agent and an additional therapeutically active component to a subject in a single dosage form, or in separate dosage forms administered to the subject within about 30 minutes or less of each other. If administered in separate dosage forms, each dosage form may be administered via the same route (*e.g.*, both the immunoglobulin protein or other reversal agent and the additional therapeutically active component may be administered intravenously, etc.); alternatively, each dosage form may be administered via a different route (*e.g.*, the immunoglobulin protein or other reversal agent may be administered intravenously, and the additional therapeutically active component may be administered orally). In any event, administering the components in a single dosage from, in separate dosage forms by the same route, or in separate dosage forms by different routes are all considered "concurrent administration," for purposes of the present disclosure. For purposes of the present disclosure, administration of an immunoglobulin protein or other reversal agent "prior to", "concurrent with," or "after" (as those terms are defined herein above) administration of an additional therapeutically active component is considered administration of an immunoglobulin protein or other reversal agent "in combination with" an additional therapeutically active component.

[0165] The present disclosure includes pharmaceutical compositions in which an immunoglobulin protein or other reversal agent of the present disclosure is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Diagnostic Uses of the Immunoglobulin Proteins

[0166] The rescue agents of the present disclosure may be used to detect and/or measure anti-NPR1 antibody in a sample, *e.g.*, for diagnostic purposes. Exemplary diagnostic assays for an anti-NPR1 antibody may comprise, *e.g.*, contacting a sample obtained from a patient with a rescue agent of the disclosure, wherein the rescue agent is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate the anti-NPR1 antibody from patient samples. Alternatively, an unlabeled rescue agent can be used in diagnostic applications in

combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure anti-NPR1 antibody in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0167] Samples that can be used in anti-NPR1 antibody diagnostic assays according to the present disclosure include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either anti-NPR1 antibody protein, or fragments thereof, after administration of the same to a subject. Generally, levels of anti-NPR1 antibody protein in a particular sample obtained from a healthy patient (*e.g.*, a patient who has not received anti-NPR1 antibody) will be measured to initially establish a baseline, or standard, level of anti-NPR1 antibody. This baseline level of anti-NPR1 antibody can then be compared against the levels of anti-NPR1 antibody measured in samples obtained from individuals suspected of having been administered an anti-NPR1 antibody.

[0168] The immunoglobulin proteins specific for anti-NPR1 antibody protein may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

EXAMPLES

[0169] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the disclosure, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.,* amounts, temperature, etc.), but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, room temperature is about 25°C, and pressure is at or near atmospheric.

Example 1: Generation of Human Antibodies to Agonist Antibodies that Specifically Bind Natriuretic Peptide Receptor 1 (NPR1)

Generation of anti-R5381 Antibodies

[0170] Human antibodies to anti-NPR1 antibody protein were generated in a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The mice were immunized with anti-NPR1 antibody R5381 (described elsewhere herein).

[0171] The antibody immune response was monitored by an anti-R5381-specific immunoassay. When a desired immune response was achieved, splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce anti-R5381-specific antibodies. The cell lines were used to obtain several anti-R5381 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains).

[0172] Anti-R5381 antibodies were also isolated directly from antigen-positive mouse B cells without fusion to myeloma cells, as described in U.S. Patent 7,582,298, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-R5381 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained.

[0173] Exemplary antibodies generated as disclosed above were designated as mAb36312 and mAb36313.

Generation of 'One-armed' anti-R5381 antibodies

[0174] Selected antibodies as generated above were used to produce monovalent or 'one-armed' antibodies or antigen-binding molecules. Such monovalent antigen-binding molecules include a single R5381-binding portion comprising a HCVR and LCVR. Monovalent antibodies comprising a full-length heavy chain, a full-length light chain and an additional Fc domain polypeptide were constructed using standard methodologies (see WO2010151792) , wherein the heavy chain constant region differs from the Fc domain polypeptide by at least two amino acids. Such modifications are useful in purification of the monovalent antibodies (see WO2010151792).

[0175] Exemplified one-armed antibodies were manufactured having an IgG4 Fc domain and were designated as REGN9035 and REGN9037.

[0176] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2: Heavy and Light Chain Variable Region Amino Acid and Nucleotide Sequences

[0177] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-R5381 antibodies of the disclosure.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
mAb36312	2	4	6	8	10	12	14	16
mAb36313	22	24	26	28	30	32	34	36

[0178] The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
mAb36312	1	3	5	7	9	11	13	15
mAb36313	21	23	25	27	29	31	33	35

[0179] Antibodies referred to herein typically have fully human variable region(s) but may have human or mouse constant regions. As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 2 – will remain the same, and the binding properties to antigen are expected to be identical or substantially similar regardless of the nature of the Fc domain. In certain embodiments, selected antibodies with a mouse IgG1 Fc are converted to antibodies with human IgG4 Fc. In one embodiment, the IgG4 Fc domain comprises 2 or more amino acid changes as disclosed in US20100331527. In one embodiment, the human IgG4 Fc comprises a serine to proline mutation in the hinge region (S108P) to promote dimer stabilization. Unless indicated otherwise, all antibodies used in the following examples comprise a human IgG4 isotype.

[0180] An exemplary bivalent antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/10 is REGN6580. REGN6580 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 18 and a light chain comprising the amino acid sequence of SEQ ID NO: 20.

[0181] An exemplary bivalent antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 22/30 is REGN6581. REGN6581 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 40.

[0182] Selected anti-R5381 antibodies were used in the construction of one-armed antibodies which comprise an anti-R5381 binding arm and an additional Fc polypeptide (or truncated heavy chain). In certain embodiments, the anti-R5381 binding arm comprises a heavy chain constant region of IgG1, IgG2, IgG3, IgG4 isotypes or a variant thereof. In one embodiment, the additional Fc polypeptide is of IgG1 isotype or a variant thereof. In one embodiment, the additional Fc polypeptide is of IgG4 isotype of a variant thereof.

[0183] Tables 3A, 3B, and 3C list the HCVR, LCVR, CDRs and heavy chain and light sequence identifiers of selected one-armed antibodies.

Table 3A: Amino acid sequences of variable regions and CDRs of selected antibodies

Antibody Identifier	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
REGN9035	2	4	6	8	10	12	14	16
REGN9037	22	24	26	28	30	32	34	36

Table 3B: Heavy chain and light chain amino acid sequence identifiers of selected antibodies

Antibody Identifier	Anti-R5381 Heavy Chain	Truncated Heavy Chain	Light Chain
REGN9035	SEQ ID NO: 42	SEQ ID NO: 46	SEQ ID NO: 20
REGN9037	SEQ ID NO: 44	SEQ ID NO: 46	SEQ ID NO: 40

Table 3C: Heavy chain and light chain nucleic acid sequence identifiers of selected antibodies

Antibody Identifier	Anti-R5381 Heavy Chain	Truncated Heavy Chain	Light Chain
REGN9035	SEQ ID NO: 41	SEQ ID NO: 45	SEQ ID NO: 19
REGN9037	SEQ ID NO: 43	SEQ ID NO: 45	SEQ ID NO: 39

[0184] Unless indicated otherwise, all antibodies used in the following examples comprise a human IgG4 isotype.

Example 3: Biacore Binding Kinetics of Selected Antibodies

[0185] Equilibrium dissociation constants (K_D) for binding of selected anti-R5381 antibodies (mAbs) to H2aM22033N (parental hybridoma of R5381) were determined

using a real-time surface plasmon resonance (SPR) based Biacore T200 biosensor. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25°C and 37°C. The Biacore CM5 sensor chip surface was first derivatized by amine coupling with anti-mouse Fc specific antibody (GE Healthcare, # BR100838) to capture H2aM22033N. Different concentrations of mAbs (100nM – 3.7nM, 3-fold serial dilution) prepared in HBS-ET running buffer were injected at a flow rate of 50µL/min for 3 minutes. The dissociation of different mAbs bound to H2aM22033N was monitored for 10 minutes in HBS-ET running buffer. At the end of each cycle, the H2aM22033N capture surface was regenerated using a 60sec injection of 10mM Glycine-HCl, pH 1.5. The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life (t½) were calculated from the kinetic rates as:

$$K_D\ (M) = \frac{kd}{ka}, \quad \text{and} \quad t½\ (min) = \frac{\ln(2)}{60 * kd}$$

[0186] Binding kinetics parameters for different mAbs binding to H2aM22033N of the disclosure at 25°C and 37°C are shown in Table 4 and Table 5, below, respectively.

Table 4: Binding kinetics parameters of different mAbs binding to H2aM22033N at 25°C

mAb Captured	Injected Analyte	Construct details of Injected Analyte	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM22033N	REGN6580	Bivalent hIgG4	179 ± 1.4	110	1.78E+05	9.42E-05	5.28E-10	123
	REGN9035	Monovalent hIgG4	148 ± 0.7	47	7.51E+04	1.32E-04	1.76E-09	87
	REGN6712	Fab	174 ± 0.3	60	8.50E+04	2.47E-04	2.90E-09	47
	REGN6581	Bivalent hIgG4	177 ± 0.5	65	1.27E+05	7.11E-05	5.61E-10	163
	REGN9037	One-arm hIgG4	144 ± 0.5	31	6.51E+04	9.89E-05	1.52E-09	117
	REGN6713	Fab	173 ± 0.5	39	5.91E+04	1.66E-04	2.81E-09	70

Table 5: Binding kinetics parameters of different mAbs binding to H2aM22033N at 37°C

mAb Captured	Injected Analyte	Construct details of Injected Analyte	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM22033N	REGN6580	Bivalent hIgG4	217 ± 0.7	160	4.64E+05	4.24E-04	9.14E-10	27
	REGN9035	Monovalent hIgG4	166 ± 1.6	81	1.49E+05	8.60E-04	5.78E-09	13
	REGN6712	Fab	210 ± 0.4	85	2.18E+05	1.09E-03	5.02E-09	11
	REGN6581	Bivalent hIgG4	215 ± 0.6	104	3.17E+05	3.67E-04	1.16E-09	31
	REGN9037	One-arm hIgG4	158 ± 0.6	55	1.10E+05	7.38E-04	6.71E-09	16
	REGN6713	Fab	209 ± 0.5	66	1.64E+05	8.09E-04	4.95E-09	14

Example 4: Cross-competition Between Different anti-R5381 antibodies

[0187] Binding competition between anti-R5381 antibodies (mAbs) was determined using a real time, label-free bio-layer interferometry (BLI) assay on the Octet HTX biosensor platform (Pall ForteBio Corp.). The entire experiment was performed at 25°C in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, 1mg/mL BSA, pH 7.4 (HBS-EBT) buffer with the plate shaking at a speed of 1000rpm. To assess whether 2 mAbs are able to compete with one another for binding to their respective epitopes on R5381, ~0.47nm of R5381 was first captured onto anti-human antibody (AHC) coated Octet biosensor tips (Fortebio Inc, # 18-5064) by submerging the biosensor tips for 1 minute in wells containing 1.7 µg/mL solution of R5381. The R5381 captured biosensor tips were then saturated with the first anti-R5381 mAb (subsequently referred to as mAb-1) by dipping into wells containing 50 µg/mL solution of mAb-1 for 4 minutes. The biosensor tips were then subsequently dipped into wells containing 50 µg/mL solution of second anti-R5381 mAb (subsequently referred to as mAb-2) for 3 minutes. The biosensor tips were washed in HBS-ETB buffer in between every step of the experiment. The real-time binding response was monitored during the entire course of the experiment and the binding response at the end of every step was recorded. The response of mAb-2 binding to R5381 pre-complexed with mAb-1 was compared, and competitive/non-competitive behavior of different anti-R5381 mAbs was determined. There was cross-competition between the anti-R5381 mAbs.

Example 5: pH sensitivity of anti-R5381 antibody binding to R5381

[0188] The dissociation rate constants (k_d) for different anti-R5381 mAbs in pH 7.4, pH 6.5, pH 6.0, and pH 5.0 buffers were determined using a real-time surface plasmon resonance (SPR) based Biacore 4000 biosensor. All binding studies were performed at 37°C using four running buffers, (i) PBS, 0.05% v/v Surfactant Tween-20, pH 7.4 (PBS-T-pH 7.4), (ii) PBS, 0.05% v/v Surfactant Tween-20, pH 6.5 (PBS-T-pH 6.5), (iii) PBS, 0.05% v/v Surfactant Tween-20, pH 6.0 (PBS-T-pH 6.0), and (iv) PBS, 0.05% v/v Surfactant Tween-20, pH 5.0 (PBS-T-pH 5.0). The Biacore CM5 sensor chip surface was first derivatized by amine coupling with anti-mouse Fc specific antibody (GE Healthcare, # BR100838) to capture H2aM22033N (parental hybridoma of R5381). Different concentrations of anti-R5381 mAbs (100nM – 11.11nM, 3-fold serial dilution) prepared in PBS-T-pH7.4 buffer were injected at a flow rate of 30µL/min for 3 minutes followed by the dissociation of bound anti-R5381 mAb in PBS-T-pH 7.4, PBS-T-pH 6.5, PBS-T-pH 6.0 or PBS-T PBS-T-pH 5.0 running buffers for 10 minutes.

[0189] The dissociation rate constants (k_d) in four pH running buffers were determined by fitting the real-time binding sensorgrams to a 1:1 binding model using Scrubber 2.0c curve-fitting software. The dissociative half-life (t½) was calculated from the k_d values as:

$$t½ \text{ (min)} = \frac{\ln(2)}{60 * kd}$$

[0190] The k_d and t½ values for selected anti-R5381 mAbs binding to H2aM22033N in PBS-T, pH 7.4 followed by dissociation in PBS-T-pH 7.4, PBS-T-pH 6.5, PBS-T-pH 6.0 or PBS-T-pH 5.0 of the disclosure at 37°C are shown in Table 6, Table 7, Table 8, and Table 9, respectively.

Table 6: Binding of selected anti-R5381 mAbs to H2aM22033N in PBS-T-pH 7.4 buffer and the dissociation in PBS-T-pH 7.4 buffer at 37°C

mAb Captured	Injected Analyte	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_d (1/s)	t½ (min)
H2aM22033N	REGN9035	159 ± 0.9	84	9.96E-04	12
	REGN9037	155 ± 1	55	8.67E-04	13

Table 7: Binding of selected anti-R5381 mAbs to H2aM22033N in PBS-T-pH 7.4 buffer and the dissociation in PBS-T-pH 6.5 buffer at 37°C

mAb Captured	Injected Analyte	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_d (1/s)	t½ (min)
H2aM22033N	REGN9035	173 ± 0.4	97	1.42E-03	8
	REGN9037	172 ± 0.3	63	7.92E-04	15

Table 8: Binding of selected anti-R5381 mAbs to H2aM22033N in PBS-T-pH 7.4 buffer and the dissociation in PBS-T-pH 6.0 buffer at 37°C

mAb Captured	Injected Analyte	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_d (1/s)	t½ (min)
H2aM22033N	REGN9035	171 ± 0.8	95	2.94E-03	3.9
	REGN9037	168 ± 0.4	61	8.11E-04	14

Table 9: Binding of selected anti-R5381 mAbs to H2aM22033N in PBS-T-pH 7.4 buffer and the dissociation in PBS-T-pH 5.0 buffer at 37°C

mAb Captured	Injected Analyte	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_d (1/s)	t½ (min)
H2aM22033N	REGN9035	165 ± 0.5	84	1.97E-02	0.6
	REGN9037	162 ± 0.3	54	6.64E-04	17

[0191] A comparison of the dissociative half-life (t½) of R5381 anti-idiotype monoclonal antibodies REGN9035 and REGN9037 in pH 7.4, pH 6.5, pH 6.0, and pH 5.0 buffers is shown in Figure 1.

Example 6: Inhibition of R5381-induced NPR1 activation

[0192] In order to assess the regulation of human NPR1 (hNPR1), a stable HEK293 cell line stably expressing hNPR1 with a C-term myc and FLAG tag was developed. The cell line was sorted for high expression of hNPR1, HEK293/hNPR1.MycDDK HS or abbreviated as HEK29/hNPR1, and maintained in DMEM containing 10% FBS, NEAA, pen/strep/glut, and 500 µg/mL G418 sulfate. Binding of ligand to NPR1 activates the receptor's guanylate cyclase domain, which catalyzes the production of

cGMP from GTP (Zois, *et al.*, 2014 *Nature* 11(7):403-412). A homogeneous time-resolved fluorescence (HTRF) assay that measures cGMP levels was used to assess NPR1 activity.

[0193] For the cGMP assay, HEK293/hNPR1 cells were plated in 96-well half-area plates at 20,000 cells/well with 50 µl of complete growth media and cultured overnight. The next day, activation of hNPR1 was induced by replacing the media with 10 µl of dilution buffer (OptiMEM with 0.1% FBS) followed by the addition of 10 µl of 2X agonist at a range of concentrations including a sample without any agonist (see Table 10, below) made in dilution buffer. To evaluate the reversal of R5381-induced hNPR1 activation, cells were treated with 10 µl of 2x anti-R5381 at a range of concentrations (see Table 10) made in dilution buffer followed by the addition of 10 µl of 70 nM R5381 made in dilution buffer alone or containing 40 pM ANP or 80 pM BNP. The treated cells were incubated at 37°C for 30 min. HTRF assay was performed using a cGMP HTRF kit from Cisbio according to manufacturer's protocol (#62GM2PEH). Briefly, 20 µl of 1x cGMP series dilution was added to empty wells for cGMP standard curve. For measuring cGMP concentration in samples with test articles, 10 µl of cGMP-d2 and 10 µl of anti-cGMP antibody conjugated with cryptate diluted in lysis buffer were added in order to each well for 60 min at RT in dark. The fluorescence intensity was detected using an EnVision multilabel plate reader (excitation = 320 nm, emission = 620 nm/665 nm, Perkin Elmer), and the fluorescence resonance energy transfer (FRET) ratio was calculated using the equation described below:

$$FRET\ ratio = \frac{Signal\ at\ 665\ nm}{Signal\ at\ 620\ nm} \times\ 10\text{^}4$$

[0194] The FRET ratios were converted to cGMP concentrations according to the cGMP standard curve and analyzed using a 4-parameter logistic equation over an 11 point dose-response curve to obtain the half maximal effective concentration (EC_{50}) values for the tested agonists and the half maximal inhibitory concentration (IC_{50}) values for the tested antagonists using GraphPad Prism 8. The maximum inhibition was calculated with the equation described below:

$$\%\ Maximum\ inhibition = \frac{[cGMP, nM]_{70nM\ REGN5381} - [cGMP, nM]_{test\ antibody}}{[cGMP, nM]_{70nM\ REGN5381} - [cGMP, nM]_{baseline}} \times\ 100\ \%$$

[0195] In this equation, $[cGMP, nM]_{baseline}$, $[cGMP, nM]_{test\ antibody}$ and $[cGMP, nM]_{70nM\ R5381}$ are the cGMP concentration values from the cells treated with dilution buffer, the highest concentration of the anti-R5381 antibody, and 70nM R5381 with or without 40pM ANP or 80pM BNP, respectively.

