

ANTAGONIST ANTI-NPR1 ANTIBODIES AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application is being filed on December 6, 2022, as a PCT International Patent Application and claims priority to U.S. Provisional Patent Application Nos. 63/286,476, filed December 6, 2021, and 63/310,078, filed February 14, 2022, the entire content of each of which is incorporated by reference herein.

REFERENCE TO A SEQUENCE LISTING XML

[002] This application contains a Sequence Listing which has been submitted electronically in XML format. The Sequence Listing XML is incorporated herein by reference. Said XML file, created on November 24, 2022, is named 40848_0112WOU1_SL.xml and is 82,654 bytes in size.

FIELD OF THE DISCLOSURE

[003] The present disclosure is related to antagonist antibodies and antigen-binding fragments of antibodies that specifically bind to natriuretic peptide receptor 1 (NPR1), and therapeutic and diagnostic methods of using those antibodies.

BACKGROUND OF THE DISCLOSURE

[004] Natriuretic peptide receptor 1 (NPR1; also known as NPR-A) is a membrane-bound guanylate cyclase that mediates the intracellular conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP) (Martinez-Rumayor, *et al.*, 2008 *Am J Cardiol* 101(3a):3-8). NPR1 is broadly expressed, including in the 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). NPR1 agonism results in alterations of systemic blood pressure (BP) through cGMP-mediated effects on intravascular volume, vasorelaxation, natriuresis, and diuresis.

[005] Monoclonal antibodies to NPR1 were first described by Kitano, *et al.*, in 1995 (*Immunol. Lett.* 47: 215-22). Activating or agonist anti-NPR1 antibodies are disclosed in, for example, US Patent/Publication Nos. 9090695, 20160168251, and 20200123263, and in WO2010065293.

[006] Hypotension, or low blood pressure, can be a relatively benign, asymptomatic condition, but it can become of concern, when the pumping pressure is not sufficient to perfuse key organs with oxygenated blood (Sharma, *et al.*, updated 2021 *Hypotension*, available from <https://www.ncbi.nlm.nih.gov/books/NBK499961/>). Complications of untreated hypotension with poor cardiac output are severe and can even lead to death.

[007] Disorders characterized by hypotension have a high unmet medical need for safe, long-acting therapies. Relatively few drugs increase blood pressure and intravascular volume. Most existing drugs have limitations, for example, oral agents have a short duration of action, necessitating multiple doses/day, and intravenous vasopressors require infusion in an ICU with frequent monitoring due to a narrow therapeutic index.

BRIEF SUMMARY OF THE DISCLOSURE

[008] In one aspect, the present disclosure provides antagonist antibodies and antigen-binding fragments thereof that specifically bind the natriuretic peptide receptor 1 (NPR1) protein. In one embodiment, an isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein is provided, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1. In a further embodiment, blocking NPR1 comprises inhibiting and/or blocking NPR1's signaling and/or activity. In certain embodiments, the anti-NPR1 antibodies are fully human antibodies that bind to NPR1 with high affinity and block NPR1. The antibodies of the present disclosure are useful, *inter alia*, for blocking or reducing NPR1 signaling and/or the hypotensive activity of NPR1 protein. In certain embodiments, the antibodies are useful in preventing, treating, or ameliorating at least one symptom or indication of a NPR1-associated disease or disorder, including hypotension, in a subject. In certain embodiments, the antibodies may be administered prophylactically or therapeutically to a subject having or at risk of having a NPR1-associated disease or disorder. In specific embodiments, the antibodies are used to increase systemic blood pressure in a subject suffering from low blood pressure. Such antibodies can be used as therapy for a disorder or condition associated with hypotension when administered to a subject in need thereof.

[009] The antagonist antibodies disclosed herein bind to NPR1 with high affinity and have improved pharmacokinetic properties (as compared to standard-of-care drugs). A single dose of an antibody of the present disclosure led to sustained increase in blood pressure. Indeed, the antibodies disclosed herein are efficacious in increasing the blood pressure and maintaining the increased pressure for as long as 28 days when administered as a single dose. Such antibodies can be used to provide superior

efficacy, along with less frequent dosing, in a subject with a NPR1-associated disease or disorder (e.g., hypotension).

[010] The antibodies of the disclosure can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, F(ab')₂ or scFv fragment), and may be modified to affect functionality, e.g., to increase persistence in the host or to eliminate residual effector functions (Reddy, *et al.*, 2000, *J. Immunol.* 164:1925-1933). In certain embodiments, the antibodies may be bispecific.

[011] In a first aspect, the present disclosure provides isolated recombinant monoclonal antagonist antibodies or antigen-binding fragments thereof that bind specifically to NPR1.

[012] In some embodiments, the antibodies are fully human monoclonal antibodies.

[013] Exemplary anti-NPR1 antibodies of the present disclosure are listed in Tables 1 and 2 herein. 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 antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary antibodies.

[014] The present disclosure provides 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.

[015] The present disclosure also provides 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.

[016] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain variability region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55.

[017] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain variability region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63.

[018] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising three complementarity determining regions (CDRs) contained within a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55; and three CDRs contained within a light chain variable region (LCVR) selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63.

[019] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodiments, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-NPR1 antibodies listed in Table 1. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from one of SEQ ID NOs: 2/10 (e.g., mAb38067), 22/30 (e.g., mAb38072), 38/46 (e.g., mAb38090), and 55/63 (e.g., mAb22034).

[020] The present disclosure also provides 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 anti-NPR1 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 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.

[021] 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.

[022] 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.

[023] 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.

[024] 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.

[025] 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.

[026] 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.

[027] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising:

- (a) a heavy chain determining region (HCDR)1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 24, 40, and 57;
- (b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 26, 42, and 59;
- (c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 28, 44, and 61;

- (d) a light chain determining region (LCDR)1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 48, and 65;
- (e) a LCDR2 domain having an amino acid sequence selected from the group consisting of AAS and GAS; and
- (f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, and 69.

[028] The present disclosure also provides 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 anti-NPR1 antibodies 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 (*e.g.*, mAb38067), 28/32 (*e.g.*, mAb38072), 44/16 (*e.g.*, mAb38090), and 61/69 (*e.g.*, mAb22034).

[029] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a HCVR and a LCVR, said HCVR comprising HCDR1 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, HCDR2 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, and HCDR3 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid. In certain embodiments, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising a HCVR and a LCVR, said LCVR comprising LCDR1 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, LCDR2 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, and LCDR3 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid. For example, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising a HCVR and a LCVR, said HCVR comprising HCDR1 comprising an amino acid sequence of SEQ ID NO: 24 or an amino acid sequence differing from SEQ ID NO: 24 by 1 amino acid, HCDR2 comprising an amino acid sequence of SEQ ID NO: 26 or an amino acid sequence differing from SEQ ID NO: 26 by 1 amino acid, and HCDR3 comprising an amino acid sequence of SEQ ID NO: 28 or an amino acid sequence differing from SEQ ID NO: 28 by 1 amino acid. In

another exemplary embodiment, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising a HCVR and a LCVR, said LCVR comprising LCDR1 comprising an amino acid sequence of SEQ ID NO: 12 or an amino acid sequence differing from SEQ ID NO: 12 by 1 amino acid, LCDR2 comprising an amino acid sequence of AAS or an amino acid sequence differing from AAS by 1 amino acid, and LCDR3 comprising an amino acid sequence of SEQ ID NO: 32 or an amino acid sequence differing from SEQ ID NO: 32 by 1 amino acid.

[030] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary antibodies listed in Table 1. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NO: 4-SEQ ID NO: 6-SEQ ID NO: 8-SEQ ID NO: 12-AAS-SEQ ID NO: 16 (*e.g.*, mAb38067), SEQ ID NO: 24-SEQ ID NO: 26-SEQ ID NO: 28-SEQ ID NO: 12-AAS-SEQ ID NO: 32 (*e.g.*, mAb38072), SEQ ID NO: 40-SEQ ID NO: 42-SEQ ID NO: 44-SEQ ID NO: 48-AAS-SEQ ID NO: 16 (*e.g.*, mAb38090), and SEQ ID NO: 57-SEQ ID NO: 59-SEQ ID NO: 61-SEQ ID NO: 65-GAS-SEQ ID NO: 69 (*e.g.*, mAb22034).

[031] In a related embodiment, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary antibodies listed in Table 1. For example, the present disclosure includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 8/16 (*e.g.*, mAb38067), 28/32 (*e.g.*, mAb38072), 44/16 (*e.g.*, mAb38090), and 61/69 (*e.g.*, mAb22034). In a related embodiment, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain variable region (HCVR)/light chain variable region (LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 38/46, and 55/63.

[032] Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. 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.

[033] In certain embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain and light chain, wherein the light chain is selected from the group consisting of SEQ ID NOs: 20, 36, 53, and 73.

[034] In additional embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain is selected from the group consisting of SEQ ID NOs: 18, 34, 51 and 71; and the light chain is selected from the group consisting of SEQ ID NOs: 20, 36, 53, and 73.

[035] In additional embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof binds to residues in the lower lobe of the extracellular domain of NPR1. In still further embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof interacts with at least one of the NPR1 residues selected from the group consisting of Arg143, Leu144, Glu384, Leu401, Val402, Ala103, Ser405, Gly406, Arg407, Lys408, Trp411, Leu413, Gly414, Tyr415, and Pro416.

[036] In additional embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 18; and the light chain comprises an amino acid sequence of SEQ ID NO: 20.

[037] In additional embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 34; and the light chain comprises an amino acid sequence of SEQ ID NO: 36.

[038] In additional embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain and a light chain,

wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 51; and the light chain comprises an amino acid sequence of SEQ ID NO: 53.

[039] In additional embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 71; and the light chain comprises an amino acid sequence of SEQ ID NO: 73.

[040] In certain embodiments, the present disclosure includes an antibody or antigen-binding fragment thereof that binds specifically to NPR1, 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) and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR), wherein the HCVR comprises: (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55; (ii) an amino acid sequence having at least 90% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55; (iii) an amino acid sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55; or (iv) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55, said amino acid sequence having no more than 12 amino acid substitutions; and the LCVR comprises: (a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63; (b) an amino acid sequence having at least 90% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63; (c) an amino acid sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63; or (d) an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63, said amino acid sequence having no more than 10 amino acid substitutions.

[041] In certain preferred embodiments, the present disclosure includes antibodies that bind specifically to NPR1 in an antagonist manner, *i.e.*, block or reduce NPR1 binding and/or activity.

[042] The present disclosure includes anti-NPR1 antibodies 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).

[043] In certain embodiments, the present disclosure provides antibodies and antigen-binding fragments thereof that exhibit pH-dependent binding to NPR1. For example, the present disclosure includes antibodies and antigen-binding fragment thereof that bind NPR1 with higher affinity at neutral pH than at basic pH (*i.e.*, reduced binding at basic pH).

[044] The present disclosure also provides for antibodies and antigen-binding fragments thereof that compete for specific binding to NPR1 with an antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[045] The present disclosure also provides antibodies and antigen-binding fragments thereof that cross-compete for binding to NPR1 with a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[046] The present disclosure also provides antibodies and antigen-binding fragments thereof that bind to the same epitope as a reference antibody or antigen-binding fragment thereof comprising three CDRs of a HCVR and three CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[047] In certain embodiments, the antibodies or antigen-binding fragments of the present disclosure are bispecific comprising a first binding specificity to a first epitope of NPR1 and a second binding specificity to a second epitope of NPR1 wherein the first and second epitopes are distinct and non-overlapping.

[048] In certain embodiments, the present disclosure provides an isolated antagonist anti-NPR1 antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof has one or more of the following characteristics: (a) is a fully human monoclonal antibody; (b) binds to human NPR1 at 25°C and at 37°C with a dissociation constant (K_D) of less than 1.7nM, as measured in a surface plasmon resonance assay; (c) binds to monkey NPR1 at 25°C and 37°C with a K_D of less than 1.99nM, as measured in a surface plasmon resonance assay; (d) binds to human NPR1 in the presence of ANP at 25°C and at 37°C with a K_D of less than 1.52nM, as measured in a surface plasmon resonance assay; (e) inhibits ligand-induced NPR1 activation (for example, induced by ANP or BNP), as measured by cGMP accumulation assay; (f) binds to human NPR1 in the presence or absence of ANP or

BNP with an EC_{50} of less than 2.9nM, as measured by electrochemiluminescence-based immunoassay; (g) binds to monkey NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 4.2nM, as measured by electrochemiluminescence-based immunoassay; (h) increases the systemic blood pressures (including systolic, diastolic, mean arterial, and pulse pressures) when administered to normotensive and hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose; (i) increases the systemic blood pressures when administered to ANP overexpression-induced hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose; (j) increases systemic blood pressures in LPS-induced hypotensive mice; and (k) comprises a HCVR comprising an amino acid sequence selected from the group consisting of HCVR sequence listed in Table 1 and a LCVR comprising an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

[049] In a second aspect, the present disclosure provides nucleic acid molecules encoding anti-NPR1 antibodies or portions thereof. In certain embodiments, an isolated polynucleotide molecule is provided comprising a polynucleotide sequence that encodes a heavy chain variable region (HCVR) of an isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1. In additional embodiments, an isolated polynucleotide molecule is provided comprising a polynucleotide sequence that encodes a light chain variable region (LCVR) of an isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1. 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.

[050] 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.

[051] The present disclosure also 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 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.

[052] 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.

[053] 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.

[054] 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.

[055] 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.

[056] 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.

[057] The present disclosure also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3),

wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary antibodies listed in Table 1.

[058] The present disclosure also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary antibodies listed in Table 1.

[059] The present disclosure also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of 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 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, and a polynucleotide sequence selected from any of the LCVR nucleic 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. In certain embodiments according to this aspect of the disclosure, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-NPR1 antibody listed in Table 1.