Table 10: Concentration of reagents used in NPR1 cGMP assay

NPR1 agonist	Concentration Range [nM]
ANP	2 – 0.0020
BNP	4 – 0.0039
R5381	300 – 0.29
Anti-R5381 (±R5381, ±ANP or ±BNP)	300 – 0.29

Results

[0196] R5381 activated hNPR1 expressed in HEK293/hNPR1 cells to stimulate cGMP accumulation with EC50 values of 1.78 – 31.2 nM in the presence or absence of ANP or BNP, where the basal levels of cGMP without antibody were increased due to the constant amount of ANP or BNP (Table 11, below). Isotype control, REGN1945, did not show any measurable activation in dilution buffer. The EC50 values for ANP and BNP were not calculated due to the limit of quantitation at high concentration compared to the cGMP standard curve in this assay.

[0197] All the one-armed and bivalent anti-R5381 antibodies blocked 70nM R5381-induced hNPR1 activation in the presence or absence of 40pM ANP or 80pM BNP with IC50 values of 15.5 – 57.5 nM and maximum inhibition of 97% - 106% (Table 11). Isotype control, REGN1945, did not show any significant inhibition of 70 nM R5381-induced hNPR1 activation in the presence or absence of ANP or BNP (Table 11).

[0198] Taken together, all the anti-R5381 antibodies showed significant inhibition of R5381-induced hNPR1 activation in the presence or absence of endogenous ligand as measured by cGMP accumulation.

Table 11. One-armed and bivalent anti-R5381 antibodies significantly inhibited R5381-induced human NPR1 activation as measured by cGMP accumulation in the presence or absence of endogenous ligand

R5381 ± ligand, EC50 [M]	**No Ligand**		**ANP**		**BNP**	
			40pM ANP		**80pM BNP**	
	3.12E-08		2.07E-08		1.78E-09	
Anti-R5381 @70nM	**No Ligand**		**40pM ANP**		**80pM BNP**	
	IC50 [M]	**Max Inh. (%)**	**IC50 [M]**	**Max Inh. (%)**	**IC50 [M]**	**Max Inh. (%)**
REGN6580	1.55E-08	99	2.23E-08	102	3.32E-08	99
REGN6581	1.80E-08	100	2.06E-08	104	3.50E-08	99
REGN9035	4.60E-08	100	2.66E-08	104	4.13E-08	97
REGN9037	3.63E-08	99	1.76E-08	101	3.81E-08	97
Isotype control	ND	27	ND	28	ND	6

ND: not determined; Max Inh.: maximum inhibition.

[0199] Anti-R5381 antibodies were found to inhibit R5381-induced hNPR1 activation in the absence of endogenous ligand or in the presence of 40 pM ANP, or 80 pM BNP. Cells were incubated with increasing concentrations of ANP, BNP, R5381 or Isotype control (REGN1945) alone, or 70 nM R5381 in the presence or absence of constant concentration of ANP or BNP. The fluorescence intensity was detected using an EnVision (excitation = 320 nm, emission = 620 nm/665 nm) and the FRET ratio and cGMP concentration were calculated as described in the experimental procedure.

Example 7. Blocking ELISA assay

[0200] hNPR1.ecto.mmh (of which amino acids 1-441: human NPR1 amino acid G32-E473 from translation of NM_000906.3, and amino acids 442-469: Myc-Myc-hexahistidine tag; SEQ ID NO: 47), at 1.0 µg/mL in PBS, was coated on 96-well microtiter plates and incubated overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS (assay buffer). Anti-R5381 mAbs and isotype control mAb, were three-fold serially diluted from 500nM to 8.46pM in assay buffer. In a 96-well dilution plate, 285pM Biotin-R5381 was mixed with the three-fold serially diluted antibodies and allowed to pre-bind at room temperature (RT) for 1 hour. The final concentrations of anti-R5381 and isotype control mAbs ranged from 333.33nM to 5.64pM and the final concentration of Biotin-R5381 was 95pM. After 1 hour, incubation at RT, the pre-bind reaction mix was transferred to microtiter plates coated with hNPR1.ecto.mmh. The microtiter plates were incubated at RT for 1 hour and then washed with plate washing solution. Biotin-R5381 binding was detected using Poly-HRP Streptavidin protein. The plates were incubated with detection protein for 1 hour at RT and then washed with plate washing solution. The assay plates were developed with TMB colorimetric substrates according to the manufacturer's recommended procedure.

[0201] The absorbance at 450nm for each well was recorded and plotted as the function of the concentration of antibody. Data was analyzed in GraphPad Prism software using a four-parameter logistic equation over an 11-point dose response curve and IC_{50} values were calculated. The calculated IC_{50} value, defined as the concentration of antibody required to reduce 50% binding of Biotin-R5381 to immobilized hNPR1.ecto.mmh, was used as an indicator of blocking potency. Percent blocking at highest anti-R5381 concentration was calculated as an indicator of the ability of the antibodies to block binding of Biotin-R5381 to NPR1 relative to the baseline of the assay. The baseline signal of the assay, defined as 0% binding of Biotin-R5381, was determined from OD450nm readings from Poly-HRP Streptavidin

binding in wells with assay buffer alone. Binding signal of 95pM Biotin-R5381 in absence of the anti-R5381 was defined as 100% binding or 0% blocking.

Results

[0202] The ability of anti-R5381 antibodies to block Biotin-R5381 binding to human NPR1 was assessed using a blocking ELISA assay. The blocking results are summarized in Table 12, below. The percent blockade calculated at highest antibody concentration (333.33nM) tested, is reported for all antibodies. REGN9035 blocked binding of 95pM Biotin-R5381 with IC_{50}[M] of 2.04nM and demonstrated 99.29% blockade at highest tested concentration. REGN9037 blocked binding of 95pM Biotin-R5381 with IC_{50}[M] of 2.40nM and demonstrated 99.05% blockade at highest tested concentration.

[0203] Anti-R5381 bivalent antibodies REGN6580 and REGN6581, blocked 95pM Biotin-R5381 binding with IC_{50}[M] values of 381pM and 543pM respectively and both mAbs demonstrated 100% blockade at highest concentration. The isotype control mAb (REGN1945), did not show any blocking of Biotin-R5381, under identical assay conditions.

Table 12: Summary of IC50[M] values for selected anti-R5381 antibodies blocking 95pM Biotin-R5381 binding to hNPR1.ecto.mmh (REGN3037)

	Anti-R5381 (single arm and bivalent) mAbs blocking 95pM Biotin-R5381 binding to ELISA plate-coated 1ug/ml hNPR1.ecto.mmh	
REGN#	**IC_{50}[M]**	**% Blocking with 333.33nM antibody**
REGN9035	2.04E-09	99.29
REGN6580	3.81E-10	100
REGN9037	2.40E-09	99.05
REGN6581	5.43E-10	100
Isotype control	not calculated	No blocking

100% Blocking= OD_{450nm} value of wells with HRP-conjugated secondary protein in assay buffer alone (no Biotin-R5381 binding)

0% Blocking= OD_{450nm} value of wells with HRP-conjugated secondary protein in assay buffer in presence of constant amount of Biotin-R5381 (without Anti-R5381).

$$\textbf{Maximum \% Blocking} = 100 - \frac{[\text{Experimental Signal}_{(\text{Anti-REGN5381+Biotin-REGN5381})} - \text{Background Signal}_{(\text{buffer only})}]}{[\text{Maximum Signal}_{(\text{Biotin-REGN5381 Alone})} - \text{Background Signal}_{(\text{buffer only})}]} \times 100$$

Experimental Signal = OD_{450} of Biotin-R5381 binding observed at tested Anti-R5381 concentration

Maximum Signal = OD_{450} of 95pM Biotin-R5381 binding in absence of Anti-R5381

Background Signal = OD_{450} of Poly-HRP Streptavidin binding in assay buffer only control

[0204] ELISA-based methods were used to assess the blocking of Biotin-R5381 binding to ELISA plate coated hNPR1.ecto.mmh (SEQ ID NO: 47) in presence of a range of concentrations of one armed anti-R5381 mAbs, REGN9035 and REGN9037. As controls, respective bivalent mAbs REGN6580 and REGN6581, and isotype control mAb were also tested. Percent blocking at highest mAb concentration (333.33 nM) and blocking IC_{50}[M] values of the mAbs are tabulated in Table 12, above. Molarity [M] indicates antibody concentration for mAbs.

Example 8. Circulating immune complex formation between R5381 and anti-R5381

[0205] The potential to form circulating immune complexes (CIC) between R5381 and anti-R5381 antibodies was tested using Microvue C1q-CIC kit developed by Quidel. This assay was performed according to the manufacturer's instructions. Antigen and antibody samples were combined at either 1:1 or 1:10 ratios and incubated at 37°C for 30 minutes to initiate complex formation. The antibody-antigen samples, as well as positive and negative heat aggregated gamma globin (HAGG) controls, were then diluted 1:50 into C1q coated test plates. Kit standards were added directly into C1q coated test plates. Test plates were then incubated at RT for 1 hour. Unbound antibodies, antigens or complexes were washed from the plates using a 1X wash buffer. An HRP-conjugated detection antibody was added to test plates and incubated at RT for 30 minutes after which unbound HRP-conjugated detection antibody was washed from plates using 1X wash buffer. HRP substrate was added to the test plates and incubated at RT for 30 minutes. An acidic stopping solution was then applied to inactivate the HRP enzyme. The plates were then read on a Perkin Elmer Victor X5 plate reader at 405nM.

[0206] The raw data was background subtracted, and MicroVue C1q-CIC kit standards were used to plot a linear standard curve that was analyzed by linear regression. The sample and HAGG standards values (µg Eq/mL) were then calculated using a linear regression equation.

Results

[0207] HAGG High and low controls were included on each plate. According to the manufacturer's instructions any sample with a value lower than 4.0 µg Eq/mL is considered negative. The potential for R5381 to form CICs with anti-R5381 antibodies

was investigated using the MicroVue C1q-CIC kit. The results are summarized in Tables 13A and 13B, below. The final value and presence of CIC is indicated. No CIC was detected in any of the experimental tested conditions.

Table 13A: Clq-CIC assay

Plate 1			
Antibody (REGN#)	**Antigen (REGN#)**	**Final Value (ug Eq/mL)**	**Result (Pos/Neg)**
1μM REGN6580	100nM R5381	0.20	Neg
100nM REGN6580	100nM R5381	0.22	Neg
1μM REGN9035	100nM R5381	0.29	Neg
100nM REGN9035	100nM R5381	0.18	Neg
1μM Isotype control	100nM R5381	0.30	Neg
100nM Isotype control	100nM R5381	0.31	Neg
1μM REGN6580	100nM Isotype control	0.16	Neg
100nM REGN6580	100nM Isotype control	0.11	Neg
1μM REGN9035	100nM Isotype control	0.20	Neg
100nM REGN9035	100nM Isotype control	0.15	Neg
1μM Isotype control	100nM Isotype control	0.17	Neg
100nM Isotype control	100nM Isotype control	0.16	Neg
-	100nM R5381	0.08	Neg
-	100nM Isotype control	0.18	Neg
1μM REGN6580	-	0.21	Neg
100nM REGN6580	-	0.22	Neg
1μM REGN9035	-	0.25	Neg
100nM REGN9035	-	0.16	Neg
1μM Isotype control	-	0.25	Neg
100nM Isotype control	-	0.20	Neg
High HAGG control	-	**23.9**	
Low HAGG control	-	**1.81**	

Table 13B: Clq-CIC assay

Plate 2			
Antibody (REGN#)	**Antigen (REGN#)**	**Final Value (ug Eq/mL)**	**Result (Pos/Neg)**
1μM REGN6581	100nM R5381	0.53	Neg
100nM REGN6581	100nM R5381	0.65	Neg
1μM REGN9037	100nM R5381	0.42	Neg
100nM REGN9037	100nM R5381	0.64	Neg
1μM Isotype control	100nM R5381	0.46	Neg
100nM Isotype control	100nM R5381	0.55	Neg
1μM REGN6581	100nM Isotype control	0.30	Neg
100nM REGN6581	100nM Isotype control	0.35	Neg
1μM REGN9037	100nM Isotype control	0.55	Neg
100nM REGN9037	100nM Isotype control	0.37	Neg
1μM Isotype control	100nM Isotype control	0.36	Neg
100nM Isotype control	100nM Isotype control	0.41	Neg
-	100nM R5381	0.39	Neg
-	100nM Isotype control	0.36	Neg
1μM REGN6581	-	0.62	Neg
100nM REGN6581	-	0.37	Neg
1μM REGN9037	-	0.38	Neg
100nM REGN9037	-	0.58	Neg
1μM Isotype control	-	0.41	Neg
100nM Isotype control	-	0.43	Neg
High HAGG control	-	**21.2**	
Low HAGG control	-	**1.92**	

Example 9. Pharmacokinetic assessment of anti-R5381 antibodies in NPR1$^{hu/hu}$ mice

[0208] Evaluation of the pharmacokinetics of two one-armed anti-R5381 antibodies, REGN9035 and REGN9037 and their respective bivalent parental counterparts, REGN6580 and REGN6581, were conducted in humanized NPR1 mice (mice homozygous for the humanized NPR1 allele, NPR1$^{hu/hu}$). Cohorts contained 5 mice per tested antibody. Mice dosed with REGN6580 and REGN6581 received a single

sub-cutaneous (SC) 1 mg/kg dose. Mice dosed with REGN9035 and REGN9037 received a single normalized SC dose based on molar equivalence (0.67 mg/kg) to their parental counterparts. Blood samples were collected at 6 hours and 1, 2, 3, 7, 10, 14, 21 and 30-days post dosing. Blood was processed into serum and frozen at -80 °C until analyzed. The total and functional hIgG serum concentrations of REGN9035, REGN9037, REGN6580 and REGN6581 were measured using the GyroLab xPlore platform (Gyros).

[0209] Gyros technology uses an affinity flow-through format for automated immunoassays with laser-induced fluorescence detection. Samples are loaded onto a compact disc (CD) which contains multiple radially arranged nanoliter-scale affinity capture columns. Liquid flow is controlled by centrifugal and capillary forces.

[0210] For the measurement of total and functional REGN9035, REGN9037, REGN6580 and REGN6581 concentrations in serum, a test article-specific biotinylated capture reagent (**Error! Reference source not found.**4, below) was added onto a Gyrolab Bioaffy 200 CD containing affinity columns preloaded with streptavidin-coated beads (Dynospheres). The standards used for calibration (Table 14) were run at concentrations ranging from 0.488 – 2000 ng/mL. Serial dilutions of serum samples were prepared in phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA). Serial dilutions of standards were prepared in PBS + 0.5% BSA containing 2% normal mouse serum (NMS). Singlets of serum samples diluted at 1:50 and duplicates of standards were added onto the capture reagent-coated affinity columns at room temperature. Captured human IgG was detected using Alexa-647-conjugated mouse anti-human IgG1/hIgG4 monoclonal antibody (@ 0.5 µg/mL) diluted in Rexxip F buffer (Gyros); the resultant fluorescent signal was recorded in response units (RU) by the GyroLab xPlore instrument. The respective assay's lower limit of quantitation (LLOQ) of 0.05 µg/mL was defined as the lowest concentration on the standard curve for which a Quality Control (QC) sample was determined to consistently deviate less than 25% from the expected concentration (Table 14). Sample concentrations were determined by interpolation from a standard curve that was constructed using a 4-parameter logistic curve fit in Gyrolab Evaluator Software. Average concentrations from 2 replicate experiments were used to calculate final concentrations.

Table 14. Assay Conditions For Gyros ImmunoAssays for Human IgG

Detected human IgG	Capture Reagent	Concentration of Capture Reagent	Standard
REGN9035 (Total)	Biotin-conjugated mouse anti-human kappa light chain constant region mAb	20 µg/mL	REGN9035
REGN9037 (Total)			REGN9037
REGN6580 (Total)			REGN6580
REGN6581 (Total)			REGN6581
REGN9035 (Functional)	Biotin-conjugated Fab of R5381	75 µg/mL	REGN9035
REGN9037 (Functional)			REGN9037
REGN6580 (Functional)			REGN6580
REGN6581 (Functional)			REGN6581

Fab, antibody binding fragment

[0211] PK parameters were determined by non-compartmental analysis (NCA) using Phoenix®WinNonlin® software Version 6.3 (Certara, L.P., Princeton, NJ) and an extravascular dosing model. Using the respective mean concentration values (total hIgG) for each antibody, all PK parameters including observed maximum concentration in serum (C_{max}), estimated half-life observed ($t_{1/2}$), area under the concentration curve versus time up to the last measurable concentration (AUC_{last}), and antibody clearance rates (Cl) were determined using a linear trapezoidal rule with linear interpolation and uniform weighting.

Results

[0212] Following 1 mg/kg (or dose equivalent) SC administration of the anti-R5381 Abs in NPR1$^{hu/hu}$ mice, REGN9035, REGN9037, REGN6580, and REGN6581 exhibited similar dose normalized maximum concentrations of total hIgG in serum ($C_{max/D}$ = 10.3, 9.23, 9.3, and 11.4 mg/mL, respectively). In addition, REGN9035, REGN9037, REGN6580, and REGN6581 also exhibited similar half-life values ($T_{1/2}$=18.1, 17.1, 16.1, and 15.4 days, respectively), dose normalized drug exposure values ($AUC_{last/D}$= 204, 174, 165, and 205 (d*mg/mL)/(mg/kg), respectively) and

clearance rates (Cl = 5.0, 6.1, 4.9, and 3.7 mL/day/kg, respectively). Furthermore, total and functional human IgG concentrations of REGN9035, REGN9037, REGN6580, and REGN6581 were comparable over all measured timepoints. No measurable differences were seen in the PK profiles of the one-armed antibodies, REGN9035 and REGN9037 in comparison to their bivalent counterparts, REGN6580 and REGN6581 in NPR1$^{hu/hu}$ mice.

[0213] A summary of the data for total and functional antibody concentrations are summarized in Table 15, below, mean PK parameters are described in Table 16, below, and mean total antibody concentrations versus time are shown in Figure 2.

Table 15: Mean Serum Concentrations (± SEM) of Total and Functional hIgG Following a Single 1 mg/kg (or Dose Equivalent) Subcutaneous Injection of REGN9035, REGN9037, REGN6580 or REGN6581 Antibodies in NPR1$^{hu/hu}$ Mice Over Time

		Total hIgG Concentration		Functional hIgG Concentration	
		1 mg/kg (0.67 mg/kg dose normalized)			
Antibody	**Time (d)**	**Mean (µ/mL)**	**+/- SEM**	**Mean (µ/mL)**	**+/- SEM**
REGN9035	0.25	2.2	0.18	2.2	0.17
	1	6.5	0.25	6.3	0.24
	2	6.8	0.23	7.4	0.29
	3	6.7	0.17	7.0	0.24
	7	5.9	0.27	5.9	0.25
	10	5.2	0.26	5.1	0.26
	14	3.9	0.71	3.8	0.76
	21	2.9	0.70	2.8	0.70
	30	1.9	0.49	1.8	0.44
REGN9037	0.25	1.8	0.18	1.7	0.16
	1	6.2	0.26	5.5	0.13
	2	6.2	0.14	5.7	0.08
	3	5.9	0.32	5.5	0.21
	7	5.1	0.15	4.8	0.05
	10	4.5	0.05	4.2	0.09
	14	4.2	0.18	3.7	0.10
	21	3.0	0.10	2.8	0.10
	30	2.0	0.14	1.7	0.13
REGN6580	0.25	1.6	0.20	1.7	0.21
	1	7.5	0.30	7.8	0.56
	2	9.0	0.45	8.6	0.40
	3	8.6	0.53	8.4	0.48
	7	7.5	0.52	7.0	0.50
	10	6.7	0.56	6.4	0.53
	14	6.1	0.56	5.6	0.50
	21	4.6	0.57	4.3	0.55
	30	2.2	0.57	2.1	0.55

		Total hIgG Concentration		Functional hIgG Concentration	
		1 mg/kg (0.67 mg/kg dose normalized)			
Antibody	**Time (d)**	**Mean (µ/mL)**	**+/- SEM**	**Mean (µ/mL)**	**+/- SEM**
REGN6581	0.25	2.4	0.38	2.4	0.36
	1	9.7	0.68	9.4	0.60
	2	11.2	0.66	10.7	0.39
	3	10.6	0.47	9.9	0.53
	7	9.3	0.56	8.1	0.52
	10	8.4	0.37	7.4	0.45
	14	7.2	0.38	6.6	0.37
	21	5.3	0.39	4.8	0.31
	30	3.0	0.32	2.9	0.32

Abbreviations: Time = Time in days post single-dose injection; d = Day of study; SEM = Standard Error of the Mean PK parameters were derived from mean concentration versus time profiles of total hIgG concentrations. $T_{1/2}$ and AUC_{last} are based on concentrations out to day 30. The mean ± SEM value for each PK parameter is shown for all dose groups.

Table 16: Summary of Pharmacokinetic Parameters

Parameter	Units	1 mg/kg (0.67 mg/kg dose normalized)			
		REGN9035	REGN9037	REGN6580	REGN6581
C_{max}	µg/mL	6.9 ± 0.2	6.5 ± 0.2	9.3 ± 0.5	11.4 ± 0.7
$C_{max/D}$	µg/mL/ mg/kg	10.3 ± 0.3	9.23 ± 0.5	9.3 ± 0.5	11.4 ± 0.7
$T_{1/2}$	d	18.1 ± 1.1	17.1± 1.2	16.1 ± 2.3	15.4 ± 1.1
AUC_{last}	d* µg/mL	136 ± 4	117 ± 3.1	165 ± 19	205 ± 11
$AUC_{last/D}$	(d*µg/mL)/ (mg/kg)	204 ± 5.9	174 ± 4.6	165 ± 19	205 ± 11
Cl	mL/day/kg	5.0 ± 0.3	6.1± 0.3	4.9 ± 1.0	3.7 ± 0.3

Abbreviations: AUC_{last} = area under the curve from the time of dosing to the last measurable concentration; $AUC_{last/D}$ = AUC last dose normalized to 1 mg/kg dosing; $t_{½}$ = terminal half-life of elimination; C_{max} = peak concentration; $C_{max/d}$ = C_{max} dose normalized to 1 mg/kg dosing; Cl = clearance rate of antibody over time.; SEM = standard error of the mean.

Example 10. Serum concentration analysis of total R5381 (anti-NPR1 mAb) after rescue by anti-R5381 antibodies

[0214] Sample concentration analysis of serum from an *in vivo* study evaluating the effectiveness of bivalent anti-R5381 mAbs, REGN6580 and REGN6581 in comparison

to an isotype control antibody, REGN1945, at reversing the blood pressure lowering effects of R5381. The study was performed in telemetered normotensive humanized NPR1 (mice homozygous for the humanized NPR1 allele, $NPR1^{hu/hu}$) mice. In short, cohorts contained 5 mice per tested antibody. All mice were dosed with a single sub-cutaneous (SC) 5 mg/kg dose of R5381. Three days later, mice received either a single intravenous (IV) 50 mg/kg dose of REGN6580, REGN6581 or isotype control, REGN1945. Blood samples were collected at 7 and 22-days post initial R5381 dosing. Blood was processed into serum and frozen at -80 °C until analyzed. The serum concentrations of total R5381 were measured using the GyroLab xPlore platform (Gyros).

[0215] Gyros technology uses an affinity flow-through format for automated immunoassays with laser-induced fluorescence detection. Samples are loaded onto a compact disc (CD) which contains multiple radially arranged nanoliter-scale affinity capture columns. Liquid flow is controlled by centrifugal and capillary forces.

[0216] For the measurement of total R5381 in serum, an immunoassay was run. Mice dosed with R5381 followed by dosing with an anti-R5381 mAb are presumed to form antibody: anti-idiotype complexes in serum. (R5381: REGN6580 or R5381: REGN6581). In order to accurately measure the total R5381 antibody concentrations of these samples, a dissociation step was performed at the start of the assay. Briefly, to measure total R5381, a test article- specific biotinylated capture reagent, REGN6712 at a concentration of 75 µg/mL, was pre-incubated for 4 hours at 37°C with serum samples diluted at 1:250 or standards diluted at concentrations ranging from 0.244 to 1000 ng/mL. Dilutions of serum samples were prepared in phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) (+ capture reagent), and serial dilutions of standards (R5381) were prepared in PBS + 0.5% BSA containing 0.4% normal mouse serum (NMS) (+ capture reagent). Following a 4-hour 37°C pre-incubation of capture reagents with samples or standards, diluted singlets of serum samples (+capture reagent) and diluted duplicates of standards (+ capture reagent) were added onto a Gyrolab Bioaffy 200 CD containing affinity columns preloaded with streptavidin-coated beads (Dynospheres). Captured human IgG was detected using 0.5 µg/mL Alexa-647-conjugated mouse anti-human IgG1/hIgG4 monoclonal antibody diluted in Rexxip F buffer (Gyros); the resultant fluorescent signal was recorded in response units (RU) by the GyroLab xPlore instrument. The respective assay's lower limit of quantitation (LLOQ) of 0.1 µg /mL was defined as the lowest concentration on the standard curve for which a pre-complexed Quality control

(QC) (R5381: REGN6580, R5381: REGN6581) sample was determined to consistently deviate less than 25% from the expected concentration.

[0217] For the measurement of total R5381concentrations in serum from mice dosed with R5381 followed by a non-binding control antibody (REGN1945), a dissociation step was not necessary. Total R5381 concentrations were measured as follows. Briefly, a test article- specific biotinylated capture reagent, mAb36313 at 50 µg/mL was added onto the Gyrolab Bioaffy 200 CD containing affinity columns preloaded with streptavidin-coated beads (Dynospheres) at room temperature. The standard (R5381) used for calibration in this assay was diluted at concentrations ranging from 0.488 to 2000 ng/mL. Serial dilutions of standards were prepared in PBS + 0.5% BSA containing 1% normal mouse serum (NMS). Singlets of serum samples diluted at 1:100 and duplicates of standards were added onto the capture reagent coated affinity columns at room temperature. Captured human IgG was detected using 0.5 µg/mL Alexa-647-conjugated mouse anti-human IgG1/hIgG4 monoclonal antibody diluted in Rexxip F buffer (Gyros); the resultant fluorescent signal was recorded in RU by the GyroLab xPlore instrument. The respective assay's LLOQ of 0.05 µg /mL was defined as the lowest concentration on the standard curve for which a QC sample was determined to consistently deviate less than 25% from the expected concentration.

[0218] Sample concentrations were determined by interpolation from a standard curve that was constructed using a 4-parameter logistic curve fit in Gyrolab Evaluator Software. Average concentrations from 2 replicate experiments were used to calculate final concentrations.

Calculation of Mean Concentrations

[0219] Individual and mean concentrations below the LLOQ (<LLOQ) are reported as below the limit of quantitation (BLQ). If >50% of the individual values are BLQ, the mean value for that time point is reported as BLQ. If ≤50% of the individual values within a treatment group are BLQ and, using zero as the BLQ value, the mean value is arithmetically BLQ, then the mean value is reported as BLQ. If ≤50% of the individual values within a treatment group are BLQ and, using zero as the BLQ value, the mean value is arithmetically ≥LLOQ, then this arithmetic value is reported.

Results

[0220] Seven days post 5 mg/kg dosing of R5381 in $NPR1^{hu/hu}$ mice followed three days later by administration of 50 mg/kg of rescue reagents REGN6580, REGN6581, or isotype control mAb (REGN1945), resulted in lower average serum concentrations of total R5381 in mice receiving the anti-idiotype antibodies as compared to mice receiving a dose of isotype control. This indicates that anti-idiotype antibodies,

REGN6580 and REGN6581, were responsible for the faster clearance of R5381 seen in mice when given these anti-idiotype mAbs as compared to mice dosed with isotype control.

[0221] Mice dosed with R5381 and followed three days later (7 days post initial R5381 dosing) by a dose of REGN6580 or REGN6581 had total R5381 concentrations of 3.6 or 7.4 µg/mL, respectively. In comparison, mice dosed 3 days later with Isotype control resulted in total R5381 concentrations of 30.5 µg/mL. Analysis of serum taken 22 days post R5381 dosing followed by dosing of the anti-idiotype antibodies, resulted in undetectable concentrations of total R5381, whereas serum concentrations of total R5381 in the Isotype control dosed group were still approximately 6 µg/mL.

[0222] A summary of the data for total R5381 serum concentrations in mice rescued by IV dosing of REGN6580, REGN6581, or isotype control, REGN1945, are found in Table 17, below.

Table 17: Mean Serum Concentrations (± SEM) of Total IgG Following a Single 5 mg/kg Subcutaneous Injection of R5381 Followed Three Days Later by a Single 50 mg/kg Intravenous Injection of REGN6580 or REGN6581 Anti-Idiotype Antibodies or Isotype Control in NPR1hu/hu Mice at Days 7 and 22

Antibody Group	Time (d)	Total hIgG Concentration	
R5381 + REGN6580		Total R5381	
		Mean (µg/mL)	+/- SEM
	7	3.6	0.4
	22	BLQ	NA
R5381 + REGN6581		Total R5381	
		Mean (µg/mL)	+/- SEM
	7	7.4	2.7
	22	BLQ	NA
R5381 + Isotype control		Total R5381	
		Mean (µg/mL)	+/- SEM
	7	30.5	3
	22	5.9	2.8

Abbreviations: Time, Time in days post single-dose injection; d, Day of study; BLQ, Below the limit of quantitation; NA, not applicable; SEM = Standard Error of the Mean

Example 11. Evaluation of reversal of R5381-induced blood pressure lowering using a single 50 mg/kg intravenous dose of bivalent anti-R5381 mAbs in normotensive NPR1hu/hu mice

[0223] In order to assess the effects of bivalent anti-R5381 antibodies at reversing the blood pressure lowering induced by R5381 in telemetered normotensive $NPR1^{hu/hu}$ mice, male $NPR1^{hu/hu}$ mice (n=20) aged ~18-20 weeks were implanted with PA-C10 telemeters (DSI, St. Paul, MN) and allowed to recover for at least 7 days. Animals were stratified into groups (Groups 1-4) based on pre-study systolic blood pressures and body weight. Animals were individually housed under standard conditions (Temperatures of 64°F to 84°F (18°C to 29°C); relative humidity of 30% to 70%) and a 12-hour light/12-hour dark cycle was maintained. Food (Research Diets Standard pellet chow) and water were provided ad libitum.

[0224] The test proteins were administered to the appropriate animals by single subcutaneous injection on Day 0. The rescue agents were administered to the appropriate animals by single intravenous injection on Day 3. The dose volume for each animal was based on the most recent body weight measurement.

Table 18: Summary of Doses and Dose Groups

Group No.	Test Article	Dose (mg/kg s.c.)	Rescue Article	Dose (mg/kg i.v.)	Number of Animals
					Males
1	PBS	0	PBS	0	5
2	REGN5381	5	IgG4P isotype control mAb	50	5
3			REGN6580		5
4			REGN6581		5

[0225] Systolic pressure, diastolic pressure, pulse pressure, mean arterial pressure and heart rate were collected for 10 seconds every 10 minutes for the duration of the testing period. Data were binned and assessed accordingly for acute (hourly bins) and chronic (24-hr bins) reversal of R5381-induced blood pressure lowering. Day 21/22 cyclic guanosine monophosphate (cGMP) concentrations in urine were assessed by ELISA. Gross absolute and relative heart weights were collected at necropsy. All data are presented as mean±SEM.

Results

[0226] The *in vivo* screen of bivalent anti-R5381 antibodies REGN6580 and REGN6581 demonstrated rapid and persistent reversal of the blood pressure lowering effects of R5381 (Figure 3). Both REGN6580 and REGN6581, when administered intravenously 3 days after initial dosing of R5381, were able to increase pressures

back to baseline levels. The initial drop in pressure of 6-8 mmHg (Table 19, below) compared to time-matched controls was reversed within 3 days following administration of either REGN6580 or REGN6581 (Figure 3).

Table 19: Day 1-2 - Mean Blood Pressures and Heart Rates Prior to Administration of Rescue Agent

Group	Test Article:	Systolic (mmHg)	Diastolic (mmHg)	Pulse Pressure (mmHg)	Mean Arterial (mmHg)	Heart Rate (BPM)
1	PBS	129±1	96±1	33±0	114±1	600±7
2	R5381	123±0**	97±1	26±0***	111±1	629±3*
3		121±0***	92±0*	28±0***	107±0**	629±1*
4		121±1***	94±1	27±0***	108±1**	593±8

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS as described in Table 18. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Dunnett's; *p<0.05 vs. PBS; **p<0.01 vs. PBS; ***p<0.001 vs. PBS.*

[0227] The durability of reversal was maintained for the 22-day duration of the study, with statistically significant differences in all hemodynamic parameters for animals dosed with REGN6580 or REGN6581 when compared to animals administered R5381 and isotype control mAb (Table 20, below).

Table 20: Day 4-20 Mean Blood Pressures and Heart Rates Following Administration of Rescue Agent

Group	Test Article	Rescue Article	Systolic (mmHg)	Diastolic (mmHg)	Pulse Pressure (mmHg)	Mean Arterial (mmHg)	Heart Rate (BPM)
1	PBS	PBS	132±0****	97±0****	35±0****	115±0****	567±4**
2	R5381	isotype control mAb	122±0	93±0	29±0	108±0	582±4
3		REGN6580	128±0****	95±1*	33±1****	112±0****	582±3
4		REGN6581	129±0****	97±0****	33±0****	114±0****	562±2***

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS on study day 0 followed by either isotype control mAb or anti-R5381 on study day 3 as described in Table 18. All values are mean ± SEM, n=4-5 per group. Statistics – one way ANOVA with Dunnett's; *p<0.05 vs. Isotype control; **p<0.01 vs. Isotype Control; ***p<0.001 vs. Isotype Control; ****p<0.0001 vs. Isotype Control.*

[0228] When compared to PBS control animals, no statistically significant differences were noted (Table 20, Figures 3-6) following administration of either REGN6580 or REGN6581, indicating full and persistent reversal of R5381-induced blood pressure lowering effects.