[060] In a related aspect, the present disclosure provides a vector comprising a polynucleotide molecule encoding an HCVR of an isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1. In another related aspect, the present disclosure provides a vector comprising a polynucleotide molecule encoding an LCVR of an isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1. In yet another related aspect, the present disclosure provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy and/or light chain variable region of an antibody. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 2. In certain embodiments, the present disclosure provides expression vectors comprising: (a) a nucleic acid molecule comprising a nucleic acid sequence encoding a HCVR of an antibody that binds NPR1, wherein the HCVR comprises an amino acid sequence selected from the group

consisting of sequences listed in Table 1; and/or (b) a nucleic acid molecule comprising a nucleic acid sequence encoding a LCVR of an antibody that binds NPR1, wherein the LCVR comprises an amino acid sequence selected from the group consisting of sequences listed in Table 1. Also included within the scope of the present disclosure are host cells comprising a vector according to the disclosure, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced. In certain embodiments, the method of producing according to the disclosure further comprises formulating the antibody or antigen-binding fragment thereof as a pharmaceutical composition comprising an acceptable carrier. In certain embodiments, the host cells comprise 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. In certain embodiments, the present disclosure provides methods of producing an antibody or antigen-binding fragment thereof of the disclosure, the methods comprising introducing into a host cell an expression vector comprising a nucleic acid sequence encoding a HCVR and/or LCVR of an antibody or antigen-binding 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 antibody or antigen-binding fragment thereof from the culture medium and/or host cell. The isolated antibody or antigen-binding fragment thereof may be purified using any of the methods known in prior art.

[061] In a third aspect, the disclosure provides a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1, and a pharmaceutically acceptable carrier. In another embodiment, the pharmaceutical composition comprises a therapeutically effective amount of at least one recombinant monoclonal antibody or antigen-binding fragment thereof that specifically binds NPR1 and a pharmaceutically acceptable carrier. In a related aspect, the disclosure features a composition that is a combination of an anti-NPR1 antibody and a second therapeutic agent or therapy. In one embodiment, the second therapeutic agent or therapy is any agent or therapy that is advantageously combined with an anti-NPR1 antibody. Exemplary agents or therapies that may be advantageously combined with an antagonist anti-NPR1 antibody include, without limitation, other agents that bind and/or block NPR1 signaling and/or activity (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do not directly bind NPR1 but nonetheless treat or ameliorate at

least one symptom or indication of a NPR1-associated disease or disorder (disclosed elsewhere herein). Additional combination therapies and co-formulations involving the anti-NPR1 antibodies of the present disclosure are disclosed elsewhere herein.

[062] In a fourth aspect, the disclosure provides therapeutic methods for treating a disease or disorder associated with NPR1 in a subject using an anti-NPR1 antibody or antigen-binding portion of an antibody of the disclosure, wherein the therapeutic methods comprise administering a pharmaceutical composition comprising an therapeutically effective amount of an antibody or antigen-binding fragment of an antibody according to the disclosure to the subject in need thereof. In certain embodiments, the disorder treated is any disease or condition that is improved, ameliorated, inhibited or prevented by blocking of NPR1 activity (e.g., hypotension). In certain embodiments, the NPR1-associated disease or disorder is selected from the group consisting of hypotension, circulatory shock, septic shock, neurogenic orthostatic hypotension, postural orthostatic tachycardia syndrome (POTS), heart failure, cardiogenic shock, obesity, renal failure, chronic kidney disease, macular edema, glaucoma, stroke, lung disorders, pulmonary fibrosis, inflammation, asthma, skeletal growth disorders, bone fractures, diabetes, hypoglycemia, and cancer.

[063] In certain embodiments, the disclosure provides methods to prevent, or treat a NPR1-associated disease or disorder comprising administering a therapeutically effective amount of an anti-NPR1 antibody or antigen-binding fragment thereof of the disclosure to a subject in need thereof. In some embodiments, the antibody or antigen-binding fragment thereof may be administered prophylactically or therapeutically to a subject (for example, to a subject having or at risk of having a NPR1-associated disease or disorder). In certain embodiments, the antibody or antigen-binding fragment, or the composition comprising an antibody or antigen-binding fragment thereof, according to the disclosure, is administered in combination with a second therapeutic agent or therapy. The second therapeutic agent or therapy may, in certain embodiments, be selected from the group consisting of an angiogenesis inhibitor, a vasoconstrictor/vasopressor, an immunosuppressant, ascorbic acid, a calcineurin inhibitor, a corticosteroid, a VEGF inhibitor, a decongestant, an antidepressant, hormonal birth control, a stimulant (including cardiac stimulant), caffeine, extracorporeal membrane oxygenation, ventricular assist device, intra-aortic balloon pump, a lifestyle modification, a dietary supplement, an anti-microbial drug, insulin, and an anti-inflammatory drug. In certain embodiments, the second therapeutic agent or therapy may be an agent or therapy that helps to counteract or reduce any possible side effect(s) associated with an antibody or antigen-binding fragment thereof of the disclosure, if such side effect(s) should occur.

The antibody or fragment thereof, or the composition comprising an antibody or antigen-binding fragment thereof, according to the disclosure, may be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, or intramuscularly. The antibody or fragment thereof may be administered at a dose of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight of the subject. In certain embodiments, an antibody of the present disclosure may be administered at one or more doses comprising between 10mg to 600mg.

[064] The present disclosure also includes use of an anti-NPR1 antibody or antigen-binding fragment thereof of the disclosure in the manufacture of a medicament for the treatment of a disease or disorder that would benefit from the blocking of NPR1 binding and/or activity.

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

BRIEF DESCRIPTION OF THE FIGURES

[066] Figures 1A and 1B show that anti-NPR1 antibodies inhibited (Fig. 1A) ANP and (Fig. 1B) BNP induced hNPR1 activation. Cells were pre-treated with increasing concentrations of anti-NPR1 antibodies, control mAb, or dilution buffer alone for 15 minutes at 37°C, followed by increasing concentrations of ANP, BNP, 0.2 nM ANP or 0.7 nM BNP for 30 minutes at 37°C. Experiment was performed in duplicate. Open symbols indicate conditions when no test article or only constant concentration of ANP or BNP was added, and closed symbols indicate conditions when the test article was added in a range of concentrations; dilution buffer: OptiMEM with 0.1% FBS.

[067] Figures 2A-2E show the non-competitive inhibition of ANP-mediated activation of NPR1 by (Fig. 2A) H4H22034N, (Fig. 2B) REGN7541, (Fig. 2C) REGN7544, and (Fig. 2D) REGN7548 with HEK293/hNPR1 cells. The data of the panel of Figs. 2A to 2D were analyzed in Schild plot analysis (Fig. 2E) to evaluate the Schild slope for each of the anti-NPR1 antibodies. The detection of fluorescence intensity and cGMP concentration were calculated as described in the experimental procedure. Experiment was performed in duplicate. No ligand stimulation for each indicated antibody concentration was plotted at 0.1 pM and included in the Schild plot analysis. Open symbols indicate conditions of ANP alone without test article; closed symbols indicate conditions when the test article was added at indicated concentrations of 50 nM, 150 nM, or 450 nM with ANP in a range of concentrations of 1 pM to 1 μ M; dilution buffer: OptiMEM with 0.1% FBS; CR: concentration ratio.

[068] Figures 3A-3C show that anti-NPR1 antibodies induced NPR1 internalization as measured by cytotoxicity assay with secondary ADC (Fig. 3A) in the absence of ligand

or in the presence of (Fig. 3B) 100 nM ANP or (Fig. 3C) 100nM BNP. HEK293/hNPR1 cells were pre-treated with increasing concentrations of anti-NPR1 antibodies, control mAb, or dilution buffer alone in the presence or absence of 100 nM ANP or 100 nM BNP for 5 minutes at 37°C, followed with secondary ADC treatment for 3 days at 37°C. Experiment was performed in duplicate. Open symbols indicate conditions when no test article or only constant concentration of ANP or BNP was added, and closed symbols indicate conditions when the test article was added in a range of concentrations; dilution buffer: OptiMEM with 0.1% FBS.

[069] Figure 4 shows the acute effects of NPR1 antagonist mAb's on pulse pressures in normotensive NPR1^{hu/hu} mice – single 25 mg/kg dose. Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 25 mg/kg intravenous injection of an NPR1 antagonist mAb or PBS as described in Table 25. All values are mean ± SEM, n=6 per group.

[070] Figure 5 shows the effects of NPR1 antagonist mAb's on systolic blood pressures in normotensive NPR1^{hu/hu} mice – single 25 mg/kg dose. Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 25 mg/kg intravenous injection of an NPR1 antagonist mAb or PBS as described in Table 25. All values are mean ± SEM, n=6 per group.

[071] Figure 6 show the effects of NPR1 antagonist mAb's on systolic blood pressures in normotensive NPR1^{hu/hu} Mice – single 1 mg/kg dose. Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 1 mg/kg subcutaneous injection of an NPR1 antagonist mAb or single 25 mg/kg injection of IgG4P isotype control mAb as described in Table 28. All values are mean ± SEM, n=4-5 per group.

[072] Figure 7 shows the effects of NPR1 antagonist mAb's on systolic blood pressures in normotensive NPR1^{hu/hu} mice – single 25 mg/kg dose. Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 25 mg/kg subcutaneous injection of an NPR1 antagonist mAb or IgG4P isotype control mAb as described in Table 28. All values are mean ± SEM, n=4-5 per group.

[073] Figure 8 shows the effects of NPR1 antagonist mAb's on systolic blood pressures in ANP overexpression-induced hypotensive NPR1^{hu/hu} mice. Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 50 ug HDD dose of control or ANP plasmid followed by a single 25 mg/kg intravenous injection of NPR1 antagonist mAb or isotype control as described in Table 31. All values are mean ± SEM, n=4-7 per group.

[074] Figures 9A and 9B show the effects of NPR1 antagonist mAb's on absolute (Fig. 9A) and relative heart weight (Fig. 9B) following overexpression-induced hypotensive NPR1^{hu/hu} mice. Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 25 mg/kg subcutaneous injection of an NPR1 antagonist mAb or IgG4P isotype control mAb as described in Table 31. All values are mean $\pm$ SEM, n=5-8 per group.

[075] Figure 10 shows the effects of NPR1 antagonist mAb's on pulse pressures in LPS-induced hypotensive NPR1^{hu/hu} mice – single 25 mg/kg preventative or therapeutic intravenous dose. Telemetered NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 5 mg/kg intraperitoneal injection of LPS or saline and a single intravenous injection of an NPR1 antagonist mAb or PBS ~24hours pre- or 8 hours post-LPS dose as described in Table 35. All values are mean $\pm$ SEM, n=7-10 per group.

[076] Figure 11 shows the mean change in pulse pressure normalized to baseline for each treatment group between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment. Data are expressed as the group mean $\pm$ standard error of the mean.

[077] Figure 12 shows the mean change in systolic blood pressure normalized to baseline for each treatment group between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment. Data are expressed as the group mean $\pm$ standard error of the mean. Vertical stippled lines indicate administration of drug indicated by route of administration (*i.e.*, SC or PO).

DETAILED DESCRIPTION

[078] 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.

[079] 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

[080] 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. NPR1 is the receptor for both atrial (ANP) and brain (BNP) natriuretic peptides and undergoes conformational changes in the extracellular domain upon ligand binding (Ogawa, *et al.*, 2004 *J. Biol. Chem.* 279: 28625-31). 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. 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 such as ROR1 (for example, SEQ ID NOs: 74-78).

[081] The term "antibody", as used herein, 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. 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.

[082] 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).

[083] 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.

[084] The fully human anti-NPR1 monoclonal 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"). 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.

[085] The present disclosure also includes fully human anti-NPR1 monoclonal 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 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.

[086] The term "human antibody", or "fully human antibody", 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.

[087] 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.

[088] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is

relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about 1×10^{-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™, which bind specifically to NPR1. Moreover, multi-specific antibodies that bind to one domain in NPR1 and one or more additional antigens or a bi-specific that binds to two different regions of NPR1 are nonetheless considered antibodies that “specifically bind”, as used herein.

[089] The term “high affinity” antibody refers to those mAbs having a binding affinity to NPR1, 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.

[090] By the term “slow off rate”, “Koff” or “kd” is meant an antibody that dissociates from NPR1, with a rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or less, preferably $1 \times 10^{-4} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, e.g., BIACORE™.

[091] 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 bind to NPR1 protein.

[092] In specific embodiments, antibody or antibody fragments of the disclosure may be conjugated to a moiety such a ligand or a therapeutic moiety (“immunoconjugate”), a second anti-NPR1 antibody, or any other therapeutic moiety useful for treating a NPR1-associated disease or disorder.

[093] 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 NPR1, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than NPR1).

[094] An “antagonist antibody”, as used herein (or an “antibody that blocks or reduces NPR1 activity”), is intended to refer to an antibody whose binding to NPR1 results in the blocking or reduction of NPR1 signaling and/or at least one biological activity of NPR1. For example, an antagonist anti-NPR1 antibody may increase systemic blood pressure upon administration to a subject in need thereof. The anti-NPR1 antibodies disclosed herein are antagonist antibodies.

[095] An "activating antibody" or an "agonist antibody", as used herein (or an "antibody that increases or potentiates NPR1 activity" or "an antibody that stabilizes the activated conformation"), is intended to refer to an antibody whose binding to NPR1 results in activation of at least one biological activity of NPR1. For example, an activating anti-NPR1 antibody or an agonist anti-NPR1 antibody may decrease systemic blood pressure upon administration to a subject in need thereof.

[096] 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.).

[097] The term " K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[098] 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. A single antigen may have more than one epitope. Thus, different 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.

[099] 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 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).

[0100] 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.

[0101] 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.

[0102] 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.

[0103] 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*). As used herein, the phrase refers to an amount that blocks NPR1 (*e.g.*, NPR1 signaling, NPR1 activity) and/or that increases systemic blood pressure.

[0104] As used herein, the term "subject" refers to an animal, preferably a mammal, more preferably a human, in need of amelioration, prevention and/or treatment of a NPR1-associated disease or disorder such as hypotension. The term includes human subjects who have or are at risk of having such a disease or disorder.

[0105] 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 NPR1-associated disease or disorder due to the administration of a therapeutic agent

such as an antagonist antibody of the present disclosure 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. The disorder or disease may include hypotension and/or a disorder or disease associated with hypotension and/or hypotension associated with a disease or disorder, for example, septic shock and neurodegenerative disease. The disorder or disease may also include postural orthostatic tachycardia syndrome (POTS).

[0106] The terms "prevent", "preventing" or "prevention" refer to inhibition of manifestation of a NPR1-associated disease or disorder such as hypotension or any symptoms or indications of such a disease or disorder upon administration of an antibody of the present disclosure.

[0107] As used herein, the phrase "blood pressure" may refer to any one of systolic blood pressure, diastolic blood pressure, mean arterial pressure (area under the arterial pressure/time curve, divided by the cardiac cycle duration), and pulse pressure (difference between systolic and diastolic pressures). Methods for measurement of blood pressure are known in the art. Blood pressure is measured in units of millimeters of mercury (mm Hg) and is usually expressed in terms of systolic (blood) pressure over diastolic (blood) pressure. Measurement methods include auscultatory, oscillometric, ultrasound, finger cuff methods. It can generally be measured, for example, using a digital blood pressure monitor or a sphygmomanometer.

Antigen-Binding Fragments of Antibodies

[0108] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. 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 NPR1 protein. 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.

[0109] 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.

[0110] 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.

[0111] 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)).

[0112] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (e.g., bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present disclosure using routine techniques available in the art.

Preparation of Human Antibodies

[0113] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present disclosure to make human antibodies that specifically bind to NPR1.

[0114] An immunogen comprising any one of the following can be used to generate antibodies to NPR1 protein. In certain embodiments, the antibodies of the disclosure are obtained from mice immunized with a full length, native NPR1 protein (See, for example, UniProtKB/Swiss-Prot accession number P16066.1) 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.

[0115] In some embodiments, the immunogen may be a recombinant NPR1 protein or fragment thereof expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells (for example, SEQ ID NOs: 74-78)

[0116] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to NPR1 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.

[0117] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0118] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. 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

[0119] The antagonist anti-NPR1 antibodies and antibody fragments 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 NPR1 protein. Such variant antibodies and antibody fragments 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 antibodies. Likewise, the antibody-encoding DNA sequences of the present disclosure 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 antibody or antibody fragment of the disclosure.

[0120] 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.

[0121] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, or potency.

[0122] In one embodiment, two antigen-binding 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.

[0123] In one embodiment, two antigen-binding 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.

[0124] 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 antibody 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 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 antibody.

[0125] Bioequivalent variants of the 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. 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 antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-NPR1 Antibodies Comprising Fc Variants

[0126] According to certain embodiments of the present disclosure, anti-NPR1 antibodies are provided comprising an Fc domain comprising one or more mutations which 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 anti-NPR1 antibodies comprising 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 antibody 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.

[0127] For example, the present disclosure includes anti-NPR1 antibodies comprising 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.

[0128] The present disclosure also includes anti-NPR1 antibodies comprising 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 antibodies 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 Antibodies

[0129] In general, the antagonist antibodies of the present disclosure function by binding to NPR1 protein and blocking its signaling and/or activity. For example, the present disclosure includes antibodies and antigen-binding fragments of antibodies that bind to human NPR1 at 25°C and at 37°C with a dissociation constant (K_D) of less than 1.7nM, as measured in a surface plasmon resonance assay, *e.g.*, using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind human NPR1 with a K_D of less than about 1.27nM, less than about 0.34nM, less than about 0.08nM, less than about 0.06nM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0130] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that bind to monkey NPR1 at 25°C and at 37°C with a dissociation constant (K_D) of less than 1.99nM, as measured in a surface plasmon resonance assay, *e.g.*, using the assay format as defined in Example 3 herein. In

certain embodiments, the antibodies or antigen-binding fragments thereof bind monkey NPR1 with a K_D of less than about 1.23nM, less than about 0.32nM, less than about 0.1nM, less than about 0.07nM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0131] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that bind to human NPR1 in the presence of ANP at 25°C and at 37°C with a K_D of less than 1.52nM, as measured in a surface plasmon resonance assay, *e.g.*, using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind human NPR1 in the presence of ANP at 25°C and at 37°C with a K_D of less than about 1.1nM, less than about 0.8nM, less than about 0.6nM, and less than about 0.5nM, as measured in a surface plasmon resonance assay, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0132] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that inhibit ligand-induced NPR1 activation (for example, induced by ANP or BNP), as measured by cGMP accumulation assay, *e.g.*, using the assay format as defined in Example 6 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof inhibit ligand-induced NPR1 activation by at least about 80%, at least about 90%, at least about 92%, at least about 99% and at least about 100%, as measured by cGMP accumulation assay, *e.g.*, using the assay format as defined in Example 6 herein, or a substantially similar assay.

[0133] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that bind to human NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 2.9nM, as measured by electrochemiluminescence-based immunoassay, *e.g.*, using the assay format as defined in Example 7 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind human NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than about 2.1nM, less than about 1.2 nM, and less than about 0.6nM, as measured by electrochemiluminescence-based immunoassay, *e.g.*, using the assay format as defined in Example 7 herein, or a substantially similar assay.

[0134] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that bind to monkey NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 4.2nM, as measured by electrochemiluminescence-based immunoassay, *e.g.*, using the assay format as defined in Example 7 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind monkey NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than about

2.9nM, less than about 2.1 nM, and less than about 0.7nM, as measured by electrochemiluminescence-based immunoassay, *e.g.*, using the assay format as defined in Example 7 herein, or a substantially similar assay.

[0135] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that increases the systemic blood pressures (including systolic, diastolic, mean arterial, and pulse pressures) when administered to normotensive and hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose, *e.g.*, as described in Example 9 herein.

[0136] The present disclosure also includes antibodies and antigen-binding fragments of antibodies that increase the systemic blood pressures when administered to ANP overexpression-induced hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose, *e.g.*, as described in Example 10 herein.

[0137] The present disclosure also includes antibodies and antigen-binding fragments thereof that increase systemic blood pressures in LPS-induced hypotensive mice, as described in Example 11 herein.

[0138] In one embodiment, the present disclosure provides an isolated recombinant antibody or antigen-binding fragment thereof that binds specifically to NPR1 protein in the presence or absence of ANP or BNP and reduces or blocks the signaling and/or activity of NPR1, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (a) is a fully human monoclonal antibody; (b) binds to human NPR1 at 25°C and at 37°C with a dissociation constant (K_D) of less than 1.7nM, as measured in a surface plasmon resonance assay; (c) binds to monkey NPR1 at 25°C and 37°C with a K_D of less than 1.99nM, as measured in a surface plasmon resonance assay; (d) binds to human NPR1 in the presence of ANP at 25°C and at 37°C with a K_D of less than 1.52nM, as measured in a surface plasmon resonance assay; (e) inhibits ligand-induced NPR1 activation, as measured by cGMP accumulation assay; (f) binds to human NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 2.9nM, as measured by electrochemiluminescence-based immunoassay; (g) binds to monkey NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 4.2nM, as measured by electrochemiluminescence-based immunoassay; (h) increases the systemic blood pressures when administered to normotensive and hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose; (i) increases the systemic blood pressures when administered to ANP overexpression-induced hypotensive mice, wherein the increase in systemic blood pressures lasts for up to

about 28 days upon administration of a single dose; (j) increases systemic blood pressures in LPS-induced hypotensive mice; 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.

[0139] The antibodies of the present disclosure may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Other biological characteristics of the antibodies 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

[0140] The present disclosure includes antagonist anti-NPR1 antibodies that interact with one or more amino acids found within one or more regions of the NPR1 protein molecule. The epitope to which the antibodies 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 NPR1 protein 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 aforementioned domains of the protein molecule (e.g. a conformational epitope).

[0141] 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., Ehrling, (1999) *Analytical Biochemistry* 267: 252-259; Engen and Smith, (2001) *Anal. Chem.* 73: 256A-265A.

[0142] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. 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.

[0143] 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.

[0144] In certain embodiments, the present disclosure includes antagonist anti-NPR1 antibodies and antigen-binding fragments thereof that interact with one or more epitopes found within the extracellular domain of NPR1. 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 NPR1. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within NPR1.

[0145] The present disclosure includes antagonist anti-NPR1 antibodies that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary antibodies listed in Table 1. Likewise, the present disclosure also includes antagonist

anti-NPR1 antibodies that compete for binding to NPR1 protein or a fragment thereof with any of the specific exemplary antibodies listed in Table 1. For example, the present disclosure includes antagonist anti-NPR1 antibodies that cross-compete for binding to NPR1 protein with one or more antibodies listed in Table 1.

[0146] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-NPR1 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-NPR1 antibody of the disclosure, the reference antibody is allowed to bind to a NPR1 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the NPR1 protein molecule is assessed. If the test antibody is able to bind to NPR1 following saturation binding with the reference anti-NPR1 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-NPR1 antibody. On the other hand, if the test antibody is not able to bind to the NPR1 protein following saturation binding with the reference anti-NPR1 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-NPR1 antibody of the disclosure.

[0147] To determine if an antibody competes for binding with a reference anti-NPR1 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a NPR1 protein under saturating conditions followed by assessment of binding of the test antibody to the NPR1 molecule. In a second orientation, the test antibody is allowed to bind to a NPR1 molecule under saturating conditions followed by assessment of binding of the reference antibody to the NPR1 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the NPR1 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to NPR1. 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.

[0148] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. 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.

[0149] 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.

[0150] In certain embodiments, the present disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein and blocks NPR1.

Immunoconjugates

[0151] The disclosure encompasses a human antagonist anti-NPR1 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), to treat a NPR1-associated disease or disorder (e.g., hypotension). As used herein, the term "immunoconjugate" refers to an antibody 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 antibody 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. 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 anti-NPR1 antibody and 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.

Multi-specific Antibodies

[0152] The antagonist antibodies of the present disclosure may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt, *et al.*, 1991, *J. Immunol.* 147:60-69; Kufer, *et al.*, 2004, *Trends Biotechnol.* 22:238-244.

[0153] Any of the multi-specific antigen-binding molecules of the disclosure, or variants thereof, may be constructed using standard molecular biological techniques

(e.g., recombinant DNA and protein expression technology), as will be known to a person of ordinary skill in the art.

[0154] In some embodiments, NPR1-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct domains of NPR1 protein are linked together to confer dual-domain specificity within a single binding molecule. Variable regions with specificity for individual domains, (e.g., segments of the N-terminal domain), or that can bind to different regions within one domain, are paired on a structural scaffold that allows each region to bind simultaneously to the separate epitopes, or to different regions within one domain. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one domain are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second domain to identify non-cognate V_L partners that can be paired with an original V_H without disrupting the original specificity for that V_H . In this way, a single V_L segment (e.g., V_{L1}) can be combined with two different V_H domains (e.g., V_{H1} and V_{H2}) to generate a bi-specific comprised of two binding "arms" (V_{H1} - V_{L1} and V_{H2} - V_{L1}). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, US2011/0195454 and US2010/0331527).

[0155] Alternatively, antibodies that bind more than one domains and a second target, such as, but not limited to, for example, a second different anti-NPR1 antibody, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct regions may be linked together with variable regions that bind to relevant sites on, for example, the extracellular domain of NPR1, to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. Variable regions with specificity for the extracellular domain are combined with a variable region with specificity for outside the extracellular domain and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[0156] Other exemplary bispecific formats that can be used in the context of the present disclosure include, without limitation, e.g., scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (e.g., common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab² bispecific formats (see, e.g., Klein, *et al.*, 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific

antibodies can also be constructed using peptide/nucleic acid conjugation, e.g., wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (See, e.g., Kazane, *et al.*, *J. Am. Chem. Soc.* [Epub: Dec. 4, 2012]).

Therapeutic Administration and Formulations

[0157] The disclosure provides therapeutic compositions comprising the antagonist anti-NPR1 antibodies or antigen-binding fragments thereof 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.

[0158] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When an antibody of the present disclosure is used for treating a disease or disorder in an adult patient, or for preventing such a disease, it is advantageous to administer the antibody of the present disclosure normally at a single dose of about 0.1 to about 100 mg/kg body weight. In one embodiment, the antibody according to the disclosure is administered at a single dose of about 25 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 antibody or antigen-binding fragment thereof 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, about 10 to about 400 mg, or about 100 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody 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.

[0159] 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).

[0160] The use of nanoparticles to deliver the antibodies 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.

[0161] 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.

[0162] 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.

[0163] 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.

[0164] 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 antibody 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 antibody 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 Antibodies

[0165] The antagonist antibodies of the present disclosure are useful for the treatment, and/or prevention of a disease or disorder or condition associated with NPR1 and/or for ameliorating at least one symptom associated with such disease, disorder, or condition. In certain embodiments, an antibody or antigen-binding fragment thereof of the disclosure may be administered at a therapeutic dose to a patient with a disease or disorder or condition associated with NPR1.

[0166] The disorder or disease may include hypotension, and/or a disorder or disease associated with hypotension, and/or hypotension associated with a disease or disorder, for example, circulatory shock, septic shock, and neurodegenerative disease. Hypotension is a decrease in systemic blood pressure below accepted low values. Hypotension exists as a range, with no accepted standard hypotensive value, but pressures less than 90 mm Hg (systolic)/60 mm Hg (diastolic) are recognized as hypotensive. Symptoms of hypotension may include, without limitation, lightheadedness, dizziness, syncope, chest pain, shortness of breath, irregular heartbeat, elevated body temperature, headache, stiff neck, severe upper back pain, cough with sputum, diarrhea, vomiting, dysuria, acute allergic reactions, fatigue, and vision aberrations. Complications of untreated hypotension with poor cardiac output are severe and can ultimately lead to death. In impending shock or fulminant shock, untreated hypotension can lead to multi-organ failure (Sharma, *et al.*, updated 2021, *Hypotension*, available from <https://www.ncbi.nlm.nih.gov/books/NBK499961/>). In one

embodiment, an antagonistic anti-NPR1 antibody or antigen-binding fragment thereof according to the disclosure is used to treat a symptom or an indication of a type of hypotension. In another embodiment, an antagonistic anti-NPR1 antibody or antigen-binding fragment thereof according to the disclosure is used to increase the blood pressure of a subject having hypotension and/or having a disease or disorder associated with hypotension.