[0229] Both bivalent anti-R5381 antibodies REGN6580 and REGN6581 attenuated the reduction in relative heart weight as indicated by no statistically significant difference in heart weight to tibia weight in either reversal agent-dosed group compared to PBS-dosed animals (Table 21, below).

Table 21: Gross and Relative Heart Weight Following Administration of Reversal Agent

Group	Heart Weight (mg)	Brain Weight (mg)	Tibia Length (mm)	Heart Weight : Brain Weight	Heart Weight : Tibia Length
PBS + PBS	148±4	493±11	17.6±0.2	300±10	8.4±0.1
R5381 + isotype control mAb	133±4	473±20	18.2±0.2	280±9	7.3±0.2**
R5381 + REGN6580	137±2	463±5	17.4±0.2	296±5	7.9±0.1
R5381 + REGN6581	138±4	458±12	17.8±00.2	302±12	7.8±0.2

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS on study day 0 followed by either isotype control mAb or anti-R5381 on study day 3 as described in Table 18. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Tukey's; **p<0.01 vs. PBS.*

[0230] No effect on urine volume was noted with any of the test or rescue articles that were delivered (Table 22, below).

Table 22: Day 22 Urine Volumes and Urinary cGMP Levels

Group	Test Article:	Reversal Agent	Urine Volume (mL/day)g)	Urinary cGMP (pmol/mL)	Urinary cGMP (pmol/day)
1	PBS	PBS	2.1±0.1*	6568±1143	13474±2678
2	R5381 (5 mg/kg)	Isotype Control (50 mg/kg)	1.0±0.2	6855±937	6809±457
3		REGN6580 (50 mg/kg)	1.4±0.2	6389±1824	9009±3548
4		REGN6581 (50 mg/kg)	1.4±0.3	4715±907	7341±2549

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into four groups of equal body weight and given a single subcutaneous injection of R5381 followed by a dose of a reversal agent at the doses listed in Table 18. Urine was collected overnight beginning on study day 21 and ending on study day 22. All values are mean ± SEM, n=3-5 per group. Statistics – one-way ANOVA with Dunnett's; *p<0.05 vs. Group 2 R5381 + Isotype Control).*

[0231] cGMP production was not affected by administration of the reversal agents when assessed in the urine at day 22 (Table 22).

[0232] Both bivalent anti-R5381 antibodies REGN6580 and REGN6581 rapidly and persistently reversed the blood pressure-lowering effects of R5381 through study day 21 following the single intravenous injection on study day 3 to normotensive NPR1$^{hu/hu}$ mice that had received a single dose of R5381.

Example 12. Evaluation of reversal of R5381-induced blood pressure lowering using a single 50 mg/kg subcutaneous dose of bivalent anti-R5381 mAbs in normotensive NPR1$^{hu/hu}$ mice

[0233] In order to assess the effects of bivalent anti-R5381 antibodies at reversing the blood pressure lowering induced by R5381 in telemetered normotensive NPR1$^{hu/hu}$ mice, male NPR1$^{hu/hu}$ (n=20) mice aged ~10-12 weeks were implanted with PA-C10 telemeters (DSI, St. Paul, MN) and allowed to recover for at least 7 days. Animals were stratified into groups (Groups 1-4) based on pre-study systolic blood pressures and body weight. Animals were individually housed under standard conditions (Temperatures of 64°F to 84°F (18°C to 29°C); relative humidity of 30% to 70%) and a 12-hour light/12-hour dark cycle was maintained. Food (Research Diets Standard pellet chow) and water were provided ad libitum.

[0234] The test proteins were administered to the appropriate animals by single subcutaneous injection on Day 0. The rescue agents were administered to the appropriate animals by single subcutaneous injection on Day 3. The dose volume for each animal was based on the most recent body weight measurement.

Table 23: Summary of Doses and Dose Groups

Group No.	Test Article	Dose (mg/kg s.c.)	Rescue Article	Dose (mg/kg s.c.)	Number of Animals
					Males
1	PBS	0	PBS	0	5
2	REGN5381	5	IgG4P isotype control mAb	50	5
3			REGN6580		5
4			REGN6581		5

[0235] Systolic pressure, diastolic pressure, pulse pressure, mean arterial pressure and heart rate were collected for 10 seconds every 10 minutes for the duration of the testing period. Data were binned and assessed accordingly for acute (hourly bins) and chronic (24-hr bins) reversal of R5381-induced blood pressure lowering. Day 21/22 cyclic guanosine monophosphate (cGMP) concentrations in urine were assessed by ELISA. All data are presented as mean±SEM.

Results

[0236] The *in vivo* screen of bivalent anti-R5381 antibodies demonstrated persistent reversal of the blood pressure lowering effects of R5381 (Figure 7 and Table 24, below).

Table 24: Day 1-2 - Mean Blood Pressures and Heart Rates Prior to Administration of Rescue Agent

Group	Test Article:	Systolic (mmHg)	Diastolic (mmHg)	Pulse Pressure (mmHg)	Mean Arterial (mmHg)	Heart Rate (BPM)
1	PBS	126±1	91±0	34±1	109±1	522±5
2	R5381	118±0**	90±0	28±0**	104±0	538±7
3		118±1**	91±1	27±1***	105±1	539±10
4		119±1*	92±2	28±0**	106±1	521±11

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS as described in Table 23. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Dunnett's; *p<0.05 vs. PBS; **p<0.01 vs. PBS; ***p<0.001 vs. PBS.*

[0237] Both REGN6580 and REGN6581, when administered subcutaneously 3 days after initial dosing of R5381, were able to increase pressures back to baseline levels (Figure 7 and Table 25, below).

Table 25: Day 4-20 Mean Blood Pressures and Heart Rates Following Administration of Rescue Agent

Group	Test Article:	Rescue Article	Systolic (mmHg)	Diastolic (mmHg)	Pulse Pressure (mmHg)	Mean Arterial (mmHg)	Heart Rate (BPM)
1	PBS	PBS	130±1****	95±0****	35±0****	113±1****	523±4
2	R5381	isotype control mAb	116±0	88±0	28±0	102±0	524±3
3		REGN6580	126±1****	92±0****	34±0****	109±1****	501±3****
4		REGN6581	127±1****	93±1****	34±0****	111±1****	494±4****

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or isotype control mAb as described in Table 23. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Dunnett's; ****p<0.0001 vs. Isotype Control.*

[0238] The durability of reversal was maintained for the 22-day duration of the study, with statistically significant differences in all hemodynamic parameters for animals dosed with REGN6580 or REGN6581 when compared to animals administered R5381 and isotype control mAb (Figure 7 and Table 25). When compared to PBS control

animals, no statistically significant differences were noted (Table 25, Figure 7) following administration of either REGN6580 or REGN6581, indicating full and persistent reversal of R5381-induced blood pressure-lowering effects.

[0239] Both REGN6580 and REGN6581 demonstrated attenuation of NPR1 signaling as indicated by the statistically significant reduction of cGMP levels in the urine following subcutaneous administration of either REGN6580 or REGN6581 22 days later (Table 26, below).

Table 26: Day 22 Urine Volumes and Urinary cGMP Levels

Group	Test Article:	Reversal Agent	Urine Volume (mL/day)g)	Urinary cGMP (pmol/mL)	Urinary cGMP (pmol/day)
1	PBS	PBS	1.3±0.2	2699±223*	3844±969*
2	R5381 (5 mg/kg)	Isotype Control (50 mg/kg)	1.7±0.2	6781±2079	11500±3795
3		REGN6580 (50 mg/kg)	1.1±0.1	2932±435*	3312±738*
4		REGN6581 (50 mg/kg)	1.9±0.5	3165±370**	6305±2367

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into four groups of equal body weight and given a single subcutaneous injection of R5381 followed by a subcutaneous dose of a reversal agent at the doses listed in Table 23. Urine was collected overnight beginning on study day 21 and ending on study day 22. All values are mean ± SEM, n=3-5 per group. Statistics – one-way ANOVA with Dunnett's; *p<0.05 vs. Group 2 R5381 + Isotype Control).*

[0240] A trending non-statistically significant reduction in absolute and relative heart weight was observed with administration of R5381 and isotype control (Table 27, below).

Table 27: Gross and Relative Heart Weight Following Administration of Reversal Agent

Group	Heart Weight (mg)	Brain Weight (mg)	Tibia Length (mm)	Heart Weight : Brain Weight	Heart Weight : Tibia Length
PBS + PBS	126±8	427±7	17.6±0.2	0.294±0.015	0.007±0.0004
R5381 + isotype control mAb	120±15	442±3	18.0±0.1	0.271±0.032	0.007±0.0009
R5381 + REGN6580	126±8	437±4	17.8±0.2	0.288±0.018	0.007±0.0004
R5381 + REGN6581	126±6	452±7	17.9±0.2	0.280±0.012	0.007±0.0004

Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or isotype control as

described in Table 23. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Dunnett's.

[0241] This change is likely attributed to the hemodynamic effects of R5381. Although not significant, animals that received R5381 followed by either REGN6580 or REGN6581 had absolute and relative heart weights closer to control animals, aligned with mitigation of R5381-induced hemodynamic effects.

[0242] Following a single subcutaneous injection on study day 3, both bivalent anti-R5381 antibodies, REGN6580 and REGN6581, rapidly and persistently reversed the blood pressure-lowering effects of R5381 as assessed in telemetered normotensive $NPR1^{hu/hu}$ mice. Both agents also functioned to inhibit NPR1-induced cGMP production through study day 22.

Example 13. Evaluation of reversal of R5381-induced blood pressure lowering using a single 50 mg/kg dose of monovalent anti-R5381 mAbs in normotensive $NPR1^{hu/hu}$ mice

[0243] In an effort to assess the effects of monovalent anti-R5381 antibodies at reversing the blood pressure lowering induced by R5381 in telemetered normotensive $NPR1^{hu/hu}$ mice, male $NPR1^{hu/hu}$ (n=48) mice aged ~13-14 weeks were implanted with PA-C10 telemeters (DSI, St. Paul, MN) and allowed to recover for at least 7 days. Animals were stratified into groups (Table 28, below) (Groups 1-8) based on pre-study systolic blood pressures and body weight (Table 29, below).

Table 28: Summary of Doses and Dose Groups

Group No.	Test Article	Dose (mg/kg s.c.)	Rescue Article	Rescue Dose (mg/kg)	Rescue Dose Route	Number of Animals
						Males
1	PBS	0	PBS	0	i.v.	6
2	REGN5381	5	IgG4P isotype control mAb	50	s.c.	6
3			REGN6580			6
4			REGN9035			6
5			REGN9037		i.v.	6
6			REGN6580			6
7			REGN9035			6
8			REGN9037			6

Table 29: Day 1-2 - Mean Blood Pressures and Heart Rates Prior to Administration of Rescue Agent

Group	Test Article:	Systolic (mmHg)	Diastolic (mmHg)	Pulse Pressure (mmHg)	Mean Arterial (mmHg)	Heart Rate (BPM)
1	PBS	123±0	89±0	34±0	107±0	545±5
2	R5381	120±0***	90±0	30±0***	106±0*	582±2****
3		120±0***	91±1	29±0***	106±0	556±3*
4		116±0****	88±0	28±0****	103±0****	574±0***
5		117±0****	89±0	28±0****	104±0***	565±2**
6		117±0****	88±0	28±1****	104±0***	565±1**
7		116±0****	89±1	28±0****	104±0***	555±1
8		118±0****	88±0	29±1***	104±0***	543±2

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS as described in Table 28. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Dunnett's; *p<0.05 vs. PBS; **p<0.01 vs. PBS; ***p<0.001 vs. PBS; ****p<0.0001 vs. PBS.*

[0244] Animals were individually housed under standard conditions (Temperatures of 64°F to 84°F (18°C to 29°C); relative humidity of 30% to 70%) and a 12-hour light/12-hour dark cycle was maintained. Food (Research Diets Standard pellet chow) and water were provided ad libitum.

[0245] Test proteins were administered to the appropriate animals by single subcutaneous injection on Day 0. The rescue agents were administered to the appropriate animals by single subcutaneous or intravenous injection on Day 3. The dose volume for each animal was based on the most recent body weight measurement. Overnight collection of urine was performed on study days 20 and 21.

[0246] Systolic pressure, diastolic pressure, pulse pressure, mean arterial pressure and heart rate were collected for 10 seconds every 10 minutes for the duration of the testing period. Data were binned and assessed accordingly for acute (hourly bins) and chronic (24-hr bins) reversal of R5381-induced blood pressure lowering. Day 21/22 cyclic guanosine monophosphate (cGMP) concentrations in urine were assessed by ELISA. All data are presented as mean±SEM.

Results

[0247] The *in vivo* screen of monovalent anti-R5381 antibodies demonstrated rapid and persistent reversal of the blood pressure-lowering effects of R5381 (Table 30, below; Figure 8 and Figure 9).

Table 30: Day 4-20 Mean Blood Pressures and Heart Rates Following Administration of Rescue Agent

Group	Test Article:	Rescue Article	Rescue Dose Route	Systolic (mmHg)	Diastolic (mmHg)	Pulse Pressure (mmHg)	Mean Arterial (mmHg)	Heart Rate (BPM)
1	PBS	PBS	i.v.	125±0****	90±0**	34±0****	109±0****	534±2****
2	R5381	isotype control mAb	s.c.	119±0	88±0	31±0	105±0	550±3
3		REGN6580		130±0****	94±0****	36±0****	113±0****	522±2****
4		REGN9035		125±0****	91±0****	34±0****	109±0****	550±2
5		REGN9037		126±0****	92±0****	35±0****	110±0****	531±2****
6		REGN6580	i.v.	129±1****	94±1****	34±0****	113±1****	524±2****
7		REGN9035		126±0****	92±0****	34±0****	110±0****	528±2****
8		REGN9037		129±1****	92±1****	37±0****	111±1****	514±2****

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS as described in Table 28. All values are mean ± SEM, n=4-5 per group. Statistics – one way ANOVA with Dunnett's; *p<0.05 vs. Isotype Control; **p<0.01 vs. Isotype Control; ***p<0.001 vs. Isotype Control; ****p<0.0001 vs. Isotype Control.*

[0248] Both REGN9035 and REGN9037, when administered intravenously or subcutaneously 3 days after initial dosing of R5381, were able to increase pressures back to baseline levels (Table 30; Figure 8 and Figure 9). The initial drop in absolute pressure of 3-6 mmHg (Table 29) and relative pressure of ~10 mmHg (Figure 8) compared to time-matched controls was reversed within hours following intravenous dosing of REGN9035, REGN9037 or REGN6580 (Figure 9). Subcutaneous delivery of REGN9035, REGN9037 or REGN6580 achieved full reversal within 24 hours following reversal agent administration (Figure 9). The durability of reversal was maintained for the 20-day duration of the study, with statistically significant differences in all hemodynamic parameters for animals dosed with REGN9035 or REGN9037 when compared to animals administered R5381 and isotype control mAb (Table 30, Figure 8). When compared to PBS control animals, no statistically significant difference was noted (Table 30, Figure 8) following administration of either REGN9035, REGN9037, or REGN6580 indicating full and persistent reversal of R5381-induced blood pressure lowering effects. The reduced pressures induced by R5381 resulted in smaller heart weights (Table 31, below), likely the result of reduced left ventricular afterload induced by NPR1 agonism.

Table 31: Absolute and Relative Heart Weights

Group	Test Article:	Rescue Article	Rescue Dose Route	Heart Weight (mg)	Brain Weight (mg)	Tibia Length (mm)	Heart Weight : Brain Weight	Heart Weight : Tibia Length
1	PBS	PBS	i.v.	126±0	482±0	17.4±0	0.26±0	7.2±0
2	R5381	isotype control mAb	s.c.	112±0	481±0	17.8±0	0.23±0	6.3±0
3		REGN6580		128±0	476±0	17.6±0	0.27±0*	7.3±0*
4		REGN9035		126±0	463±0	17.6±0	0.27±0*	7.1±0
5		REGN9037		133±0*	468±0	17.8±0	0.28±0**	7.5±0**
6		REGN6580	i.v.	120±0	472±1	17.7±0	0.25±1	6.8±0
7		REGN9035		125±0	476±0	17.8±0	0.26±0	7.0±0
8		REGN9037		125±0	463±1	17.4±0	0.27±1*	7.2±0

*Telemetered normotensive NPR1$^{hu/hu}$ mice were randomized into groups based on systolic blood pressures and body weight. Animals were given a single 5 mg/kg subcutaneous injection of NPR1 agonist mAb R5381 or PBS as described in Table 28. All values are mean ± SEM, n=4-5 per group. Statistics – one-way ANOVA with Dunnett's; *p<0.05 vs. Isotype Control; **p<0.01 vs. vs. Isotype Control.*

[0249] The myocardial structural changes were ameliorated (Table 31) following administration of any of the reversal agents, REGN9035, REGN9037 or REGN6580. Finally, all anti-R5381 mAbs demonstrated attenuation of NPR1 signaling as indicated by the statistically significant reduction of cGMP with subcutaneous or intravenous administration 22 days later (Figure 10).

[0250] Both bivalent and monovalent anti-R5381 antibodies REGN6580, and REGN9035 and REGN9037, respectively, rapidly and persistently reversed the blood pressure-lowering effects of R5381 and inhibited NPR1-induced cGMP production through study day 21 following the single subcutaneous or intravenous injection on study day 3 to normotensive NPR1$^{hu/hu}$ mice that had received a single dose of R5381.

Example 14. Midodrine rescue following a single intravenous dose of R5381 in telemetered Cynomolgus monkeys

[0251] An *in vivo* study was performed to evaluate the alpha-adrenergic receptor agonist, midodrine, as an effective agent to transiently reverse the blood pressure lowering effects of R5381 in cynomolgus monkeys. Animals received a single IV bolus of 25 mg/kg R5381 and 3 days later were administered 3 doses of 2.5 mg/kg midodrine by oral gavage with 3 to 4 hours between each dose. Animals were monitored for 4 days post R5381 dosing, including 1 day post midodrine dosing, to assess for hemodynamic changes; pre-dose measurements served as the baseline for each animal. Midodrine transiently reversed the R5381-induced reductions in mean

systolic blood pressure to time-matched control levels. Midodrine also transiently reversed the R5381-induced elevations in mean heart rate and resulted in a mean reduction from baseline heart rate in R5381-dosed animals.

[0252] Specifically, the evaluation of the utility of administration of a vasopressor as an agent to transiently reverse the blood pressure effects of R5381 in normotensive telemetered male cynomolgus monkeys is particularly relevant to clinical settings in which a patient on R5381 may need to have their blood pressure increased (*e.g.*, shock-induced hypotension). Prior to dose administration, each animal was surgically implanted with a radio telemetry transmitter. On Day 0, animals each received a single IV bolus of saline (PBS; n=10) or 25 mg/kg R5381 (n=13). On Day 3, animals each received three 2.5 mg/kg doses of the alpha-adrenergic receptor agonist, midodrine, (n=6 for saline group; n=7 for R5381 group) or water/vehicle (n=4 for saline group; n=6 for R5381 group) administered by oral gavage, with each dose spaced 3 to 4 hours apart. Animals were monitored for 48 hours to assess for cardiovascular hemodynamic changes. Blood pressure and heart rate measurements were collected for each animal from Day -3 pre-R5381-dose through Day 4 post-R5381-dose. Pre-dose measurements served as the baseline for each animal.

Results

[0253] Midodrine Reversed the Blood Pressure and Heart Rate Effects of R5381 in Cynomolgus Monkeys. Three doses of midodrine transiently reversed the R5381-induced reductions in mean systolic blood pressure, with the R5381-dosed animals that received midodrine exhibiting similar mean changes from baseline systolic blood pressure compared to those observed in saline-dosed animals that did not receive midodrine (Figure 11).

[0254] In addition, the 3 doses of midodrine reversed the R5381-induced elevations in mean heart rate. The administration of midodrine resulted in a mean reduction from baseline heart rate in R5381-dosed animals; a similar effect was observed in saline-dose animals that were administered midodrine (Figure 12).

[0255] The present disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An agent that reverses the hemodynamic effects of a natriuretic peptide receptor 1 (NPR1) agonist.

2. An agent that reverses a reduction in blood pressure associated with the administration of a natriuretic peptide receptor 1 (NPR1) agonist.

3. The agent of claim 1 or claim 2, wherein the agent is selected from the group consisting of an immunoglobulin protein, a vasopressor, an alpha-adrenoreceptor agonist, a steroid, an antidiuretic hormone, an angiogenesis inhibitor, and a small molecule agent that increases blood pressure.

4. The agent of any one of claims 1-3, wherein the agent is an immunoglobulin protein.

5. The agent of any one of claims 1-4, wherein the agent binds specifically to the NPR1 agonist.

6. The agent of any one of claims 1-5, wherein the NPR1 agonist is an antibody or antigen-binding fragment thereof that binds specifically to NPR1.

7. The agent of claim 6, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) comprising SEQ ID NO: 48; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising SEQ ID NO: 52.

8. The agent of claim 6 or 7, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) comprising SEQ ID NOs: 49, 50, and 51, respectively; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) comprising SEQ ID NO: 53, 54, and 55, respectively.

9. The agent of claim 8, wherein the antibody or antigen-binding fragment thereof comprises a HCVR of SEQ ID NO: 48 and a LCVR of SEQ ID NO: 52.

10. The agent of any one of claims 6-9, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody.

11. The agent of claim 10, wherein the antibody is an IgG1 antibody.

12. The agent of claim 10, wherein the antibody is an IgG4 antibody.

13. The agent of claim 10, wherein the antibody comprises a heavy chain comprising SEQ ID NO: 56 and a light chain comprising SEQ ID NO: 57.

14. The agent of any of claims 1-13, wherein the NPR1 agonist is R5381.

15. The agent of any one of claims 4-14, wherein the immunoglobulin protein comprises a monoclonal antibody or antigen-binding fragment thereof, a Fab fragment, F(ab)2 fragment, Fv fragment, Fd fragment, scFv, dAb, a bivalent monoclonal antibody, or a monovalent monoclonal antibody.

16. The agent of claim 15, wherein the immunoglobulin protein comprises at least one immunoglobulin variable domain comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR).

17. The agent of claim 15 or 16, wherein the immunoglobulin protein comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) and three light chain CDRs (LCDR1, LCDR2 and LCDR3) comprising amino acid sequences selected from SEQ ID NOs: 4, 6, 8, 12, 14 and 16; or SEQ ID NOs: 24, 26, 28, 32, 34, and 36.

18. The agent of claim 17, wherein the HCVR comprises an amino acid sequence having at least 90%, optionally 95%, 98%, or 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2 and 22.

19. The agent of claim 17 or 18, wherein the LCVR comprises an amino acid sequence having at least 90%, optionally 95%, 98%, or 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

20. The agent of any one of claims 17-19, wherein the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 22; and the LCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

21. The agent of any one of claims 17-20, wherein the HCVR comprises an amino acid sequence of SEQ ID NO: 2 and the LCVR comprises an amino acid sequence of SEQ ID NO: 10.

22. The agent of any one of claims 17-20, wherein the HCVR comprises an amino acid sequence of SEQ ID NO: 22 and the LCVR comprises an amino acid sequence of SEQ ID NO: 30.

23. The agent of any one of claims 17-22, wherein the immunoglobulin protein comprises a human monoclonal antibody comprising the at least one immunoglobulin variable domain.

24. The agent of claim 23, wherein the human monoclonal antibody is of IgG1 or IgG4 isotype.

25. The agent of claim 23 or 24, wherein the human monoclonal antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 38.

26. The agent of any one of claims 23-25, wherein the human monoclonal antibody comprises a heavy chain and a light chain, wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 20 and 40.

27. The agent of any one of claims 4-15, wherein the immunoglobulin protein comprises one immunoglobulin variable domain.

28. The agent of claim 27, wherein the one immunoglobulin variable domain comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR), wherein HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 24; HCDR2 comprises an amino acid

sequence selected from the group consisting of SEQ ID NOs: 6 and 26; HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 28; LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12 and 32; LCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14 and 34; and LCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 36.

29. The agent of claim 28, wherein HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprise amino acid sequences selected from the group consisting of (i) SEQ ID NOs: 4, 6, 8, 12, 14 and 16; and (ii) SEQ ID NOs: 24, 26, 28, 32, 34, and 36.

30. The agent of claim 28 or 29, wherein the HCVR comprises an amino acid sequence having at least 90%, optionally 95%, 98% or 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2 and 22.

31. The agent of any one of claims 28-30, wherein the LCVR comprises an amino acid sequence having at least 90%, optionally 95%, 98% or 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

32. The agent of any one of claims 28-31, wherein the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 22; and the LCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

33. The agent of any one of claims 28-32, wherein the HCVR comprises an amino acid sequence of SEQ ID NO: 2 and the LCVR comprises an amino acid sequence of SEQ ID NO: 10.

34. The agent of any one of claims 28-32, wherein the HCVR comprises an amino acid sequence of SEQ ID NO: 22 and the LCVR comprises an amino acid sequence of SEQ ID NO: 30.

35. The agent of any one of claims 26-34, wherein the one immunoglobulin variable domain is comprised within a Fab fragment.

36. The agent of any one of claims 26-35, further comprising a multimerizing component.

37. The agent of claim 36, wherein the multimerizing component comprises at least one Fc fragment.

38. The agent of claim 37, wherein the Fc fragment is of isotype IgG1, IgG4, or a variant thereof.

39. The agent of claim 38, wherein the Fc fragment is of IgG4 isotype.

40. The agent of claim 38, wherein the Fc fragment is of IgG1 isotype.

41. The agent of any one of claims 37-40 comprising a first Fc fragment and a second Fc fragment, wherein the first Fc fragment or the second Fc fragment, but not both Fc fragments, comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification.

42. The agent of claim 41, wherein the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in a Fc fragment.

43. The agent of any one of claims 37-42, wherein the multimerizing component comprises a Fc fragment comprising the amino acid sequence of SEQ ID NO: 46 and a Fc fragment comprising the amino acid sequence of SEQ ID NO: 58.

44. The agent of any one of claims 16-22 or 26-34, wherein the immunoglobulin variable domain is comprised in a monovalent monoclonal antibody.

45. The agent of claim 44, wherein the monovalent monoclonal antibody comprises a heavy chain comprising a heavy chain constant region and HCVR, and a light chain comprising a LCVR.

46. The agent of claim 45, wherein the heavy chain constant region comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification.

47. The agent of claim 46, wherein the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in the heavy chain constant region of IgG1 or IgG4 isotype.

48. The agent of any one of claims 45-47, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 42 and the light chain comprises an amino acid sequence of SEQ ID NO: 20.

49. The agent of any one of claims 45-47, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 44 and the light chain comprises an amino acid sequence of SEQ ID NO: 40.

50. The agent of any one of claims 44-49, wherein the immunoglobulin protein further comprises a Fc fragment.

51. The agent of claim 50, wherein the Fc fragment is of IgG1 or IgG4 isotype.

52. The agent of claim 50 or 51, wherein the Fc fragment comprises an amino acid sequence of SEQ ID NO: 46.

53. The agent of any one of claims 1-52, wherein the agent is REGN9035 or REGN9037.

54. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a heavy chain variable region (HCVR) of an immunoglobulin protein as set forth in any one of claims 15-53.

55. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a light chain variable region (LCVR) of an immunoglobulin protein as set forth in any one of claims 15-53.

56. A vector comprising the polynucleotide molecule of claim 54 and/or the polynucleotide molecule of claim 55.

57. A host cell expressing the vector of claim 56.

58. The host cell of claim 57, wherein the host cell is a CHO cell.

59. A method of producing an immunoglobulin protein, comprising growing the host cell of claim 57 under conditions permitting production of the protein, and recovering the protein so produced.

60. The method of claim 59, wherein the host cell is a CHO cell.

61. The agent of any one of claims 1-3, wherein the agent is a vasopressor.

62. The agent of claim 61, wherein the vasopressor is Midodrine.

63. A pharmaceutical composition comprising the agent of any one of claims 1-53 and a pharmaceutically acceptable carrier or diluent.

64. A method of reversing the hemodynamic effects of a NPR1 agonist, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of the agent of any one of claims 1-53 to a subject in need thereof.

65. A method of reversing hemodynamic changes associated with the administration of a NPR1 agonist, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of the agent of any one of claims 1-53 to a subject in need thereof.

66. The method of claim 64 or 65, wherein the composition is administered subcutaneously, intravenously, intradermally, intraperitoneally, intramuscularly, or orally to the subject.

67. The method of any one of claims 64-66, wherein the NPR1 agonist is an antibody or antigen-binding fragment thereof that binds specifically to NPR1.

68. The method of claim 67, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) comprising SEQ ID NO: 48; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising SEQ ID NO: 52.

69. The method of any one of claims 64-68, wherein the NPR1 agonist is R5381.

70. The method of any one of claims 64-69, wherein the subject has a disease or disorder selected from the group consisting of hypertension, heart failure, and chronic kidney disease.

71. An antibody or antigen-binding fragment thereof that competes for binding with the agent of any one of claims 15-53.

72. An antibody or antigen-binding fragment thereof that binds to the same epitope as an agent of any one of claims 15-53.

73. An immunoglobulin protein comprising:
(a) a single immunoglobulin variable domain comprising a heavy chain variable region (HCVR), wherein the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 22, and a light chain variable region (LCVR), wherein the LCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

74. An immunoglobulin protein comprising:
(a) a single immunoglobulin variable domain comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR), wherein HCDR1-HCDR2-HCDR3 are selected from the group consisting of SEQ ID NOs: 4, 6, and 8, and 24, 26, and 28, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within a light chain variable region (LCVR), wherein LCDR1-LCDR2-LCDR3 are selected from the group consisting of SEQ ID NOs: 12, 14, and 16, and 32, 34, and 36.

75. The immunoglobulin protein of claim 74, wherein:
(i) HCDR1 comprises the amino acid sequence of SEQ ID NO: 4, HCDR2 comprises the amino acid sequence of SEQ ID NO: 6, HCDR3 comprises the amino acid sequence of SEQ ID NO: 8, LCDR1 comprises the amino acid sequence of SEQ ID NO: 12, LCDR2 comprises the amino acid sequence of SEQ ID NO: 14, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 16; or
(ii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 24, HCDR2 comprises the amino acid sequence of SEQ ID NO: 26, HCDR3 comprises the amino acid sequence of SEQ ID NO: 28, LCDR1 comprises the amino acid sequence of SEQ ID

NO: 32, LCDR2 comprises the amino acid sequence of SEQ ID NO: 34, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 36.

76. The immunoglobulin protein of claim 74 or 75, wherein the HCVR comprises an amino acid sequence that has at least 90%, optionally 95%, 98%, or 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2 and 22.

77. The immunoglobulin protein of any one of claims 74-76, wherein the LCVR comprises an amino acid sequence that has at least 90%, optionally 95%, 98%, or 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

78. The immunoglobulin protein of any one of claims 74-77, wherein the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 22; and wherein the LCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 30.

79. The immunoglobulin protein of any one of claims 73-78, wherein:
(1) the HCVR comprises an amino acid sequence of SEQ ID NO: 2, and the LCVR comprises an amino acid sequence of SEQ ID NO: 10; or
(2) the HCVR comprises an amino acid sequence of SEQ ID NO: 22, and the LCVR comprises an amino acid sequence of SEQ ID NO: 30.

80. The immunoglobulin protein of any one of claims 73-79 further comprising a multimerizing component.

81. The immunoglobulin protein of claim 80, wherein the multimerizing component comprises at least one Fc fragment.

82. The immunoglobulin protein of claim 81, wherein the Fc fragment is of human IgG1 or IgG4 isotype.

83. The immunoglobulin protein of claim 81 or 82 comprising a first Fc fragment and a second Fc fragment, wherein the first Fc fragment or the second Fc fragment, but not both Fc fragments, comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification.

84. The immunoglobulin protein of claim 83, wherein the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in a Fc fragment.

85. The immunoglobulin protein of any one of claims 80-84, comprising a first Fc fragment that comprises the amino acid sequence of SEQ ID NO: 46 and a second Fc fragment that comprises the amino acid sequence of SEQ ID NO: 58.

86. The immunoglobulin protein of any one of claims 73-85 comprising a HCVR of SEQ ID NO: 2, a LCVR of SEQ ID NO: 10, and a multimerizing component, wherein the multimerizing component comprises a first Fc fragment comprising the amino acid sequence of SEQ ID NO: 46 and a second Fc fragment comprising the amino acid sequence of SEQ ID NO: 58.

87. The immunoglobulin protein of any one of claims 73-85 comprising a HCVR of SEQ ID NO: 22, a LCVR of SEQ ID NO: 30, and a multimerizing component, wherein the multimerizing component comprises a first Fc fragment comprising the amino acid sequence of SEQ ID NO: 46 and a second Fc fragment comprising the amino acid sequence of SEQ ID NO: 58.

88. The immunoglobulin protein of any one of claims 73-79, wherein the immunoglobulin protein comprises a heavy chain comprising the HCVR and a heavy chain constant region and a light chain comprising the LCVR and a light chain constant region, wherein the heavy chain is of human IgG1 or IgG4 isotype.

89. The immunoglobulin protein of claim 88, wherein the heavy chain constant region comprises a modification in the CH3 domain that reduces binding of the immunoglobulin protein to Protein A as compared to an immunoglobulin protein lacking the modification.

90. The immunoglobulin protein of claim 89, wherein the modification comprises a H315R substitution and a Y316F substitution (EU numbering) in the heavy chain constant region of IgG1 or IgG4 isotype.

91. The immunoglobulin protein of any one of claims 88-90, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 44.

92. The immunoglobulin protein of any one of claims 88-91, wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 20 and 40.

93. The immunoglobulin protein of any one of claims 88-92, wherein:
(i) the heavy chain comprises an amino acid sequence of SEQ ID NO: 42, and the light chain comprises an amino acid sequence of SEQ ID NO: 20; or
(ii) the heavy chain comprises an amino acid sequence of SEQ ID NO: 44, and the light chain comprises an amino acid sequence of SEQ ID NO: 40.

94. The immunoglobulin protein of any one of claims 88-93 further comprising a multimerizing component.

95. The immunoglobulin protein of claim 94, wherein the multimerizing component comprises a Fc fragment.

96. The immunoglobulin protein of claim 96, wherein the Fc fragment is of human IgG1 or IgG4 isotype.

97. The immunoglobulin protein of claim 95 or 96, wherein the Fc fragment comprises an amino acid sequence of SEQ ID NO: 46.

98. The immunoglobulin protein of any one of claims 88-97 comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 42, a light chain comprising the amino acid sequence of SEQ ID NO: 20, and a Fc fragment comprising the amino acid sequence of SEQ ID NO: 46.

99. The immunoglobulin protein of any one of claims 88-97 comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 44, a light chain comprising the amino acid sequence of SEQ ID NO: 40, and a Fc fragment comprising the amino acid sequence of SEQ ID NO: 46.

100. The immunoglobulin protein of any one of claims 73-99, wherein the immunoglobulin protein binds specifically to a NPR1 agonist.

101. The immunoglobulin protein of claim 100, wherein the NPR1 agonist is an antibody or antigen-binding fragment thereof that binds specifically to NPR1.