[0167] Septic shock is characterized by refractory hypotension, causing inadequate perfusion of tissues, and is associated with high mortality rates. The standard of care for sepsis coupled with hypotension is the administration of vasopressors, such as catecholamines or mimetics, vasopressin, or Ang II, to maintain arterial pressure and serum lactate levels in the absence of hypovolemia. However, the standard of care vasopressors have significant drawbacks, including requiring frequent titration, having a narrow therapeutic range, requiring central venous access and ICU care, and possibly even reducing capillary perfusion (causing tissue ischemia (for example, digital necrosis) at prolonged high doses). Thus, there remains a significant unmet need for addressing the (refractory) hypotension. In one embodiment, an antagonistic anti-NPR1 antibody or antigen-binding fragment thereof according to the disclosure is used to treat a symptom or an indication of septic shock, or to treat the refractory hypotension of septic shock. In another embodiment, the use of an antagonistic anti-NPR1 antibody or antigen-binding fragment thereof according to the disclosure to treat septic shock, or to treat the refractory hypotension of septic shock, allows for a reduced use of vasopressors. As a result, a reduced length of intensive care unit (ICU) stay is required.

[0168] Neurogenic orthostatic hypotension constitutes hypotension in the upright position (blood pressure drops when standing, leading to cerebral hypoperfusion) and is generally due to defects in autonomic reflexes associated with neurodegenerative disease. It complicates multiple diseases and is associated with significant morbidity, with symptom impact on daily activities rating as severe or very severe in almost 50% of patients. The two presently approved drug therapies are only modestly efficacious, both being short-acting and requiring three times daily (TID) dosing. In one embodiment, an antagonistic anti-NPR1 antibody or antigen-binding fragment thereof according to the disclosure is used to treat neurogenic orthostatic hypotension.

[0169] The disorder or disease may also include postural orthostatic tachycardia syndrome (POTS). This syndrome is marked by tachycardia plus symptoms in upright position, though without hypotension. In POTS, a heart rate increase from horizontal to standing (or as tested on a tilt table) of at least 30 beats per minute is registered in adults, measured during the first 10 minutes of standing. POTS typically afflicts young

women and causes significant morbidity. The symptoms that occur upon standing include lightheadedness, tremor, palpitations, weakness, fatigue, blurry vision, occasional syncope, and the like. A significantly reduced quality of life is reported by over 80% of POTS patients. While fludrocortisone, midodrine, and beta-blockers are commonly used, they have not exhibited significant treatment efficacy; indeed, no specific drugs to treat POTS are currently FDA-approved. In one embodiment, an antagonistic anti-NPR1 antibody or antigen-binding fragment thereof according to the disclosure is used to treat POTS.

[0170] In certain embodiments, the antagonist antibodies of the present disclosure are useful for treating or preventing at least one symptom or indication of a disease or disorder selected from the group consisting of hypotension, circulatory shock, septic shock, neurogenic orthostatic hypotension, postural orthostatic tachycardia syndrome (POTS), heart failure, cardiogenic shock, obesity, renal failure, chronic kidney disease, macular edema, glaucoma, stroke, lung disorders, pulmonary fibrosis, inflammation, asthma, skeletal growth disorders, bone fractures, diabetes, hypoglycemia, and cancer.

[0171] It is also contemplated herein to use one or more antibodies of the present disclosure prophylactically to subjects at risk for suffering from a NPR1-associated disease or disorder.

[0172] In one embodiment of the disclosure, the present antibodies are used for the preparation of a pharmaceutical composition or medicament for treating patients suffering from a disease, disorder or condition disclosed herein. In another embodiment of the disclosure, the present antibodies are used as adjunct therapy with any other agent or any other therapy known to those skilled in the art useful for treating or ameliorating a disease, disorder or condition disclosed herein.

Combination Therapies

[0173] Combination therapies may include an antagonist antibody of the disclosure and any additional therapeutic agent that may be advantageously combined with an antibody of the disclosure, or with a biologically active fragment of an antibody of the disclosure. The antibodies of the present disclosure may be combined synergistically with one or more drugs or therapy used to treat a NPR1-associated disease or disorder, including hypotension. In some embodiments, the antibodies of the disclosure may be combined with a second therapeutic agent or therapy to ameliorate one or more symptoms of said disease or condition.

[0174] Depending upon the disease, disorder, or condition, the antibodies of the present disclosure may be used in combination with one or more additional therapeutic

agents including, but not limited to, an angiogenesis inhibitor, a vasoconstrictor/vasopressor, an immunosuppressant, ascorbic acid, a calcineurin inhibitor, a corticosteroid, a VEGF inhibitor, a decongestant, an antidepressant, hormonal birth control, a stimulant (including cardiac stimulant), caffeine, extracorporeal membrane oxygenation, ventricular assist device, intra-aortic balloon pump, a lifestyle modification, a dietary supplement, an anti-microbial drug, insulin, and an anti-inflammatory drug.

[0175] 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 antagonist anti-NPR1 antibody of the present disclosure. The term "in combination with" also includes sequential or concomitant administration of an anti-NPR1 antibody and a second therapeutic agent or therapy.

[0176] The additional therapeutically active component(s) may be administered to a subject prior to administration of an anti-NPR1 antibody 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 anti-NPR1 antibody 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 anti-NPR1 antibody of the present disclosure. "Concurrent" administration, for purposes of the present disclosure, includes, *e.g.*, administration of an anti-NPR1 antibody 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 anti-NPR1 antibody 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 anti-NPR1 antibody may be administered intravenously, and the additional

therapeutically active component may be administered orally). In any event, administering the components in a single dosage form, 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 anti-NPR1 antibody "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 anti-NPR1 antibody "in combination with" an additional therapeutically active component. **[0177]** The present disclosure includes pharmaceutical compositions in which an anti-NPR1 antibody 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 Antibodies

[0178] The antagonist antibodies of the present disclosure may be used to detect and/or measure NPR1 in a sample, e.g., for diagnostic purposes. Some embodiments contemplate the use of one or more antibodies of the present disclosure in assays to detect a NPR1-associated-disease or disorder. Exemplary diagnostic assays for NPR1 may comprise, e.g., contacting a sample, obtained from a patient, with an anti-NPR1 antibody of the disclosure, wherein the anti-NPR1 antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate NPR1 from patient samples. Alternatively, an unlabeled anti-NPR1 antibody 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 ^3H , ^{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 NPR1 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0179] Samples that can be used in NPR1 diagnostic assays according to the present disclosure include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either NPR1 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of NPR1 protein in a particular sample obtained from a healthy patient (e.g., a patient not afflicted with a disease associated with NPR1) will be measured to initially establish a baseline, or standard, level of NPR1. This baseline level of NPR1 can then be compared against the levels of NPR1

measured in samples obtained from individuals suspected of having a NPR1-associated condition, or symptoms associated with such condition.

[0180] The antibodies specific for NPR1 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

[0181] 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 disclosure. 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 Natriuretic Peptide Receptor 1 (NPR1)

[0182] Human antibodies to NPR1 protein were generated in a VELOCIMMUNE® mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions. The mice were immunized with human NPR1 and mouse ANP DNA by hydrodynamic DNA delivery and boosted by extracellular domain of human NPR1 protein complexed to mouse ANP.

[0183] The antibody immune response was monitored by a NPR1-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 NPR1-specific antibodies. The cell lines were used to obtain several anti-NPR1 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains).

[0184] Anti-NPR1 antibodies were also isolated directly from antigen-positive mouse B cells without fusion to myeloma cells, as described in U.S. Patent No. 7,582,298, herein specifically incorporated by reference in its entirety. Using this method, several

fully human anti-NPR1 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained.

[0185] Exemplary antibodies generated as disclosed above were designated as mAb38067, mAb38072, mAb38090, and mAb22034.

[0186] 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

[0187] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-NPR1 antibodies of the disclosure.

Table 1: Amino Acid Sequence Identifiers

Antibody Designation	SEQ ID NOs:							
	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
mAb38067	2	4	6	8	10	12	AAS	16
mAb38072	22	24	26	28	30	12	AAS	32
mAb38090	38	40	42	44	46	48	AAS	16
mAb22034	55	57	59	61	63	65	GAS	69

[0188] The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 2: Nucleic Acid Sequence Identifiers

Antibody Designation	SEQ ID NOs:							
	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
mAb38067	1	3	5	7	9	11	gctgcatcc	15
mAb38072	21	23	25	27	29	11	gctgcatcc	31
mAb38090	37	39	41	43	45	47	gctgcatcc	49
mAb22034	54	56	58	60	62	64	ggcgcatcc	68

[0189] Antibodies referred to herein typically have fully human variable regions 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 IgG1 or a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – 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.

[0190] 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.

[0191] Exemplary antibodies mAb38067, mAb38072, mAb38090, and mAb22034 comprising a human IgG4 Fc comprising a serine to proline mutation in the hinge region (S108P) were designated as REGN7541, REGN7544, REGN7548, and H4H22034 respectively. Table 3 sets forth the nucleic acid and amino acid sequence identifiers of full-length heavy chain and light chain sequences of these antibodies.

Table 3: Full-length Heavy Chain and Light Chain Sequence identifiers

Antibody ID	Antibody Name	SEQ ID NOs:			
		HC		LC	
		DNA	PEP	DNA	PEP
mAb38067	REGN7541	17	18	19	20
mAb38072	REGN7544	33	34	35	36
mAb38090	REGN7548	50	51	52	53
mAb22034	H4H22034	70	71	72	73

Control Constructs used in the Following Examples

[0192] The following control construct (anti-NPR1 antibody) was included in the experiments disclosed herein, for comparative purposes: "Comparator 1," a monoclonal antibody against human NPR1 having VH/VL sequences of antibody "mAb5591" according to US Patent No. 20120114659 (Morphosys).

Example 3: Biacore binding kinetics of anti-NPR1 monoclonal antibodies binding to various NPR1 reagents measured at 25°C and 37°C

Experimental Procedure

[0193] The equilibrium dissociation constant (K_D) for different NPR1 reagents binding to purified anti-NPR1 monoclonal antibodies (mAbs) were determined using a real-time surface plasmon resonance (SPR) based Biacore 8k biosensor. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant P20, pH 7.4 (HBS-EP) running buffer at 25°C and 37°C. The Biacore CM5 sensor chip surface was first derivatized by amine coupling with anti-human Fc specific antibody to capture anti-NPR1 mAbs. Binding studies were performed on human NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine

(hNPR1-MMH; SEQ ID NO:74), monkey NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (mfNPR1-MMH; SEQ ID NO:75), mouse NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (mNPR1-MMH; SEQ ID NO:76), dog NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (dog_NPR1-MMH; SEQ ID NO:77), pig NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (pig_NPR1-MMH; SEQ ID NO:78), and hNPR1-MMH + 10x hANP.

[0194] Different concentrations (40nM – 0.625nM, 4-fold serial dilutions) of hNPR1-MMH, mfNPR1-MMH, hNPR1-MMH in the presence of 10-fold concentration of hANP, or a fixed concentration (40nM) of mNPR1-MMH, dog_NPR1-MMH, pig_NPR1-MMH were prepared in HBS-EP running buffer, and then injected for 150 sec at a flow rate of 30µL/min, while the dissociation of mAb bound different NPR1 reagents was monitored for 30 minutes in HBS-EP running buffer. At the end of each cycle, the anti-NPR1 mAb capture surface was regenerated using a 12sec injection of 10mM phosphoric acid. 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 Biacore insight evaluation software. Binding dissociation equilibrium constant (K_D) and dissociative half-life ($t_{1/2}$) were calculated from the kinetic rates as:

$$K_D (M) = \frac{k_d}{k_a}, \quad \text{and} \quad t_{1/2} (\text{min}) = \frac{\ln(2)}{60 \cdot k_d}$$

Binding kinetics parameters for different NPR1 reagents binding to different anti-NPR1 mAbs of the disclosure at 25°C and 37°C are shown in Tables 4 through 15.

Results

[0195] At 25°C, anti-NPR1 monoclonal antibodies bound to hNPR1-MMH with K_D values ranging from 47.4pM to 1.27nM, as shown in Table 4.

Table 4: Binding kinetics parameters of different anti-NPR1 mAbs to hNPR1-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	59 ± 0.4	34	3.26E+05	4.15E-04	1.27E-09	28
REGN7541	59 ± 0.2	26	2.02E+05	6.80E-05	3.36E-10	170
REGN7544	60 ± 0.4	29	2.81E+05	1.33E-05	4.74E-11	868
REGN7548	59 ± 0.3	38	4.22E+05	3.40E-05	8.06E-11	340
Isotype control	59 ± 0.1	0	NB*	NB	NB	NB

*NB = no binding detected

[0196] At 37°C, anti-NPR1 monoclonal antibodies bound to hNPR1-MMH with a K_D values ranging from 57.4pM to 1.7nM, as shown in Table 5.

Table 5: Binding kinetics parameters of different anti-NPR1 mAbs to hNPR1-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	71 ± 0.3	44	6.26E+05	1.06E-03	1.70E-09	11
REGN7541	69 ± 0.4	41	7.47E+05	1.34E-04	1.79E-10	86
REGN7544	72 ± 0.2	43	5.69E+05	3.27E-05	5.74E-11	353
REGN7548	71 ± 0.3	50	7.77E+05	6.60E-05	8.49E-11	175
Isotype control	73 ± 0.1	0	NB*	NB	NB	NB

*NB = no binding detected

[0197] At 25°C, anti-NPR1 monoclonal antibodies bound to mfNPR1-MMH with K_D values ranging from 69pM to 1.23nM, as shown in Table 6.