102. The immunoglobulin protein of claim 101, wherein the antibody or antigen-binding fragment thereof comprises a HCVR comprising an amino acid sequence of SEQ ID NO: 48 and a LCVR comprising an amino acid sequence of SEQ ID NO: 52.

103. The immunoglobulin protein of any one of claims 100-102, wherein the NPR1 agonist is R5381.

104. The immunoglobulin protein of any one of claims 73-103, wherein the immunoglobulin protein is REGN9035 or REGN9037.

105. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a HCVR of an immunoglobulin protein as set forth in any one of claims 73-104.

106. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a LCVR of an immunoglobulin protein as set forth in any one of claims 73-104.

107. A vector comprising the polynucleotide molecule of claim 105 and/or the polynucleotide molecule of claim 106.

108. A host cell expressing the vector of claim 107.

109. The host cell of claim 108, wherein the host cell is a CHO cell.

110. A method of producing an immunoglobulin protein, comprising growing the host cell of claim 108 under conditions permitting production of the protein, and recovering the protein so produced.

111. The method of claim 110, wherein the host cell is a CHO cell.

112. A pharmaceutical composition comprising the immunoglobulin protein of any one of claims 73-104 and a pharmaceutically acceptable carrier or diluent.

113. A method of reversing the hemodynamic effects of a NPR1 agonist, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of the immunoglobulin protein of any one of claims 73-104 to a subject in need thereof.

114. A method of reversing hemodynamic changes associated with the administration of a NPR1 agonist, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of the immunoglobulin protein of any one of claims 73-104 to a subject in need thereof.

115. The method of claim 113 or 114, wherein the composition is administered subcutaneously, intravenously, intradermally, intraperitoneally, intramuscularly, or orally to the subject.

116. The method of any one of claims 113-115, wherein the NPR1 agonist is an antibody or antigen-binding fragment thereof that binds specifically to NPR1.

117. The method of claim 116, wherein the antibody or antigen-binding fragment thereof comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 48 and a LCVR comprising the amino acid sequence of SEQ ID NO: 52.

118. The method of any one of claims 113-117, wherein the NPR1 agonist is R5381.

119. The method of any one of claims 113-118, wherein the subject has a disease or disorder selected from the group consisting of hypertension, heart failure, and chronic kidney disease.

120. An antibody or antigen-binding fragment thereof that competes for binding with an immunoglobulin protein of any one of claims 73-104.

121. An antibody or antigen-binding fragment thereof that binds to the same epitope as an immunoglobulin protein of any one of claims 73-104.

122. A composition comprising: (i) the immunoglobulin protein of any one of claims 73-104; and (ii) an NPR1 agonist.

123. The composition of claim 122, wherein the NPR1 agonist is an antibody or antigen-binding fragment thereof that binds specifically to NPR1.

124. The composition of claim 123, wherein the antibody or antigen-binding fragment thereof comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 48 and a LCVR comprising the amino acid sequence of SEQ ID NO: 52.

125. The composition of any one of claims 122-124, wherein the NPR1 agonist is R5381.

126. The composition of any one of claims 122-125 for use in a method for effective regulation of blood pressure in a subject in need thereof.

127. The composition of claim 126, wherein the subject has a NPR1-associated disease or disorder.

128. The composition of claim 127, wherein the disease or disorder is selected from the group consisting of hypertension, heart failure, and chronic kidney disease.

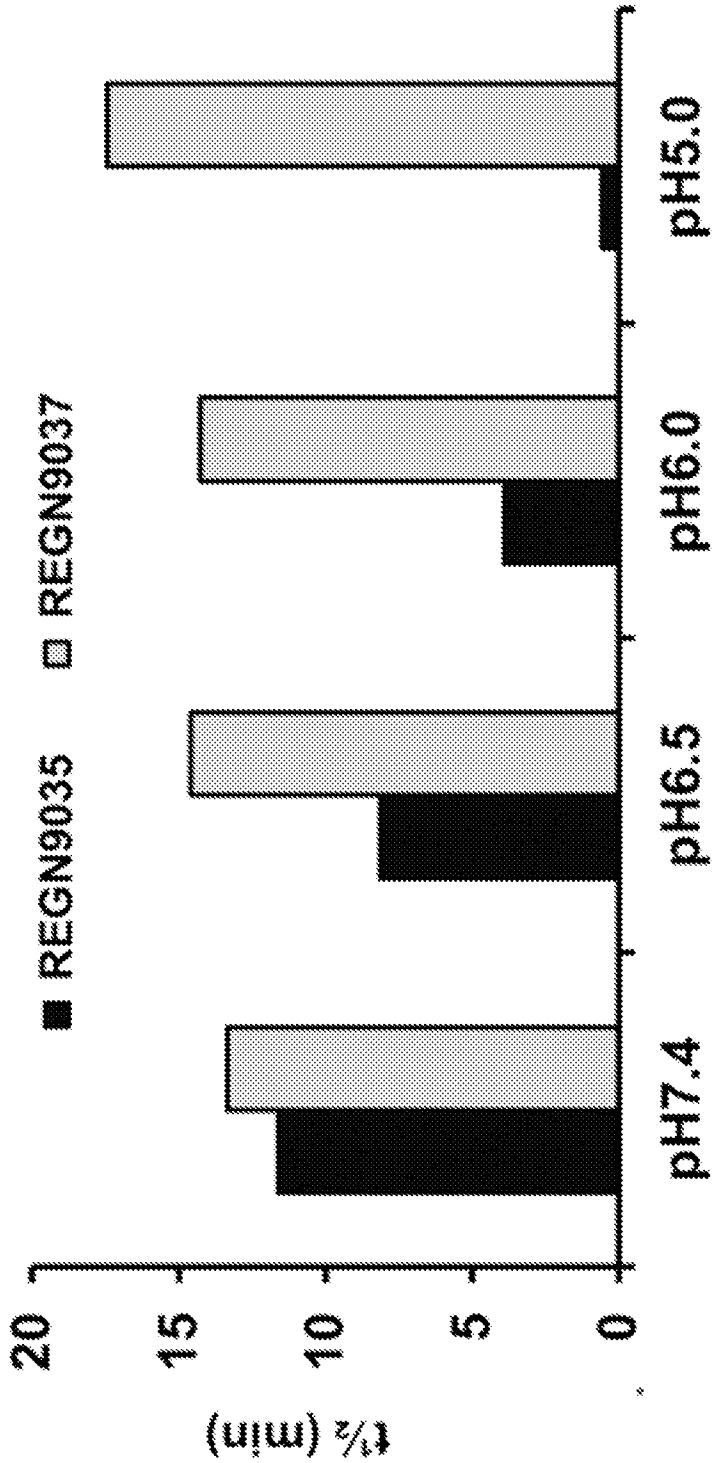

FIGURE 1

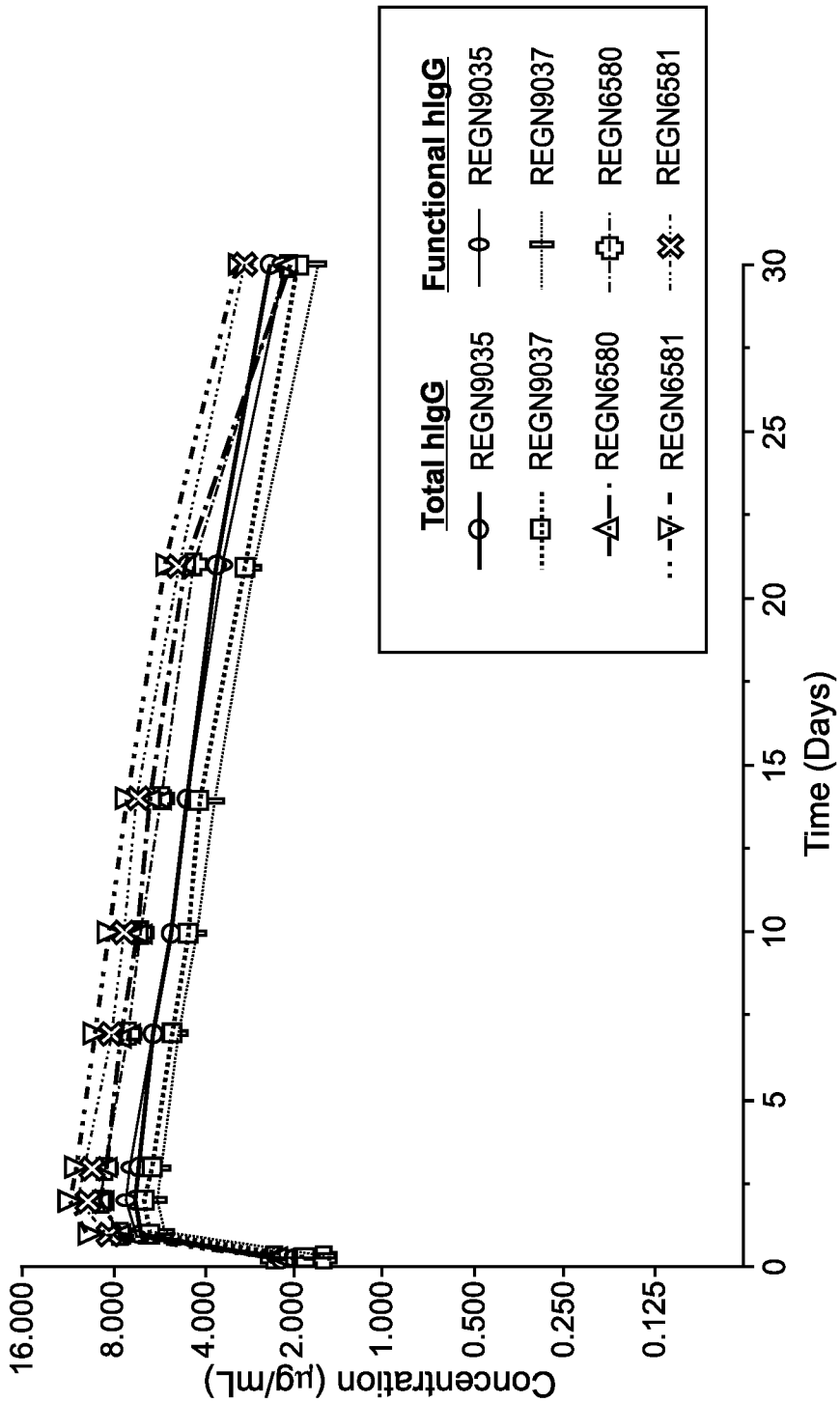
Total hIgG
REGN9035
REGN9037
REGN6580
REGN6581
Functional hIgG
REGN9035
REGN9037
REGN6580
REGN6581
Concentration (µg/mL)
16.000
8.000
4.000
2.000
1.000
0.500
0.250
0.125
0
5
10
15
20
25
30
Time (Days)

FIGURE 2

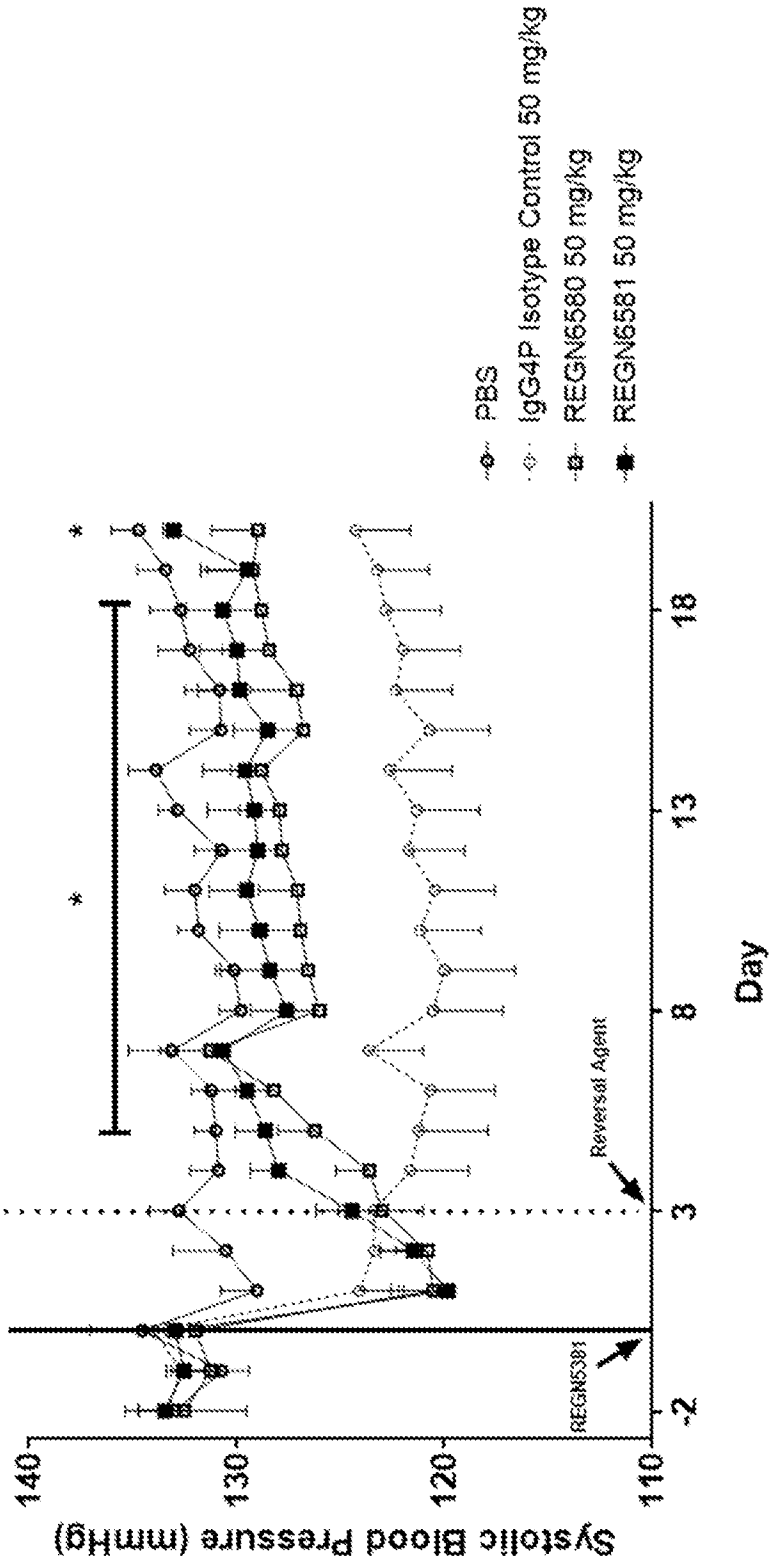
PBS
IgG4P Isotype Control 50 mg/kg
REGN6580 50 mg/kg
REGN6581 50 mg/kg
Systolic Blood Pressure (mmHg)
140
130
120
110
-2
3
8
13
18
Day
REGN5381
Reversal Agent
*
*

FIGURE 3

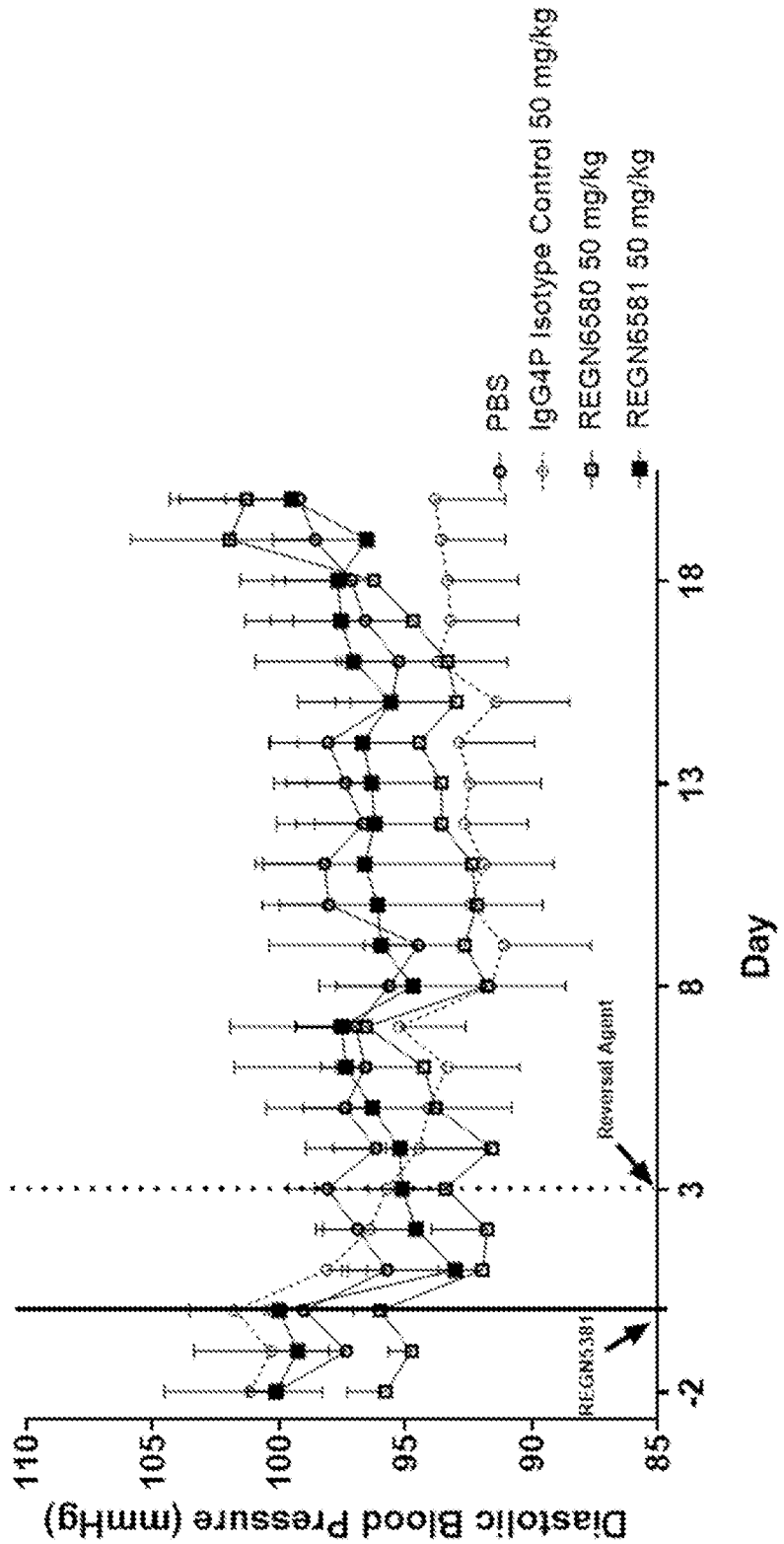
Diastolic Blood Pressure (mmHg)
110
105
100
95
90
85
-2
3
8
13
18
Day
REGN5381
Reversal Agent
PBS
IgG4P Isotype Control 50 mg/kg
REGN6580 50 mg/kg
REGN6581 50 mg/kg

FIGURE 4

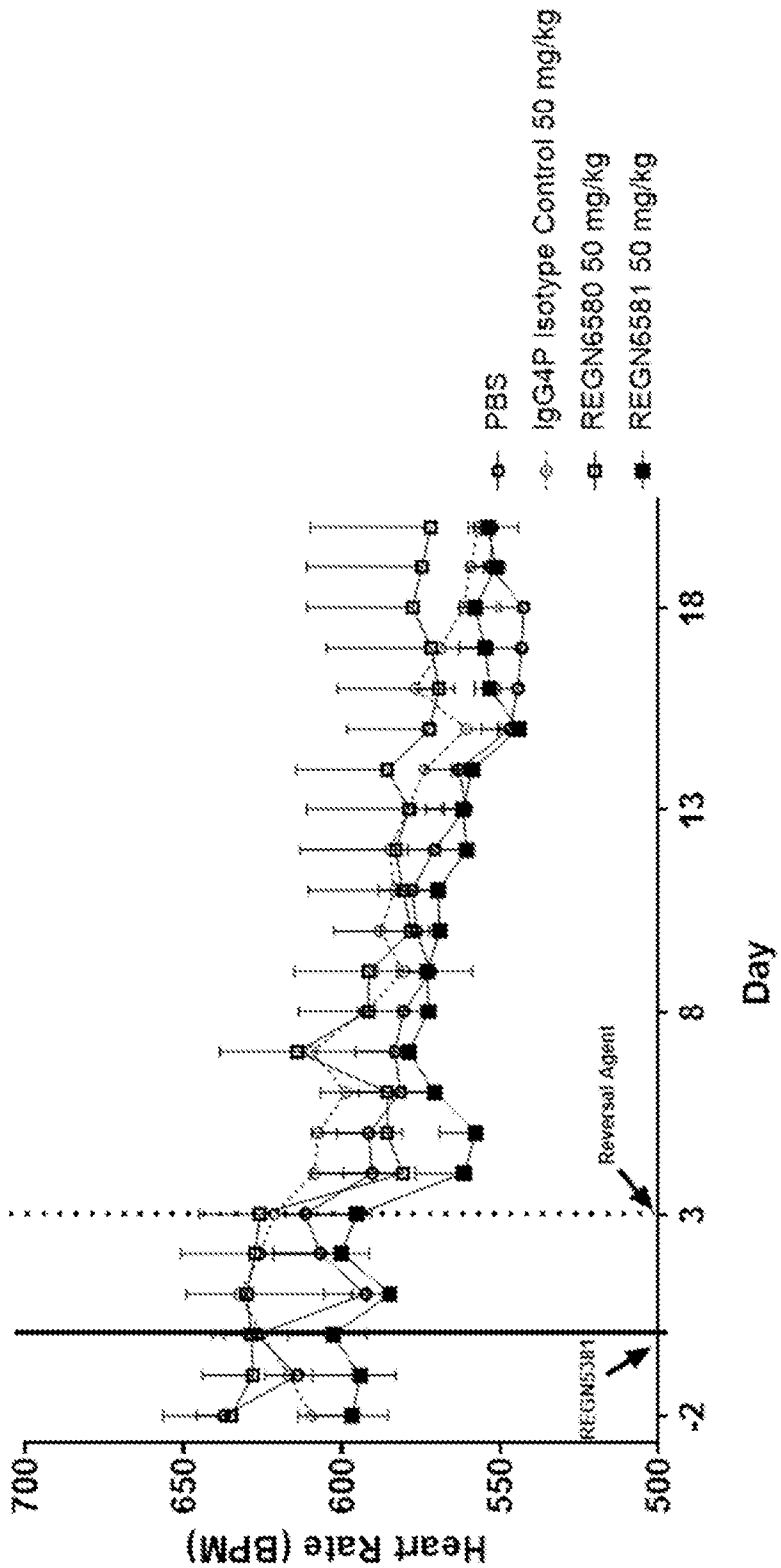
Heart Rate (BPM)
700
650
600
550
500
-2
3
8
13
18
Day
REGN5381
Reversal Agent
PBS
IgG4P Isotype Control 50 mg/kg
REGN6580 50 mg/kg
REGN6581 50 mg/kg

FIGURE 5

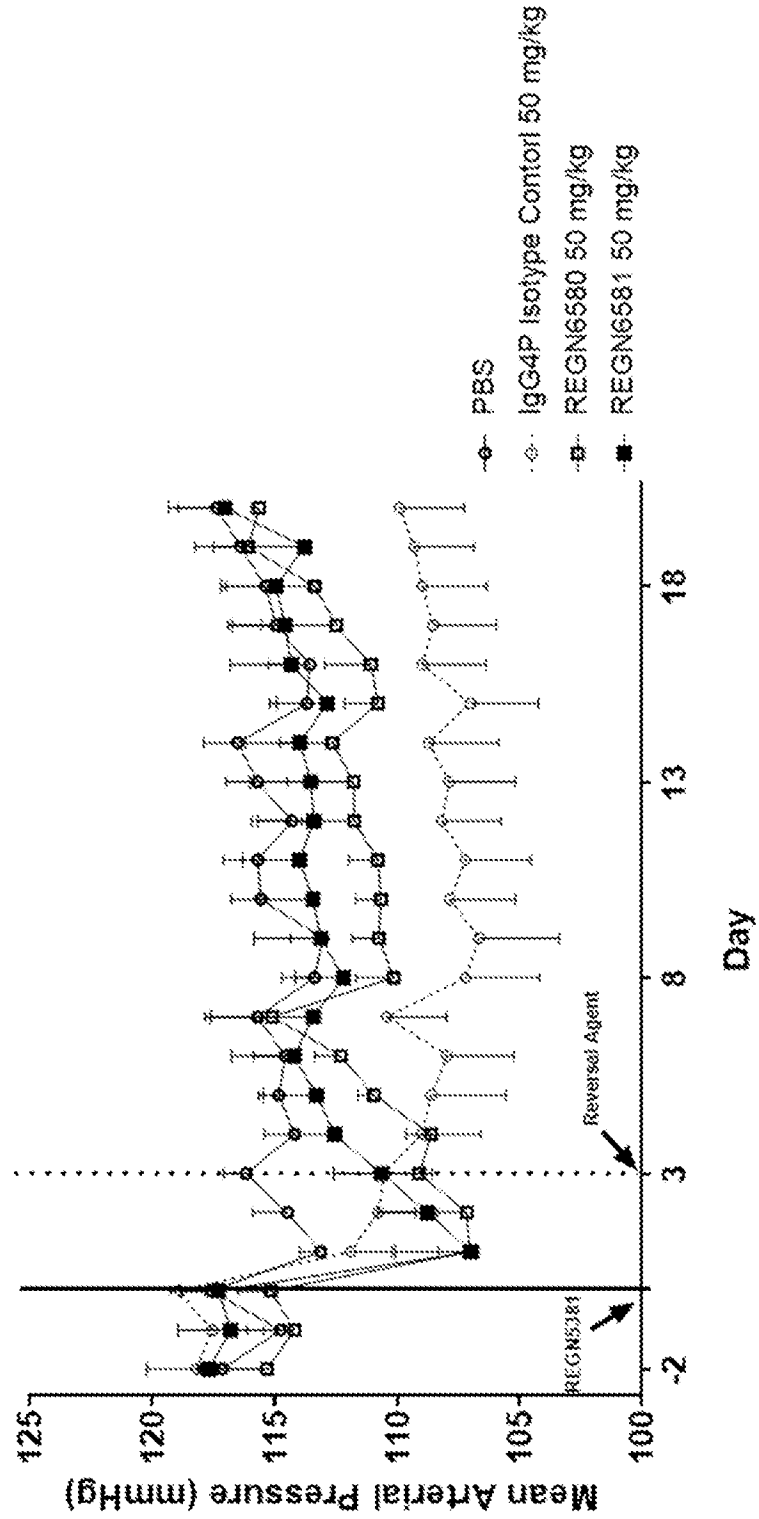
Mean Arterial Pressure (mmHg)
125
120
115
110
105
100
-2
3
8
13
18
Day
REGN6581
Reversal Agent
PBS
IgG4P Isotype Control 50 mg/kg
REGN6580 50 mg/kg
REGN6581 50 mg/kg

FIGURE 6

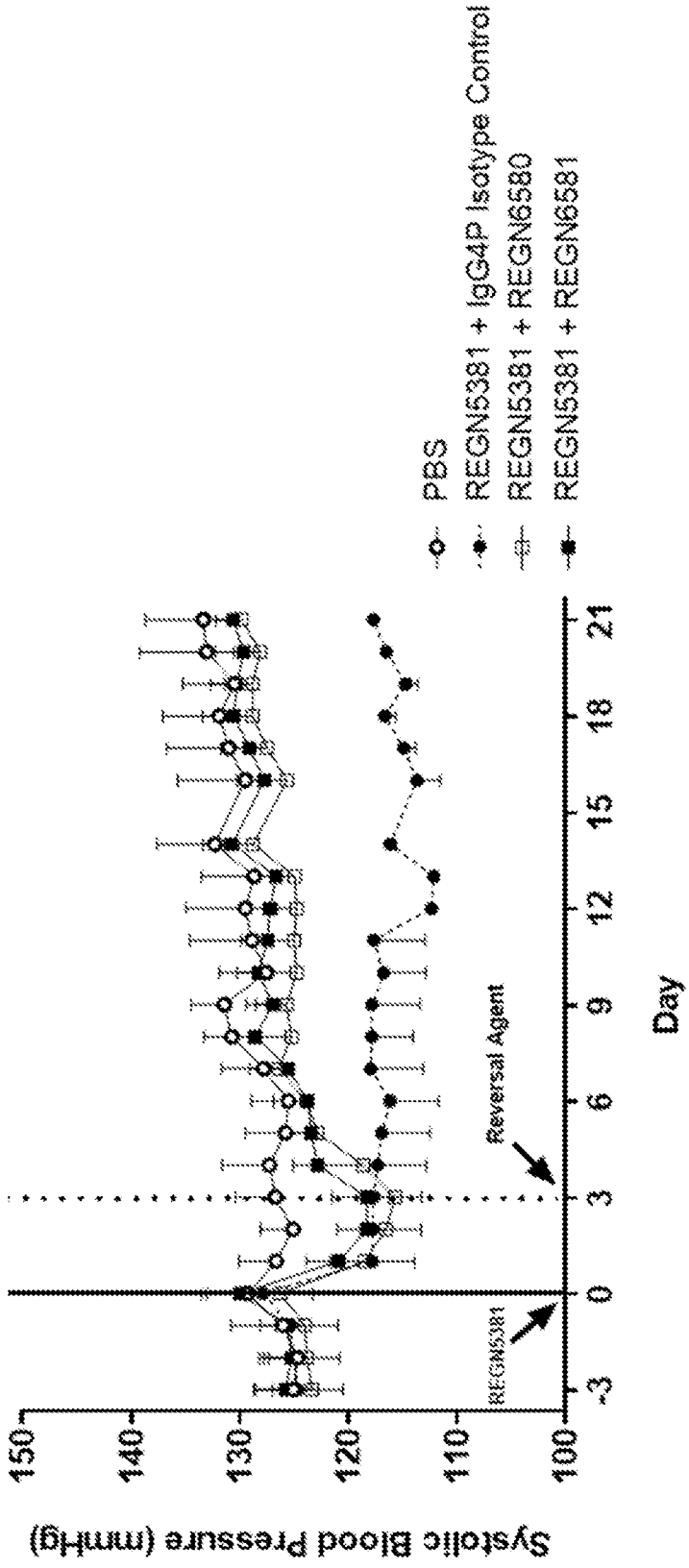
Systolic Blood Pressure (mmHg)
150
140
130
120
110
100
-3
0
3
6
9
12
15
18
21
Day
REGN5381
Reversal Agent
PBS
REGN5381 + IgG4P Isotype Control
REGN5381 + REGN6580
REGN5381 + REGN6581

FIGURE 7

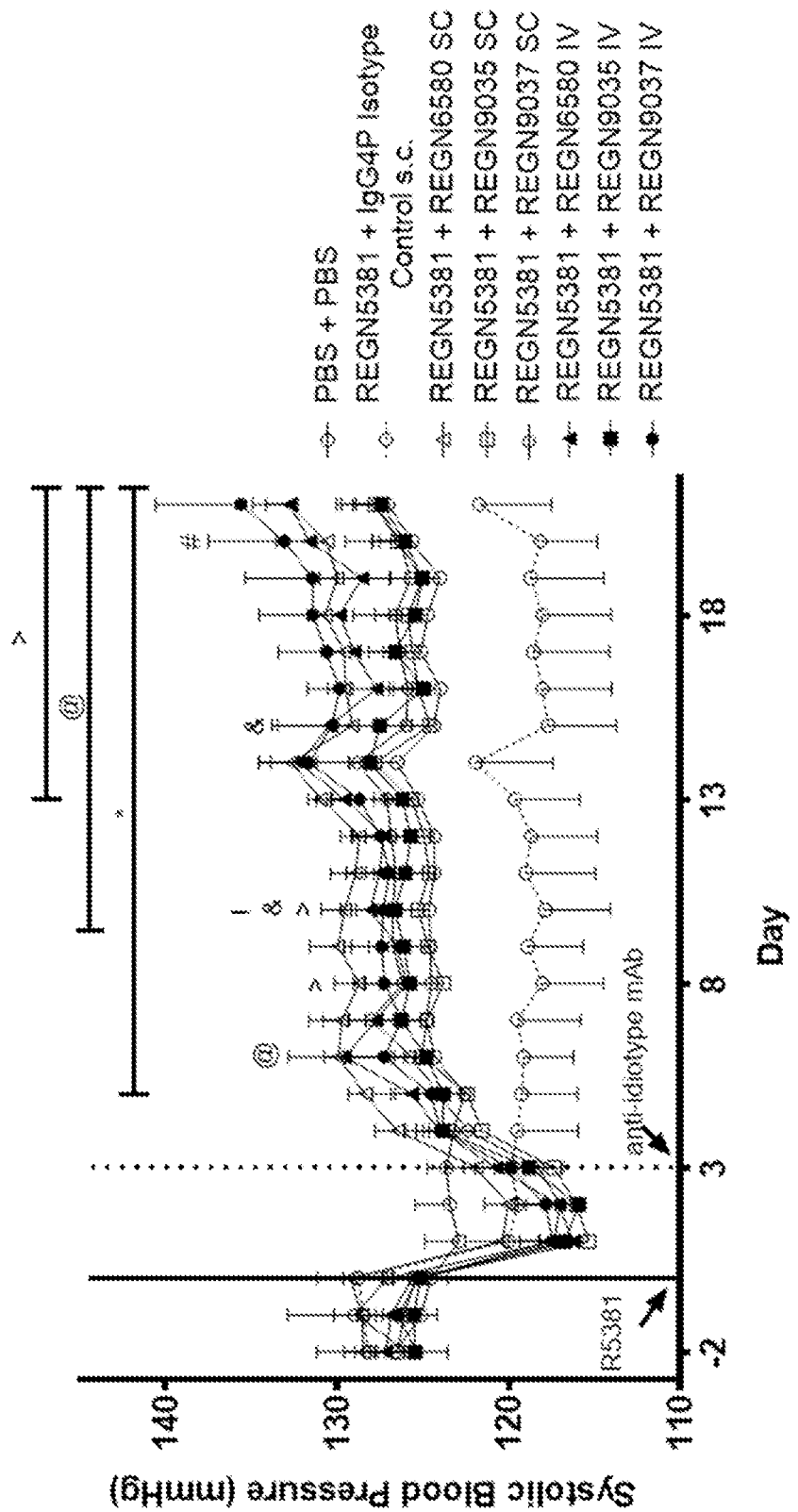
PBS + PBS
REGN5381 + IgG4P Isotype Control s.c.
REGN5381 + REGN6580 SC
REGN5381 + REGN9035 SC
REGN5381 + REGN9037 SC
REGN5381 + REGN6580 IV
REGN5381 + REGN9035 IV
REGN5381 + REGN9037 IV
Systolic Blood Pressure (mmHg)
140
130
120
110
-2
3
8
13
18
Day
R5381
anti-idiotype mAb