Table 6: Binding kinetics parameters of different anti-NPR1 mAbs to mfNPR1-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	59 ± 0.4	33	3.18E+05	3.92E-04	1.23E-09	29
REGN7541	59 ± 0.2	26	2.11E+05	6.71E-05	3.18E-10	172
REGN7544	60 ± 0.1	29	2.79E+05	2.02E-05	7.25E-11	571
REGN7548	59 ± 0.2	38	4.19E+05	2.89E-05	6.90E-11	399
Isotype control	59 ± 0.1	1	NB*	NB	NB	NB

*NB = no binding detected

[0198] At 37°C, anti-NPR1 monoclonal antibodies bound to mfNPR1-MMH with a K_D values ranging from 53.6pM to 1.99nM, as shown in Table 7.

Table 7: Binding kinetics parameters of different anti-NPR1 mAbs to mfNPR1-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	71 ± 0.3	37	5.04E+05	1.00E-03	1.99E-09	12
REGN7541	69 ± 0.4	36	6.63E+05	1.04E-04	1.57E-10	111
REGN7544	72 ± 0.1	36	5.48E+05	2.94E-05	5.36E-11	393
REGN7548	71 ± 0.2	39	6.62E+05	6.49E-05	9.81E-11	178
Isotype control	72 ± 0.1	0	NB*	NB	NB	NB

*NB = no binding detected

[0199] At 25°C, anti-NPR1 monoclonal antibodies bound to hNPR1-MMH in the presence of 10-fold concentration of hANP with K_D values ranging from 78.8pM to 500pM, as shown in Table 8.

Table 8: Binding kinetics parameters of different anti-NPR1 mAbs to hNPR1-MMH in the presence of 10-fold concentration of hANP at 25°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	60 ± 0.2	35	3.46E+05	1.73E-04	5.00E-10	67
REGN7541	60 ± 0.2	23	2.19E+05	6.07E-05	2.77E-10	190
REGN7544	59 ± 0	26	2.61E+05	4.83E-05	1.85E-10	239
REGN7548	61 ± 0	39	5.52E+05	4.35E-05	7.88E-11	266
Isotype control	57 ± 0.2	0	NB*	NB	NB	NB

*NB = no binding detected

[0200] At 37°C, anti-NPR1 monoclonal antibodies bound to hNPR1-MMH in the presence of 10-fold concentration of hANP with K_D values ranging from 642pM to 1.52nM, as shown in Table 9.

Table 9: Binding kinetics parameters of different anti-NPR1 mAbs to hNPR1-MMH in the presence of 10-fold concentration of hANP at 37°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	75 ± 0.2	42	4.71E+05	7.17E-04	1.52E-09	16
REGN7541	72 ± 0.2	32	3.49E+05	3.78E-04	1.08E-09	31

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7544	69 ± 0.2	34	4.26E+05	3.51E-04	8.25E-10	33
REGN7548	75 ± 0.1	42	5.93E+05	3.80E-04	6.42E-10	30
Isotype control	69 ± 0.1	0	NB*	NB	NB	NB

*NB = no binding detected

[0201] At 25°C, only two anti-NPR1 monoclonal antibodies bound to pig_NPR1-MMH with K_D values ranging from 35.8nM to 122nM as shown in Table 10.

Table 10: Binding kinetics parameters of different anti-NPR1 mAbs to pig_NPR1-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	60 ± 0.1	0	NB	NB	NB	NB
REGN7541	60 ± 0	0	NB	NB	NB	NB
REGN7544	59 ± 0.1	2	5.58E+04	6.79E-03	1.22E-07	1.7
REGN7548	61 ± 0.1	11	1.19E+05	4.27E-03	3.58E-08	2.7
Isotype control	57 ± 0	0	NB*	NB	NB	NB

*NB = no binding detected

[0202] At 37°C, only two anti-NPR1 monoclonal antibodies bound to pig_NPR1-MMH with K_D values ranging from 42.1nM to 98.1nM as shown in Table 11.

Table 11: Binding kinetics parameters of different anti-NPR1 mAbs to pig_NPR1-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	75 ± 0	0	NB	NB	NB	NB
REGN7541	73 ± 0	0	NB	NB	NB	NB
REGN7544	69 ± 0.3	5	9.65E+04	9.47E-03	9.81E-08	1.2
REGN7548	75 ± 0.1	14	2.20E+05	9.27E-03	4.21E-08	1.2
Isotype control	69 ± 0.1	0	NB*	NB	NB	NB

*NB = no binding detected

[0203] At 25°C or at 37°C, none of the anti-NPR1 monoclonal antibodies bound to mNPR1-MMH as shown in Table 12 and Table 13, respectively.

Table 12: Binding kinetics parameters of different anti-NPR1 mAbs to mNPR1-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	60	0	NB	NB	NB	NB
REGN7541	60	0	NB	NB	NB	NB
REGN7544	59	0	NB	NB	NB	NB
REGN7548	61	0	NB	NB	NB	NB
Isotype control	57	0	NB*	NB	NB	NB

*NB = no binding detected

Table 13: Binding kinetics parameters of different anti-NPR1 mAbs to mNPR1-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	75	0	NB	NB	NB	NB
REGN7541	73	0	NB	NB	NB	NB
REGN7544	69	0	NB	NB	NB	NB
REGN7548	75	0	NB	NB	NB	NB
Isotype control	69	0	NB*	NB	NB	NB

*NB = no binding detected

[0204] At 25°C or at 37°C, none of the anti-NPR1 monoclonal antibodies bound to dog_NPR1-MMH as shown in Table 14 and Table 15, respectively.

Table 14: Binding kinetics parameters of different anti-NPR1 mAbs to dog_NPR1-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	60 ± 0	0	NB	NB	NB	NB
REGN7541	60 ± 0.1	0	NB	NB	NB	NB
REGN7544	59 ± 0.2	0	NB	NB	NB	NB

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7548	61 ± 0	0	NB	NB	NB	NB
Isotype control	57 ± 0	0	NB*	NB	NB	NB

*NB = no binding detected

Table 15: Binding kinetics parameters of different anti-NPR1 mAbs to dog_NPR1-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	40nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H4H22034N	75 ± 0	0	NB	NB	NB	NB
REGN7541	73 ± 0.3	0	NB	NB	NB	NB
REGN7544	69 ± 0.2	0	NB	NB	NB	NB
REGN7548	75 ± 0	0	NB	NB	NB	NB
Isotype control	69 ± 0.1	0	NB*	NB	NB	NB

*NB = no binding detected

Example 4: pH sensitivity of anti-NPR1 monoclonal antibodies binding to NPR1 reagents measured at 37°C

Experimental Procedure

[0205] The dissociation rate constants (k_d) for different anti-NPR1 monoclonal antibodies (mAbs) in pH7.4, and pH6.0 buffers were determined using a real-time surface plasmon resonance (SPR) based Biacore 4000 biosensor. All binding studies were performed at 37°C using two running buffers, (i) PBS, 0.05% v/v Surfactant Tween-20, pH7.4 (PBS-T-pH7.4), and (ii) PBS, 0.05% v/v Surfactant Tween-20, pH6.0 (PBS-T-pH6.0). The Biacore CM5 sensor chip surface was first derivatized by amine coupling with anti-human Fc specific antibody to capture anti-NPR1 mAbs. Different concentrations of human NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (hNPR1-MMH; SEQ ID NO:74) or monkey NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (mfNPR1-MMH; SEQ ID NO:75) (30nM and 10nM) were prepared in PBS-T-pH7.4 buffer were injected at a flow rate of 30μL/min for 4 minutes followed by the dissociation of bound NPR1 reagents in PBS-T-pH7.4 or PBS-T-pH6.0 running buffers for 5 minutes.

[0206] The dissociation rate constants (k_d) in two 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 ($t_{1/2}$) was calculated from the k_d values as:

$$t_{1/2} \text{ (min)} = \frac{\ln(2)}{60 \cdot k_d}$$

Results

[0207] The k_d and $t_{1/2}$ values for different anti-NPR1 mAbs binding to hNPR1-MMH or mfNPR1-MMH in PBS-T, pH7.4 followed by dissociation in PBS-T-pH7.4, or PBS-T-pH6.0 of the disclosure at 37°C are shown in Table 16 through Table 19.

Table 16: Binding of different anti-NPR1 monoclonal antibodies (mAbs) to hNPR1-MMH in PBS-T-pH7.4 buffer and the dissociation in PBS-T-pH7.4 buffer at 37°C

mAb Captured	mAb Capture Level (RU)	30nM Ag Bound (RU)	k_d (1/s)	$t_{1/2}$ (min)
H4H22034N	310 ± 1.5	150	7.67E-04	15
REGN7541	326 ± 0.4	170	7.34E-05	157
REGN7544	359 ± 1.1	192	4.24E-05	273
REGN7548	356 ± 1.4	215	6.58E-05	176
Isotype control	340 ± 1.4	2	NB*	NB

*NB = no binding detected

Table 17: Binding of different anti-NPR1 monoclonal antibodies (mAbs) to hNPR1-MMH in PBS-T-pH7.4 buffer and the dissociation in PBS-T-pH6.0 buffer at 37°C

mAb Captured	mAb Capture Level (RU)	30nM Ag Bound (RU)	k_d (1/s)	$t_{1/2}$ (min)
H4H22034N	349 ± 0.6	172	1.38E-04	83
REGN7541	369 ± 0.5	193	5.31E-05	217
REGN7544	368 ± 1.1	193	1.58E-05	731
REGN7548	366 ± 1.3	219	1.65E-05	702
Isotype control	344 ± 0.9	2	NB*	NB

*NB = no binding detected

Table 18: Binding of different anti-NPR1 monoclonal antibodies (mAbs) to mfNPR1-MMH in PBS-T-pH7.4 buffer and the dissociation in PBS-T-pH7.4 buffer at 37°C

mAb Captured	mAb Capture Level (RU)	30nM Ag Bound (RU)	k_d (1/s)	$t_{1/2}$ (min)
H4H22034N	308 ± 0.2	139	7.81E-04	15
REGN7541	322 ± 0.5	158	9.48E-05	122
REGN7544	356 ± 1.2	178	3.55E-05	325
REGN7548	354 ± 0.8	203	6.22E-05	186
Isotype control	340 ± 2.2	1	NB*	NB

*NB = no binding detected

Table 19: Binding of different anti-NPR1 monoclonal antibodies (mAbs) to mfNPR1-MMH in PBS-T-pH7.4 buffer and the dissociation in PBS-T-pH6.0 buffer at 37°C

mAb Captured	mAb Capture Level (RU)	30nM Ag Bound (RU)	k_d (1/s)	$t_{1/2}$ (min)
H4H22034N	336 ± 8.2	157	1.54E-04	75
REGN7541	367 ± 0.5	179	6.94E-05	166
REGN7544	363 ± 1.9	181	1.50E-05	768
REGN7548	363 ± 0.8	207	2.67E-05	433
Isotype control	336 ± 4.4	2	NB*	NB

*NB = no binding detected

Example 5: Octet cross-competition between different anti-NPR1 monoclonal antibodies

Experimental Procedure

[0208] Binding competition between different anti-NPR1 monoclonal 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, 1mg/mL BSA, 0.02% NaN₃, 0.05% v/v Surfactant Tween-20, pH7.4 (HBS-EBT) buffer with the plate shaking at a speed of 1000rpm. To assess whether two antibodies are able to compete with one another for binding to their respective epitopes on recombinant human NPR1 extracellular domain expressed with a C-terminal myc-myc-hexahistidine (hNPR1-MMH; SEQ ID NO:74), around 0.64nm of hNPR1-MMH was first captured onto anti-Penta-His antibody coated Octet biosensor tips (ForteBio Inc, # 18-5122) by submerging the biosensor tips in wells containing 40-50µg/mL solution of hNPR1-MMH for 60 seconds.

[0209] The antigen captured biosensor tips were then saturated with a first anti-NPR1 monoclonal antibody (subsequently referred to as mAb-1) by dipping into wells containing 50µg/mL solution of mAb-1 for 4 minutes. The biosensor tips were then subsequently dipped into wells containing 50µg/mL solution of a second anti-NPR1 monoclonal antibody (subsequently referred to as mAb-2) for 3 minutes. The biosensor tips were washed in HBS-EBT 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.

Results

[0210] The response of mAb-2 binding to hNPR1-MMH pre-complexed with mAb-1 was compared to the binding response when the order was reversed (mAb-2 first antibody bound, mAb1 second antibody bound) and competitive/non-competitive behavior of different anti-NPR1 monoclonal antibodies was determined as summarized in Table 20.

Table 20. Cross-competition between different anti-NPR1 monoclonal antibodies for binding to hNPR1-MMH

mAb-1	mAb-2 competing with mAb-1
H4H22034N	H4H22034N
	REGN7541
	REGN7544
	REGN7548
REGN7541	H4H22034N
	REGN7541
	REGN7544
	REGN7548
REGN7544	H4H22034N
	REGN7541
	REGN7544
	REGN7548
REGN7548	H4H22034N
	REGN7541
	REGN7544
	REGN7548

Example 6: Assessment of NPR1 antagonism of antagonistic anti-NPR1 antibodiesExperimental Procedure

[0211] In order to assess the regulation of human natriuretic peptide receptor 1 (hNPR1), a HEK293 cell line stably expressing hNPR1 (amino acids M1 to G1061 of accession #NP_000897.3) with C-term myc and FLAG tags was generated and sorted for high hNPR1 expressing cells. The resulting cell line was named HEK293/hNPR1.MycDDK HS, abbreviated as HEK293/hNPR1, and was maintained in DMEM containing 10% FBS, NEAA, pen/strep/glutamine, 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 Natriuretic peptides in cardiometabolic regulation and disease. Nature Publishing Group, 11(7), 403–412). A homogeneous time resolved fluorescence (HTRF) assay that measures cGMP levels was used to evaluate NPR1 activity.

Analysis of NPR1 antagonism

[0212] To assess NPR1 antagonism, HEK293/hNPR1 cells were plated in 96-well half-area plates at 20,000 cells/well in complete growth media and cultured overnight. The next day, the growth media was replaced with dilution buffer (OptiMEM with 0.1% FBS) containing anti-NPR1 or isotype control antibodies over a range of concentrations (0.017 nM – 1 µM, with an additional condition without antibody) and incubated for 15 minutes at 37°C. After the pre-treatment, HEK293/hNPR1 cells were stimulated with ligand, ANP or BNP, over a range of concentrations (ANP = 0.002 – 2 nM or BNP = 0.0039 – 4 nM, with an additional condition without ligand) made in dilution buffer or dilution buffer containing 0.2 nM ANP or 0.7 nM BNP and incubated at 37°C for 30 min.

[0213] The HTRF assay was performed using a cGMP HTRF kit (Cisbio, #62GM2PEH) according to manufacturer's instructions. The fluorescence intensity was detected using an EnVision multilabel plate reader (Perkin Elmer), and the fluorescence resonance energy transfer (FRET) ratio was calculated according to manufacturer's instructions. The FRET ratios were converted to cGMP concentrations in logarithmic scale according to the cGMP standard curve and analyzed using a 4-parameter logistic equation over a 10-point concentration-response curve to obtain the half maximal inhibitory concentration (IC₅₀) values for the anti-NPR1 antibodies using GraphPad Prism 8. The half maximal effective concentration (EC₅₀) values of the ligand were not calculated due to the limit of

quantitation at high concentrations. The maximal inhibition was calculated with the equation described below:

$$\% \text{ Maximal inhibition} = \frac{[\text{Log cGMP}, M]_{0.2\text{nM ANP or } 0.7\text{nM BNP}} - [\text{Log cGMP}, M]_{1\text{ }\mu\text{M test antibody with ligand}}}{[\text{Log cGMP}, M]_{0.2\text{nM ANP or } 0.7\text{nM BNP}} - [\text{Log cGMP}, M]_{\text{baseline}}} \times 100$$

[0214] In this equation, $[\text{Log cGMP}, M]_{\text{baseline}}$, $[\text{Log cGMP}, M]_{1\text{ }\mu\text{M test antibody with ligand}}$ and $[\text{Log cGMP}, M]_{0.2\text{nM ANP or } 0.7\text{nM BNP}}$ are the cGMP concentration values in logarithmic scale from the cells treated with dilution buffer alone, the highest concentration of the anti-NPR1 antibodies at $1\text{ }\mu\text{M}$ with 0.2 nM ANP or 0.7 nM BNP , and 0.2 nM ANP or 0.7 nM BNP alone respectively.

Determination of the Mode of NPR1 Antagonism

[0215] The mode of anti-NPR1 antibody inhibition of ANP-mediated NPR1 activation was assessed by Schild analysis (Kenakin 1997 Pharmacologic Analysis of Drug–Receptor Interaction. 3rd edition. Philadelphia: Lippincott-Raven) of a cGMP accumulation assay. Conditions were optimized to have the ANP concentration response curves fall in the linear range of the cGMP standard curve. Briefly, HEK293/hNPR1 cells were lifted from the plates using an enzyme free cell dissociation solution. The cell suspension was then replated in low-volume 96-well plates at 1,000 cells/well in dilution buffer. Cells were then pre-treated with anti-NPR1 antibodies in dilution buffer at a fixed concentration of 0 nM , 50 nM , 150 nM or 450 nM for 15 minutes at room temperature. After the pre-treatment, ANP, at a range of concentrations (1.0 pM to $1\text{ }\mu\text{M}$) in dilution buffer (with an additional condition without ANP), was added to the cells and incubated at room temperature for 5 minutes.

[0216] The HTRF assay, detection of fluorescence intensity and calculation of cGMP concentration were all performed as described previously. Using 96-well low-volume plates, the volumes necessary for the cGMP standard curve and detection reagents were half of those used with 96-well half-area plates.

[0217] All assay parameters were analyzed using GraphPad Prism 8. The EC_{50} values of ANP in the presence or absence of anti-NPR1 antibodies were calculated using a 4-parameter logistic equation over a 11-point concentration-response curve. The decrease of ANP maximal response by anti-NPR1 antibodies was calculated using the equation:

$$\begin{aligned} & \% \text{ ANP Maximal response decrease} \\ &= \frac{[\text{Log cGMP}, M]_{1\mu\text{M ANP}} - [\text{Log cGMP}, M]_{1\mu\text{M ANP with 450nM antagonist}}}{[\text{Log cGMP}, M]_{1\mu\text{M ANP}} - [\text{Log cGMP}, M]_{\text{baseline}}} \times 100 \end{aligned}$$

[0218] The Schild slope was determined by Schild plot analysis derived by plotting Log(concentration ratio - 1) on the Y axis against the antagonist concentrations in logarithmic scale on the X axis. Concentration ratio is defined as the EC₅₀ values of ANP in the presence of antagonist at different concentrations divided by the EC₅₀ value of ANP in the absence of antagonist.

Analysis of anti-NPR1 antibody mediated internalization with secondary antibody-drug conjugates in a cell-based cytotoxicity assay

[0219] Internalization of anti-NPR1 antibodies was indirectly assessed by a cytotoxicity assay using a secondary antibody conjugated with cytotoxic payload, Monomethyl auristatin F (MMAF). After binding NPR1 on cells, the anti-NPR1 antibody will internalize with the receptor, resulting in co-internalization of the anti-human Fc fab secondary antibody-drug conjugate (secondary ADC), release of the conjugated cytotoxic payload and killing of cells. Therefore, the extent of cytotoxicity can be used to assess the ability of the antibody to internalize upon target engagement.

[0220] For the assay, HEK293/hNPR1 cells were plated in 96-well white plates at 1,000 cells/well in complete growth media and cultured overnight. The next day, cells were pre-treated with anti-NPR1 or control antibodies over a range of concentrations (serial dilutions from 914 fM to 6 nM in dilution buffer, with an additional condition without antibody) in the presence or absence of 100 nM ANP or 100 nM BNP for 5 minutes at 37°C. After the pre-treatment, the secondary ADC at final concentration of 20 nM was added to HEK293/hNPR1 cells. To evaluate maximal killing in the assay, digitonin at final concentration of 48 µg/ml was added to control wells. The treated cells were incubated at 37°C for three days.

[0221] To measure cell viability, Promega CellTiter-Glo was used according to manufacturer's instructions. The luminescence intensity was detected using an EnVision multilabel plate reader and analyzed using a 4-parameter logistic equation over a 9-point concentration-response curve to obtain the IC₅₀ values of the anti-NPR1 antibodies using GraphPad Prism 8. The percentage of cell viability or cell kill was calculated using the equations described below:

$$\begin{aligned} \% \text{ Viability} &= \frac{RLU_{\text{dilution buf, 100 nM ANP or 100nM BNP alone}} - RLU_{6 \text{ nM test antibody}}}{RLU_{\text{dilution buf, 100 nM ANP or 100nM BNP alone}} - RLU_{\text{digitonin}}} \times 100 \\ \% \text{ Kill} &= 100 - \% \text{ Viability} \end{aligned}$$

Results

Evaluation of the Inhibition of Ligand Mediated NPR1 activation by anti-NPR1 antibodies

[0222] Both ANP and BNP activated HEK293/hNPR1 cells, stimulating cGMP accumulation in a concentration-dependent manner (Figures 1A and 1B). All the anti-NPR1 antibodies significantly blocked 0.2 nM ANP or 0.7 nM BNP induced NPR1 activation with IC_{50} values of 7.0 – 51 nM and maximal inhibition of 82% - 107% (Figures 1A and 1B and Table 21). The isotype control antibody did not show any significant inhibition of 0.2 nM ANP or 0.7 nM BNP induced NPR1 activation (Figures 1A and 1B and Table 21).

Table 21. Anti-NPR1 antibodies significantly inhibited ligand induced NPR1 activation as measured by cGMP accumulation assay

Antibody	With 0.2 nM ANP		With 0.7 nM BNP	
	IC_{50} , M	Max inh., %	IC_{50} , M	Max inh., %
H4H22034N	2.00E-08	82	1.33E-08	99
REGN7541	4.41E-08	90	1.49E-08	100
REGN7544	5.13E-08	92	1.63E-08	107
REGN7548	1.91E-08	92	7.02E-09	103
Control mAb	ND	-1	ND	-9

ND: not determined due to lack of concentration dependent changes in signal; Max Inh.: maximal inhibition.

[0223] Taken together, H4H22034N, REGN7541, REGN7544, and REGN7548 showed significant inhibition of ligand induced NPR1 activation as measured by the cGMP accumulation assay.

Evaluation of the Mode by which anti-NPR1 antibodies Inhibit ANP Mediated NPR1 Activation

[0224] Schild analysis was performed to determine the mode of anti-NPR1 antibody inhibition on ANP-mediated NPR1 activation. In the analysis, a parallel rightward shift of agonist concentration-response curves with a Schild slope of ~1 without altering the maximal response is indicative of a competitive antagonist. In contrast, an unparallel rightward shift of agonist concentration-response curves with a Schild slope not close to one is indicative of a non-competitive antagonist. An antagonist that can change the maximal response of an agonist is defined as an insurmountable antagonist, while an antagonist that does not change the maximal response of an agonist is defined as a surmountable antagonist (Kenakin, 1997).

[0225] Using Schild analysis, H4H22034N, REGN7541, REGN7544 and REGN7548 demonstrated non-competitive inhibition of ANP agonist in a cGMP accumulation assay (Figures 2A-2E). Increasing the concentration of the four anti-NPR1 antibodies

resulted in unparallel rightward shifts of ANP concentration-response curves with Schild slopes that were not close to 1. In addition, inhibition by H4H22034N did not change the maximal response of ANP while inhibition by REGN7541, REGN7544 and REGN7548 decreased the maximal response of ANP to 10 – 62% (Figures 2A-2E and Table 22).

Table 22. Anti-NPR1 antibodies showed non-competitive antagonism in Schild analysis

Antibody	Schild slope	% ANP Max decrease
H4H22034N	0.05	0.1
REGN7541	2.0	25.5
REGN7544	2.6	61.6
REGN7548	1.4	10.0

[0226] Taken together, H4H22034N, REGN7541, REGN7544 and REGN7548 demonstrated non-competitive antagonism with various effects on the maximum ligand response.

Evaluation of anti-NPR1 antibodies Induced NPR1 internalization

[0227] Internalization of the anti-NPR1 antibodies was assessed using a secondary ADC mediated cytotoxicity assay in the presence or absence of ligand. In the absence of ligand, all the anti-NPR1 antibodies induced NPR1 internalization in a concentration-dependent manner with IC₅₀ values of 58 – 243 pM and maximal killing of 82% – 85% (Figures 3A-3C and Table 23). In the presence of 100 nM ligand, the anti-NPR1 antibodies induced NPR1 internalization with IC₅₀ values of 132 – 703 pM and maximal killing of 50% – 82%. The isotype control antibody did not show any significant NPR1 internalization in the presence or absence of ligand (Figures 3A-3C and Table 23).

Table 23. Internalization of anti-NPR1 antibodies in the presence or absence of ligand as measured by secondary ADC mediated cytotoxicity assay

Antibody	No ligand		With 100nM ANP		With 100nM BNP	
	IC ₅₀ , M	Max Kill, %	IC ₅₀ , M	Max Kill, %	IC ₅₀ , M	Max Kill, %
H4H22034N	5.84E-11	85	1.85E-10	82	1.32E-10	82
REGN7541	1.95E-10	83	6.67E-10	60	5.55E-10	78
REGN7544	2.43E-10	82	7.03E-10	50	6.77E-10	74
REGN7548	8.69E-11	85	3.97E-10	74	2.95E-10	79
Control mAb	ND	0.01	ND	-4	ND	0.5

ND: not determined due to lack of concentration dependent changes in signal.

[0228] Taken together, H4H22034N, REGN7541, REGN7544 and REGN7548 showed anti-NPR1 antibody internalization of NPR1 in the presence or absence of ligand as measured by secondary ADC mediated cytotoxicity assay.

Example 7: Potency and specificity of anti-NPR1 antibodies binding to NPR1 alone or in complex with ANP or BNP on the Cell Surface Using Electrochemiluminescence-Based Detection

Experimental Procedure

[0229] The ability of anti-human NPR1 monoclonal antibodies to bind human or monkey (*Macaca fascicularis*) NPR1 (hNPR1 or mfNPR1) expressing cells in the presence or absence of human atrial (ANP) or human brain (BNP) natriuretic peptide ligands was determined using an electrochemiluminescence (ECL) based immunoassay.

[0230] Briefly, HEK293/hNPR1 expressing cells were generated by transfecting human embryonic kidney (HEK) 293 cells with the neomycin resistant pLVX.hNPR1.myc.DDK plasmid encoding human NPR1 (amino acids M1-G1061, UniProtKB - P16066). Similarly, HEK293/mfNPR1 cells were generated by transfecting HEK293 cells with the neomycin resistant pRG984 plasmid encoding full-length monkey NPR1 (amino acids M1-G1061, accession number XP_005541809.1). The non-transfected HEK293 cell line, which showed no detectable binding of a commercial anti-hNRP1 antibody by fluorescence activated cell sorting (FACS), was included in the experiment as a non-specific binding control.

[0231] Experiments were carried out according to the following procedure. Cultures of the cell lines described above were rinsed once in 1xPBS buffer without $\text{Ca}^{2+}/\text{Mg}^{2+}$ and incubated for 10 minutes at 37°C with Enzyme Free Cell Dissociation Solution to detach cells from the flask. The collected cell pellets were washed once with and resuspended in 1xPBS with $\text{Ca}^{2+}/\text{Mg}^{2+}$ and then cells were counted with a Cellometer™ Auto T4 cell counter (Nexcelom Bioscience, Lawrence, MA). Approximately 2.0×10^4 cells per well were seeded onto 96-well carbon electrode plates (Meso Scale Diagnostics, Rockville, MD) and incubated for 1 hour at 37°C. Nonspecific binding sites were blocked using 2% BSA (w/v) in 1xPBS containing $\text{Ca}^{2+}/\text{Mg}^{2+}$ for 1 h at room temperature. Subsequently, HEK293/hNPR1 and HEK293/mfNPR1 cells were incubated for 0.5 hour at room temperature with 10nM hANP (Tocris, Minneapolis, MN), 100nM hBNP (Tocris, Minneapolis, MN), or sample dilution buffer alone, while HEK293 cells were treated with sample dilution buffer only. Without washing, serial dilutions of anti-NPR1, Comparator 1 or the isotype control antibody, ranging from 1.7pM to 100nM, or buffer containing no antibody were added to the cells followed by 1 hour incubation at room temperature. Plates were then washed to remove unbound antibodies, hANP, and hBNP using an AquaMax2000 plate washer with a cell washing head (MDS Analytical Technologies, Sunnyvale, CA). The plate-bound antibodies were detected with a SULFO-TAG™-conjugated

polyclonal goat anti-human IgG antibody specific for heavy and light chains (Jackson ImmunoResearch, West Grove, PA) that was incubated with the cells for 1 hour at room temperature.