FIGURE 8

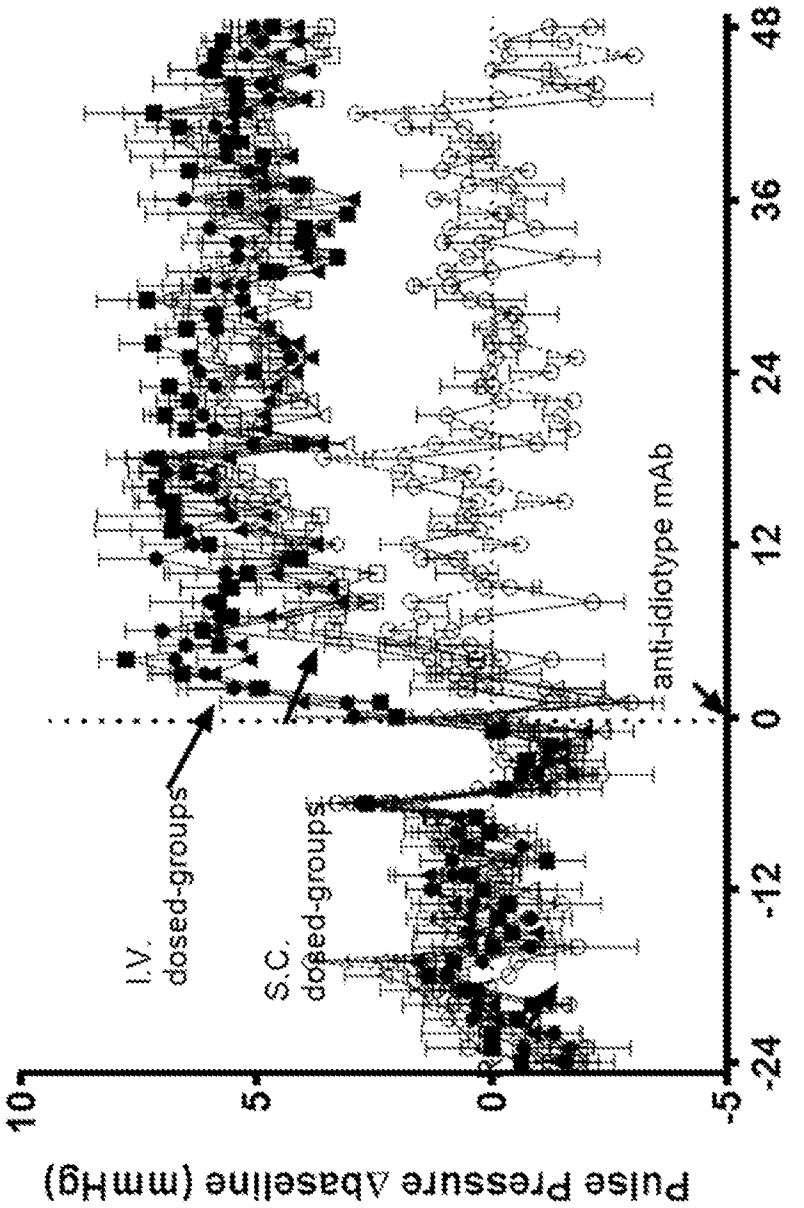
Pulse Pressure Δbaseline (mmHg)
10
5
0
-5
-24
-12
0
12
24
36
48
Hours
I.V. dosed-groups
S.C. dosed-groups
anti-idiotype mAb

FIGURE 9

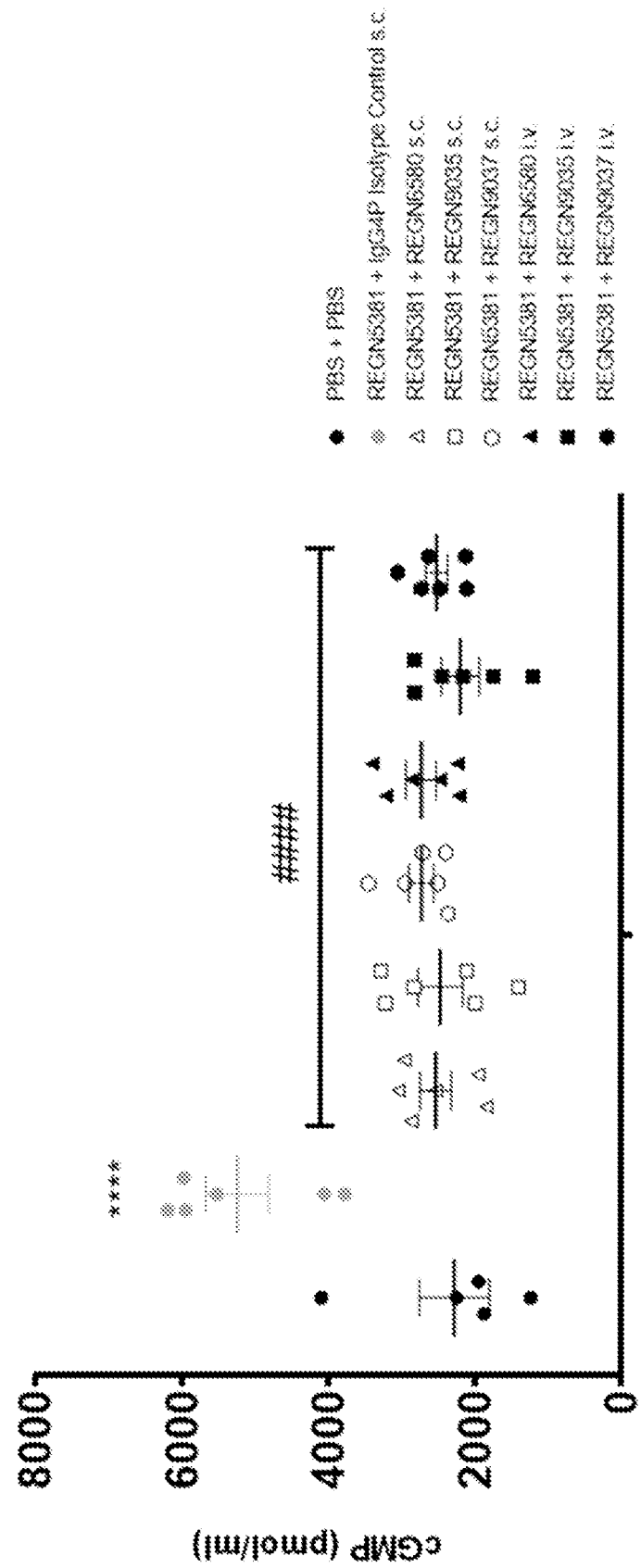
cGMP (pmol/ml)
8000
6000
4000
2000
0
Day 22
****
#####
PBS + PBS
REGN5381 + IgG4P Isotype Control s.c.
REGN5381 + REGN6380 s.c.
REGN5381 + REGN9035 s.c.
REGN5381 + REGN9037 s.c.
REGN5381 + REGN6380 i.v.
REGN5381 + REGN9035 i.v.
REGN5381 + REGN9037 i.v.

FIGURE 10

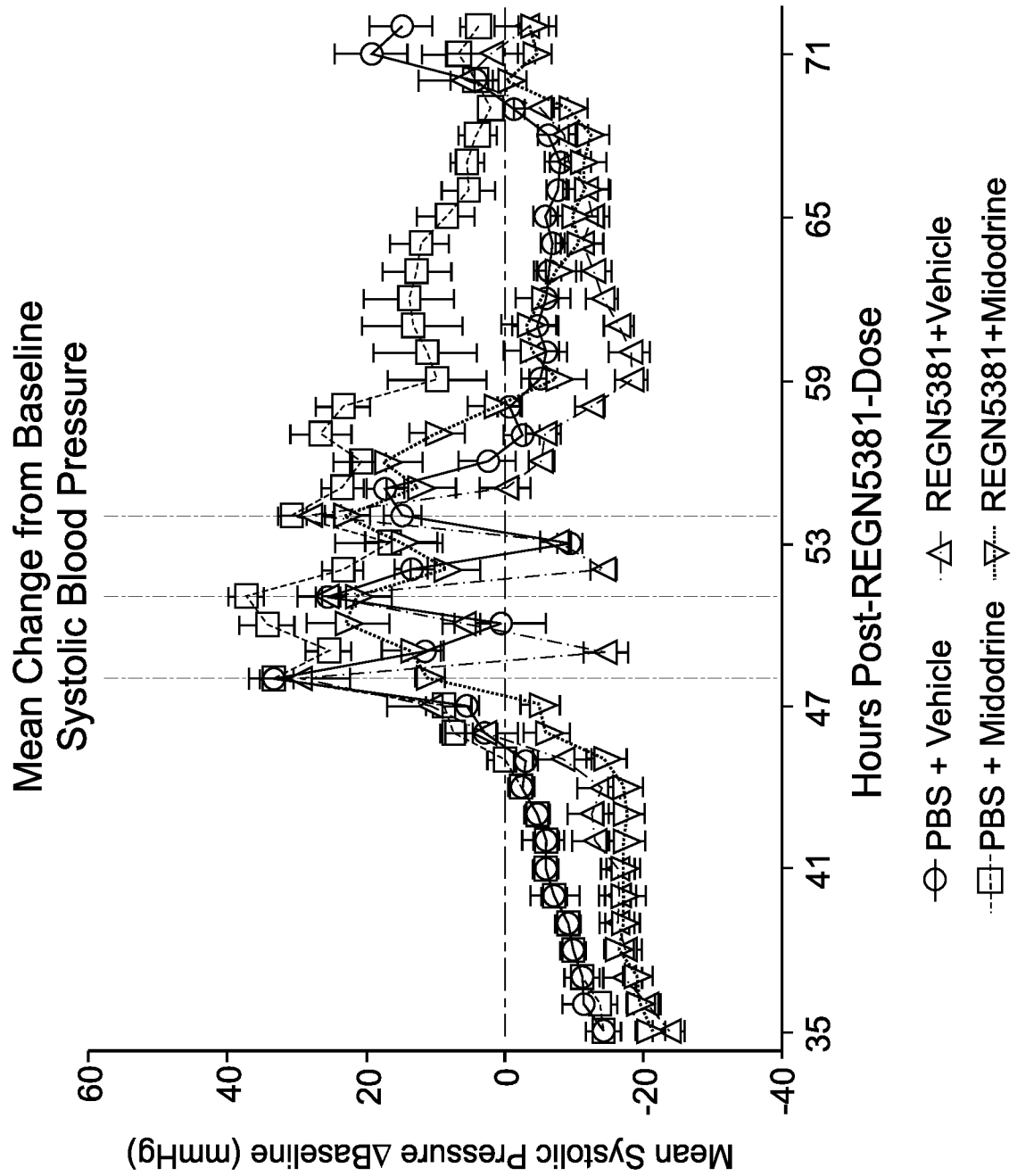
Mean Change from Baseline
Systolic Blood Pressure
Mean Systolic Pressure ΔBaseline (mmHg)
60
40
20
0
-20
-40
35
41
47
53
59
65
71
Hours Post-REGN5381-Dose
PBS + Vehicle
REGN5381+Vehicle
PBS + Midodrine
REGN5381+Midodrine

FIGURE 11

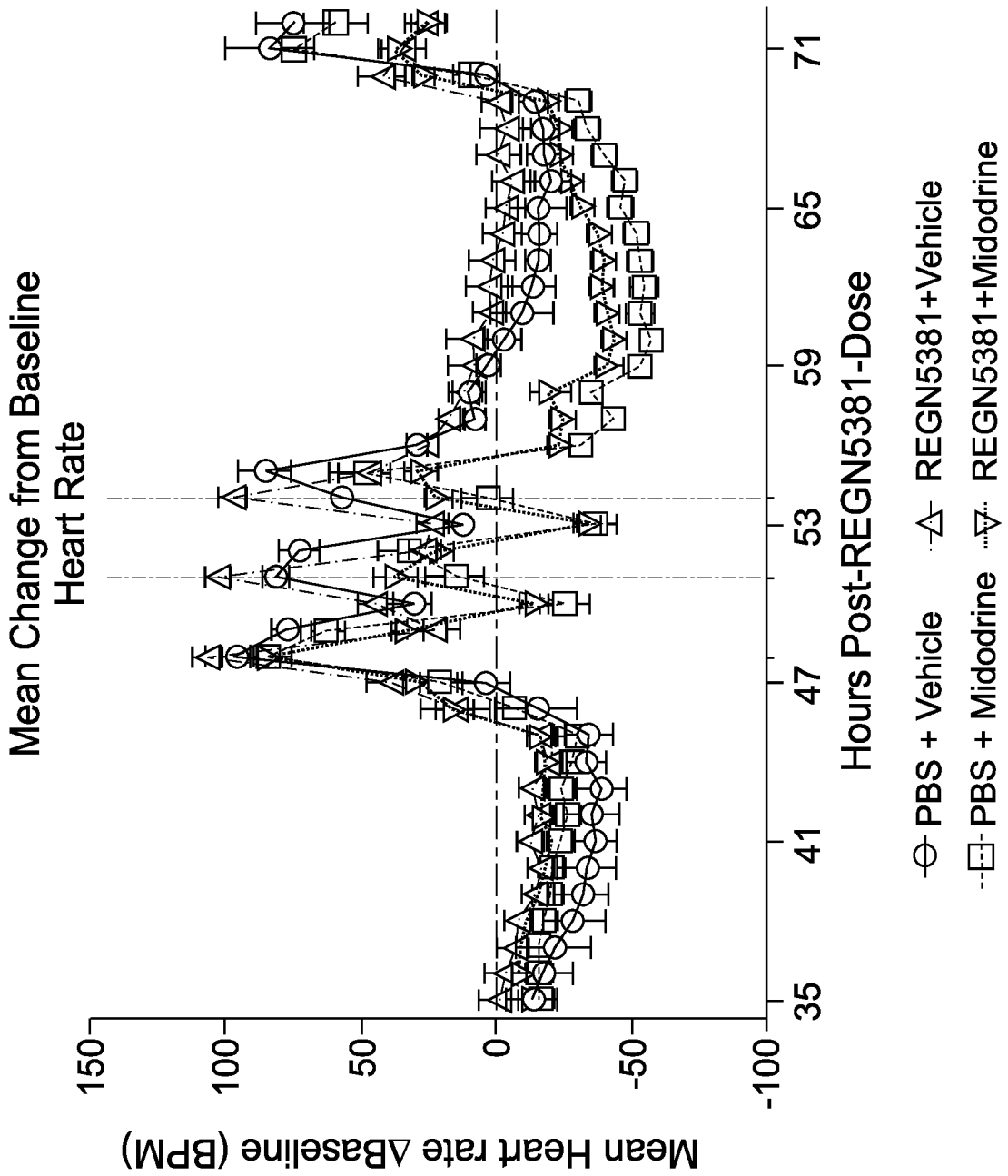
Mean Change from Baseline
Heart Rate
Mean Heart rate ΔBaseline (BPM)
150
100
50
0
-50
-100
35
41
47
53
59
65
71
Hours Post-REGN5381-Dose
PBS + Vehicle
REGN5381+Vehicle
PBS + Midodrine
REGN5381+Midodrine

FIGURE 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/064073

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/42 A61P9/12 A61K31/165
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CRUZ DINNA N: "Midodrine: a selective [alpha]-adrenergic agonist for orthostatic hypotension and dialysis hypotension", EXPERT OPIN PHARMACOTHER, vol. 1, no. 4, 1 May 2000 (2000-05-01), pages 835-840, XP055907351, London, UK ISSN: 1465-6566, DOI: 10.1517/14656566.1.4.835 the whole document ----- -/--	1-3, 61-66,70

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 April 2022	13/04/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Covone-van Hees, M

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/064073

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/064073

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 71, 72, 120, 121(completely); 1, 2(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 71, 72, 120, 121(completely); 1, 2(partially)

Present claims 1 and 2 relate to an extremely large number of possible compounds, namely a) any NPR1 agonist and b) any agent that reverses its effect. Support and disclosure in the sense of Article 6 and 5 PCT is to be found however for only a very small proportion of the compounds claimed, i.e. the once exemplified.

The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 1-2 (PCT Guidelines 9.19 and 9.23).

The search of claims 1-2 was restricted to those claimed compounds which appear to be supported namely anti-idiotypic antibodies binding the R5381 defined in the examples and midodrine exemplified in ex.14. Additionally the concept of targeting anti-NPR1 antibodies with anti-idiotypic antibodies has also been searched.

Claims 71,72,120,121 have been not searched: the epitope of the exemplified antibodies is not disclosed in the application. It is deemed undue burden for the skilled person to first have to identify the binding epitope to be able to produce further antibodies. does not comply with the requirements of Art.5 and 6 PCT. The definition of an epitope depends on the assay used to identify it and, thus, per se is not clear (Art.6 PCT, see Gershoni et al.). Most epitopes are not simple linear sequences but rather highly conformational discontinuous structures, and therefore are rarely compatible with techniques such as pepscan analysis. The most reliable technique for epitope mapping being x-ray analysis of crystals of antigen:antibody complexes. However, the latter technique is not trivial and is not readily applicable to many antigens and antibodies (e.g. Gershoni et al). Thus, it would be an undue burden for the skilled person to determine the epitope of the claimed antibodies (Art.6 PCT).

Antibodies competing with the claimed antibodies do not have necessarily the same properties of the exemplified antibodies, since competition may occur via steric hindrance or change of conformation. This definition is therefore deemed as an unusual parameter, which does not allow a direct comparison between the exemplified antibodies and the prior art.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

overcome.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2020/086406 A2 (REGENERON PHARMA [US]) 30 April 2020 (2020-04-30) cited in the application	4-19,23, 24, 27-31, 35-47, 50-52, 54-70, 74-77, 80-85, 88-97, 100-103, 105-119, 122-126
A	SEQ ID NO 194 100% identity with claimed seq.47; SEQ ID NO 2; 100%identity with claimed seq.48; SEQ ID NO 8; 100% identity with claimed seq.51; SEQ ID NO 10; 100% identity with seq.52; claims 8,13; sequences 194,2,8,10 example 3	20-22, 25,26, 32-34, 48,49, 53,73, 78,79, 86,87, 98,99, 104

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