[0232] After washes, plates were developed with Read Buffer (Meso Scale Diagnostics, Rockville, MD) according to manufacturer's recommended procedure and luminescent signals were recorded with a SECTOR Imager 600 (Meso Scale Diagnostics, Rockville, MD). The binding signals in a unit of RLU were analyzed as a function of the antibody concentration and data were fitted with a sigmoidal (four-parameter logistic) dose-response model using R statistical package (open source). The EC₅₀ value, defined as the concentration of antibody at which 50% of the maximal binding signal is detected, was determined to indicate binding potency of the anti-NPR1 antibodies to NPR1 expressing cells with or without ANP or BNP complexed to NPR1 on the cell surface.

Results

[0233] The ability of the anti-NPR1 monoclonal antibodies to bind specifically to the surface of HEK293 cells engineered to express human or monkey NPR1 in the presence or absence of ANP or BNP was assessed using an electrochemiluminescence-based immunoassay. The concentration dependence of antibody binding was analyzed and EC₅₀ values were determined. The results are summarized in Table 24.

Table 24: Potency of Anti-NPR1 Antibodies Binding to NPR1 Expressing Cells in the Presence or Absence of NPR1 Ligands.

Antibody	Experiment	Cell Binding Potency, EC ₅₀ (M)					
		HEK293/hNPR1			HEK293/mfNPR1		
		No ligand added	+10nM hANP	+100nM hBNP	No ligand added	+10nM hANP	+100nM hBNP
H4H22034	1	3.4E-10	5.6E-10	4.3E-10	3.3E-10	6.7E-10	4.2E-10
REGN7541	1	1.3E-09	2.1E-09	1.1E-09	1.4E-09	2.9E-09	9.2E-10
REGN7544	1	1.7E-09	2.9E-09	1.5E-09	1.7E-09	4.2E-09	1.7E-09
REGN7548	2	6.8E-10	1.2E-09	8.1E-10	5.6E-10	1.2E-09	5.6E-10
Comparator 1	2	NB	5.7E-10	1.8E-09	NB	INC	INC
Isotype control	2	NB	NB	NB	NB	NB	NB

Abbreviations: NB – no binding; INC – inconclusive: EC₅₀ value could not be calculated due to the data lacking sigmoidal curvature to be fitted with the sigmoidal dose-response model (4 parameters sigmoidal curve fit), but the antibody displayed marginal specific binding to mfNPR1 expressing cells in the presence of 10nM hANP or 100nM hBNP.

[0234] Four anti-NPR1 antibodies (H4H22034, REGN7541, REGN7544, REGN7548) of the disclosure bound to the human NPR1 engineered cells in the presence of 10nM hANP or 100nM hBNP. The potency of these antibodies on HEK293/hNPR1.myc.DDK cells in the presence of 10nM hANP or 100nM hBNP

ranged from EC₅₀ values of 0.56nM to 2.9nM or 0.43nM to 1.5nM, respectively (Table 24). Similar binding potencies were determined on hNPR1 expressing cells in the absence of hANP and hBNP, with EC₅₀ values ranging from 0.34nM to 1.7nM. No detectable binding was observed for these antibodies on parental HEK293 cells under the same experimental conditions. These results suggest that the antibodies are NPR1 specific binders, and that the human NPR1 binding potency for these antibodies is not affected by the presence of ANP or BNP.

[0235] In contrast, the Comparator 1 exhibited binding specificity to hNPR1 cells only when hANP or hBNP were present (10nM hANP or 100nM hBNP), with EC₅₀ values of 0.57nM and 1.8nM, respectively, and no detectable binding was observed in the absence of hANP and hBNP, hence it is classified as NPR1-ANP/BNP complex only binder.

[0236] Anti-NPR1 antibodies H4H22034, REGN7541, REGN7544, REGN7548 also bound to the monkey NPR1 expressing cells (HEK293/mfNPR1) with EC₅₀ values ranging from 0.33nM to 1.7nM. Similar to hNPR1 cells, addition of 10nM hANP or 100nM of hBNP did not affect anti-NPR1 antibody binding to HEK293/mfNPR1 cells; EC₅₀ values for antibody binding ranged from 0.67nM to 4.2nM or 0.42nM to 1.7nM in the presence of hANP and hBNP, respectively (Table 24) and no binding was detected for any of the antibodies on the parental HEK293 cells. In contrast, the Comparator 1 specifically bound to HEK293/mfNPR1 engineered cells only in the presence of 10nM hANP or 100nM hBNP, however, EC₅₀ values could not be determined due to the concentration dependent binding curve lacking sigmoidal curvature and data could not be fitted with the binding model.

[0237] In the experiment the isotype control did not bind to HEK293/hNPR1, HEK293/mfNPR1 nor HEK293 cells.

Example 8: Characterization of the acute effects on systemic blood pressure following a single 25 mg/kg intravenous dose of an NPR1 antagonist mAb in normotensive telemetered NPR1^{hu/hu} mice

Experimental Procedure

[0238] The objectives of this study were to assess the acute effects of NPR1 antagonist antibodies on baseline systemic blood pressure in telemetered normotensive NPR1^{hu/hu} mice. Male NPR1^{hu/hu} (n=30) mice aged ~14-16 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 1-5) based on body weight (Table 25). 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*.

Table 25. Summary of Doses and Dose Groups

Group No.	Test or Control Article	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Number of Animals
				Males
1	PBS	0	5	6
2	H4H22034N	25		6
3	REGN7541			6
4	REGN7544			6
5	REGN7548			6

[0239] The test proteins were administered to the appropriate animals by single intravenous injection on Day 0. The dose volume for each animal was based on the most recent body weight measurement.

[0240] Systolic pressure, diastolic pressure, mean arterial pressure, pulse pressure and heart rate were collected for 10 seconds every minute for the duration of the testing period. Telemetry data are presented as mean 60-min or 24-hour values. All data are presented as mean±SEM.

Results

[0241] The *in vivo* assessment demonstrated that when compared to PBS control, animals dosed with an NPR1 antagonist antibody had rapid and persistent increases of systemic blood pressure (Figure 4) following a single intravenous dose of any of the antibodies assessed. The pressures changes occurred almost immediately (Figure 4), and last through day 7 of the study, the duration of the experiment (Figure 5). The magnitude of acute blood pressure increase as assessed by mean (of 48-hours post-dose) systolic blood pressure change from baseline ranged from +7.64±1.13 (REGN7541) to +9.81±1.03 (H4H22034N) mmHg. The magnitude of blood pressure increase as assessed by mean (of 7-days post-dose) systolic blood pressure change from baseline ranged from +7.65±1.03 (REGN7548) to +9.55±1.16 (H4H22034N) mmHg.

[0242] Diastolic, mean arterial and pulse pressures (Table 26 and Table 27) were also significantly changed following dosing of NPR1 blocking mAbs, with magnitudes and durations of effect consistent with that observed and reported on systolic blood pressures. Heart rate responses were variable (Table 26 and Table 27), with acute changes generally trending toward a greater reduction in rate relative to baseline. These changes are consistent with the observed increase in pressure. Assessment of

heart rate out 7 days demonstrated a significant relative increase for animals that received REGN7548.

Table 26. 48-Hour Post Dose Mean Change from Baseline Blood Pressures and Heart Rates

Dose Group:	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
PBS	0	0.08±1.0	0.04±0.89	0.03±0.95	0.04±0.17	-10.02±6.85
H4H22034N	25	9.81±1.03****	4.30±0.89****	7.22±0.96****	5.51±0.21****	-22.80±6.75
REGN7541		7.64±1.13****	3.01±0.95****	5.62±1.04****	4.55±0.28****	-14.38±8.59
REGN7544		8.68±1.03****	3.79±0.94****	6.42±0.98****	4.89±0.22****	-17.91±7.22
REGN7548		7.87±1.08****	2.78±0.94****	5.55±1.01****	5.09±0.22****	-11.44±6.73

*Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 25 mg/kg intravenous injection of NPR1 antagonist mAb or PBS as described in Table 25. All values are mean ± SEM, n=6 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. PBS.*

Table 27. 7-day Post Dose Mean Change from Baseline Blood Pressures and Heart Rates

Dose Group:	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
PBS	0	-0.82±0.24	-0.26±0.17	-0.66±0.20	-0.56±0.15	-21.98±3.75
H4H22034N	25	9.55±1.16****	4.79±0.49****	7.19±0.83****	4.76±0.70****	-22.75±3.93
REGN7541		8.89±1.54****	5.44±1.12****	7.19±1.27****	3.45±0.64****	-18.41±4.51
REGN7544		7.97±1.03****	3.85±0.64****	5.96±0.80****	4.12±0.63****	-22.53±4.89
REGN7548		7.65±1.03****	3.44±0.47****	5.66±0.75****	4.21±0.62****	-9.54±3.25****

*Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 25 mg/kg intravenous injection of NPR1 antagonist mAb or PBS as described in Table 25. All values are mean ± SEM, n=6 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. PBS.*

[0243] NPR1 antagonist antibodies H4H22034N, REGN7541, REGN7544 and REGN7548 significantly and rapidly increased systemic blood pressures within hours following a single subcutaneous injection to normotensive NPR1^{hu/hu} mice. The observed hemodynamic effects lasted the duration of the 7 day experiment.

Example 9: Characterization of the effect on systemic blood pressure of a single 1 or 25 mg/kg dose of an NPR1 antagonist mAb in normotensive NPR1^{hu/hu} mice

Experimental Procedure

[0244] The objectives of this study were to assess the effects of NPR1 antagonist antibodies on baseline systemic blood pressure in telemetered normotensive NPR1^{hu/hu} mice. Male NPR1^{hu/hu} (n=54) mice aged ~20-24 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 1-10) based on body weight and baseline

systolic and pulse pressures, prior to being assigned to group (Table 28). 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.

Table 28. Summary of Doses and Dose Groups

Group No.	Test or Control Article	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Number of Animals
				Males
1	PBS	0	5	5
2	IgG4P isotype control mAb	25		5
3	H4H22034N	1		5
4	REGN7541			5
5	REGN7544			5
6	REGN7548			5
7	H4H22034N	25		6
8	REGN7541			6
9	REGN7544			6
10	REGN7548			6

[0245] The test proteins were administered to the appropriate animals by single subcutaneous injection on Day 0. The dose volume for each animal was based on the most recent body weight measurement.

[0246] Systolic pressure, diastolic pressure, mean arterial pressure, pulse pressure and heart rate were collected for 10 seconds every 10 minutes for the duration of the testing period. Telemetry data are presented as mean 24-hour values. All data are presented as mean±SEM.

Results

[0247] The *in vivo* characterization of NPR1 antagonist antibodies demonstrated that when compared to IgG4P isotype and PBS control-dosed animals, NPR1 antagonist-dosed NPR1^{hu/hu} mice presented with significant and persistent increases of systemic blood pressure following a single subcutaneous dose of 1 or 25 mg/kg of any NPR1 antagonist antibody evaluated (Table 29, Table 30, Figure 6, Figure 7). The magnitude of blood pressure increase following a single subcutaneous 1 mg/kg dose as assessed by mean (of days 0 to 14 post-dose) systolic blood pressure change from baseline ranged from +6.81±0.76 (REGN7548) to +11.22±0.93 (REGN7541) mmHg (Table 29 and Figure 6). The magnitude of blood pressure increase following a single subcutaneous 25 mg/kg dose as assessed by mean (of days 0 to 14 post-dose)

systolic blood pressure change from baseline ranged from $+8.82 \pm 0.64$ (H4H22034) to $+9.76 \pm 0.92$ (REGN7544) mmHg (Table 29 and Figure 7).

Table 29. 14-day Post Dose Mean Change from Baseline Blood Pressures and Heart Rates

Dose Group:	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
PBS	0	1.69 ± 0.23	-0.39 ± 0.14	0.57 ± 0.13	2.07 ± 0.19	-6.52 ± 2.45
IgG4P isotype control mAb	25	$-0.53 \pm 0.27^{****}$	0.26 ± 0.26	-0.03 ± 0.26	$-0.80 \pm 0.09^{****}$	-4.92 ± 1.78
H4H22034N	1	$7.10 \pm 0.60^{****}$	$3.81 \pm 0.43^{****}$	$5.55 \pm 0.51^{****}$	3.29 ± 0.30	-16.51 ± 3.24
REGN7541		$11.22 \pm 0.93^{****}$	$7.23 \pm 0.69^{****}$	$9.29 \pm 0.78^{****}$	$4.0 \pm 0.41^{***}$	-0.48 ± 2.74
REGN7544		$7.39 \pm 0.67^{****}$	$4.00 \pm 0.39^{****}$	$5.82 \pm 0.51^{****}$	$3.39 \pm 0.37^*$	-1.43 ± 2.74
REGN7548		$6.81 \pm 0.76^{****}$	$3.91 \pm 0.84^{****}$	$5.36 \pm 0.68^{****}$	2.93 ± 0.68	-9.06 ± 1.90
H4H22034N	25	$8.82 \pm 0.64^{****}$	$4.55 \pm 0.58^{****}$	$6.70 \pm 0.56^{****}$	$4.29 \pm 0.32^{****}$	-7.45 ± 2.48
REGN7541		$9.24 \pm 0.76^{****}$	$5.77 \pm 0.61^{****}$	$7.47 \pm 0.66^{****}$	3.50 ± 0.33	-12.63 ± 2.45
REGN7544		$9.76 \pm 0.92^{****}$	$3.13 \pm 0.42^{****}$	$6.51 \pm 0.64^{****}$	$6.66 \pm 0.54^{****}$	$-21.61 \pm 3.17^{****}$
REGN7548		$8.37 \pm 0.69^{****}$	$3.61 \pm 0.38^{****}$	$6.14 \pm 0.52^{****}$	$4.77 \pm 0.35^{****}$	-12.43 ± 2.32

Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 1 or 25 mg/kg subcutaneous injection of NPR1 antagonist mAb, isotype control or PBS as described in Table 28. All values are mean $\pm$ SEM, n=4-5 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. PBS.

Table 30. 15- through 27-day Post Dose Mean Change from Baseline Blood Pressures and Heart Rates

Dose Group:	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
PBS	0	3.37 ± 0.40	-0.17 ± 0.35	1.45 ± 0.36	3.55 ± 0.07	-30.93 ± 1.85
IgG4P isotype control mAb	25	$0.50 \pm 0.38^{****}$	0.44 ± 0.35	0.49 ± 0.35	$0.07 \pm 0.07^{****}$	-25.24 ± 1.95
H4H22034N	1	4.04 ± 0.30	1.50 ± 0.28	2.62 ± 0.28	2.55 ± 0.20	$-41.12 \pm 2.29^*$
REGN7541		$9.03 \pm 0.32^{****}$	$5.83 \pm 0.51^{****}$	$7.44 \pm 0.49^{****}$	3.60 ± 0.21	-24.76 ± 1.89
REGN7544		$5.20 \pm 0.54^*$	$5.02 \pm 0.42^{****}$	$4.95 \pm 0.47^{****}$	$0.16 \pm 0.32^{****}$	$-15.75 \pm 2.68^{**}$
REGN7548		4.79 ± 0.34	$3.47 \pm 0.31^{****}$	$3.92 \pm 0.30^{***}$	$1.36 \pm 0.19^*$	$-19.30 \pm 1.86^{**}$
H4H22034N	25	$10.53 \pm 0.43^{****}$	$5.38 \pm 0.42^{****}$	$7.78 \pm 0.42^{****}$	$5.17 \pm 0.15^{**}$	-29.57 ± 2.31
REGN7541		$12.05 \pm 0.32^{****}$	$9.36 \pm 0.44^{****}$	$10.29 \pm 0.35^{****}$	2.73 ± 0.23	-31.24 ± 1.97
REGN7544		$14.20 \pm 0.42^{****}$	$4.94 \pm 0.36^{****}$	$9.43 \pm 0.37^{****}$	$9.30 \pm 0.14^{****}$	$-41.43 \pm 2.15^{**}$
REGN7548		$10.58 \pm 0.29^{****}$	$4.71 \pm 0.25^{****}$	$7.52 \pm 0.26^{****}$	$5.89 \pm 0.10^{****}$	-35.53 ± 1.75

Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 1 or 25 mg/kg subcutaneous injection of NPR1 antagonist mAb, isotype control or PBS as described in Table 28. All values are mean $\pm$ SEM, n=4-5 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. PBS.

[0248] Chronic effects of these NPR1 antagonist antibodies were evaluated over 27 days (Table 30 and Figure 6 and Figure 7). Following a single subcutaneous 1 mg/kg injection on day 0, systemic blood pressure remained significantly elevated for 3 of the

4 NPR1 blocking mAb's evaluated for the duration of the testing period (Table 30 and Figure 6). The magnitude of blood pressure increase following a single subcutaneous 1 mg/kg dose as assessed by mean (of days 15 to 27 post-dose) systolic blood pressure change from baseline ranged from $+4.04 \pm 0.30$ (H4H22034) to $+9.03 \pm 0.32$ (REGN7541) mmHg. Following a single subcutaneous 25 mg/kg injection on day 0, systemic blood pressure remained significantly elevated for all NPR1 blocking mAb's evaluated. The magnitude of blood pressure increase following a single subcutaneous 25 mg/kg dose as assessed by mean (of days 15 to 27 post-dose) systolic blood pressure change from baseline ranged from $+10.53 \pm 0.43$ (H4H22034) to $+14.2 \pm 0.42$ (REGN7544) mmHg. Heart rate responses were variable (Table 29 and Table 30), with general trends of rate reductions consistent with the observed increase of systemic blood pressures. Diastolic, mean arterial and pulse pressures (Table 29 and Table 30) were also significantly changed following dosing of NPR1 blocking mAbs, with magnitudes and durations of effect consistent with that observed and reported on systolic blood pressures.

[0249] NPR1 antagonist antibodies H4H22034N, REGN7541, REGN7544 and REGN7548 significantly increased systemic blood pressures for up to 27 days following a single subcutaneous injection to normotensive NPR1^{hu/hu} mice.

Example 10: Characterization of the effect on systemic blood pressure of overexpression of ANP via hydrodynamic DNA delivery and ability to reverse effects following a single 25 mg/kg dose of an NPR1 antagonist mAb in hypotensive NPR1^{hu/hu} mice

Experimental Procedure

[0250] The objectives of this study were to assess the effects of NPR1 antagonist antibodies and their ability to reverse the blood pressure-lowering effects of ANP over expression in telemetered hypotensive NPR1^{hu/hu} mice. Male NPR1^{hu/hu} (n=48) mice aged ~12-16 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 1-6) based on body weight (Table 31). 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.

Table 31. Summary of Doses and Dose Groups

Group No.	HDD Construct	HDD Construct Dose (µg DNA, i.v.)	Test or Control Article	Dose Level (mg/kg/dose, i.v.)	Dose Volume (mL/kg)	Number of Animals
						Males
1	Control plasmid	50	Isotype control mAb	25	5	8
2	ANP plasmid		H4H22034N			8
3			REGN7541			8
4			REGN7544			8
5			REGN7548			8
6						

[0251] The HDD plasmids and test proteins were administered to the appropriate animals by single intravenous injections on Day 0 and Day 7, respectively. The dose volume for administration of test proteins was based on the most recent body weight measurement.

[0252] Systolic pressure, diastolic pressure, mean arterial pressure, pulse pressure and heart rate were collected for 10 seconds every minute for the duration of the testing period. Telemetry data are presented as mean 24-hour values. All data are presented as mean $\pm$ SEM.

Results

[0253] The *in vivo* characterization of NPR1 antagonist antibodies demonstrated that when compared to IgG4P isotype control-dosed animals, NPR1 antagonist antibodies were able to rapidly and persistently normalize the ANP overexpression-induced reductions of systemic blood pressure in NPR1^{hu/hu} mice (Table 32 and Table 33). Serum NTproANP concentrations were significantly and persistently increased for the duration of the testing period (Table 34), indicating effective overexpression of ANP. The magnitude of blood pressure increase in ANP HDD-induced hypotensive mice following a single intravenous 25 mg/kg dose of an NPR1 blocker as assessed by mean (of days 7 to 28 post-dose) systolic blood pressure change from baseline ranged from -9.27 $\pm$ 0.61 (H4H22034N) to +10.26 $\pm$ 1.48 (REGN7544) mmHg (Table 33 and Figure 8) relative to pre-ANP HDD dosing.

Table 32. 7-day Post HDD Dose Mean Change from Baseline Blood Pressures and Heart Rates

HDD Group:	Test Article	Test Article Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
Control plasmid	Isotype control mAb	25	-2.83±0.94	-0.79±0.88	-1.75±0.84	-2.05±0.43	0.36±6.65
ANP plasmid	H4H22034N		-27.31±3.53****	-14.21±1.75****	-21.65±2.72****	-13.00±1.82****	29.90±11.10****
	REGN7541		-26.27±3.57****	-12.94±1.74****	-20.13±2.69****	-13.23±1.86****	32.31±14.17****
	REGN7544		-26.92±3.58****	-13.92±1.84****	-20.70±2.77****	-12.96±1.78****	21.84±10.08*
	REGN7548		-28.18±3.73****	-14.57±1.79****	-21.99±2.80****	-13.62±2.04****	18.67±17.90
			-25.40±3.12****	-12.65±1.44****	-19.72±2.35****	-12.70±1.72****	27.46±11.94**

Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 50 ug HDD dose of control or ANP plasmid followed by a single 25 mg/kg intravenous injection of NPR1 antagonist mAb or isotype control as described in Table 31. All values are mean ± SEM, n=4-7 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. Control Plasmid (pHDD1) + Isotype control.

Table 33. 8 through 28-day Post HDD Dose Mean Change from Baseline Blood Pressures and Heart Rates

HDD Group:	Test Article	Test Article Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
Control plasmid	Isotype control mAb	25	-0.93±0.53****	0.81±0.46****	-0.18±0.47****	-1.75±0.22****	-14.04±1.86****
ANP plasmid	H4H22034N		-30.40±0.51	-15.68±0.41	-23.77±0.45	-14.64±0.18	10.49±1.77
	REGN7541		-9.27±0.61****	-6.21±0.61****	-7.67±0.57****	-3.04±0.24****	-23.86±2.40****
	REGN7544		6.02±0.85****	0.43±0.54****	3.25±0.64****	5.59±0.54****	-51.16±3.86****
	REGN7548		10.26±1.48****	2.95±0.87****	6.45±1.12****	7.32±0.72****	-65.29±5.12****
			1.44±0.80****	-1.29±0.65****	0.07±0.67****	2.73±0.21****	-41.61±2.57****

Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 50 ug HDD dose of control or ANP plasmid followed by a single 25 mg/kg intravenous injection of NPR1 antagonist mAb or isotype control as described in Table 31. All values are mean ± SEM, n=4-7 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. Control plasmid (pHDD1) + Isotype control.

Table 34. Days 14, 21 and 28 Serum NTproANP Concentrations

HDD Group:	Test Article	Test Article Dose (mg/kg)	Serum NTproANP Concentrations (nMol/L)		
			Day 14	Day 21	Day 28
Control plasmid	Isotype control mAb	25	0.61±0.07	0.85±0.15	0.49±0.08
ANP plasmid	H4H22034N		5.21±1.00****	5.54±0.55****	5.45±0.96****
	REGN7541		4.74±0.62***	4.76±0.61***	4.71±0.56***
	REGN7544		5.51±0.84****	5.26±0.85***	4.96±0.87***
	REGN7548		6.88±0.41****	5.58±0.77****	6.40±0.42****
			6.07±0.29****	5.44±0.88****	5.78±0.21****

Telemetered normotensive NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 50 ug HDD dose of control or ANP plasmid followed by a single 25 mg/kg intravenous injection of NPR1 antagonist mAb or isotype control as described in Table 31. All values are mean ± SEM, n=5-8 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. Control plasmid (pHDD1) + Isotype control.

[0254] Consistent with the significant and persistent reductions of systemic pressures and likely reductions of left ventricular afterload, absolute and relative heart weights were significantly reduced in mice that had received ANP HDD (Figures 9A and 9B). Hearts of animals that received an NPR1 blocking mAb were not different than normotensive controls and indicate an effective increase in systemic and ventricular pressures.

[0255] NPR1 antagonist antibodies H4H22034N, REGN7541, REGN7544 and REGN7548 significantly and persistently increased systemic blood pressures for up to 28 days in ANP HDD-induced hypotensive NPR1^{hu/hu} mice.

Example 11: NPR1 blockade in preventative and therapeutic dosing with LPS induced shock model in telemetered NPR1^{hu/hu} mice

Experimental Procedure

[0256] The objectives of this study were to assess the effects of NPR1 antagonist antibodies dosed preventatively or therapeutically to telemetered NPR1^{hu/hu} mice that had hypotension resulting from the administration of LPS. Male NPR1^{hu/hu} (n=58) mice aged ~12-17 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 1-6) based on body weight and systolic and pulse pressures, prior to being assigned to group (Table 35). 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*.

Table 35. Summary of Doses and Dose Groups

Group No.	Number of Animals	Challenge	Test or Control Article	Dose Level (mg/kg/ i.v.)	Dose Volume (mL/kg)
	Males				
1	8	Saline	PBS	0	5
2	10	LPS (5 mg/kg, i.p.)	REGN7544	25	
3	10		REGN7548		
4	10		*Preventative* - REGN7544		
5	10		*Preventative* - REGN7548		
6	10				

[0257] Animals were administered a single intraperitoneal injection of 5 mg/kg LPS or PBS on day 0. The test proteins were administered to the appropriate animals by

single intravenous injection ~24 hours pre- or 8 hours post-LPS dose. The dose volume for each animal was based on the most recent body weight measurement.

[0258] Systolic pressure, diastolic pressure, mean arterial pressure, pulse pressure and heart rate were collected for 10 seconds every 10 minutes for the duration of the testing period. Telemetry data are presented as mean 24-hour values. All data are presented as mean±SEM.

Results

[0259] The *in vivo* characterization of NPR1 antagonist antibodies dosed preventatively or therapeutically to LPS-induced hypotensive NPR1^{hu/hu} mice showed significant and persistent increases of systemic blood pressure (Table 36, Table 37, Figure 10). Animals dosed preventatively presented with rapid increases of pressure (Table 36). The magnitude of blood pressure increase in the preventative arm of the study following a single intravenous 25 mg/kg dose as assessed by mean (of 24 hours prior to through time of LPS dose) systolic blood pressure change from baseline ranged from +7.24±1.18 (REGN7548) to +9.07±1.10 (REGN7541) mmHg (Table 36 and Figure 10). The magnitude of blood pressure increase following a single intravenous 25 mg/kg dose administered 8 hours after LPS dosing as assessed by mean (of 8 hours to 48 hours post-LPS dose) systolic blood pressure change from baseline ranged from -1.49±2.244 (REGN7548) to -1.49±2.24 (REGN7544) mmHg (Table 37). Pulse pressures in therapeutically dosed animals as assessed by mean (of 8 hours to 48 hours post-LPS dose) pulse pressured pressure change from baseline ranged from 0.27±0.93 (REGN7548) to -0.40±0.91 (REGN7544) mmHg (Table 37, Figure 10).

Table 36. 24-hour pre to time 0 prior to LPS Dose Mean Change from Baseline Blood Pressures and Heart Rates

Challenge	Dose Group:	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
Saline	PBS	0	0.14±1.2	-0.13±1.02	0.03±1.10	0.30±0.23***	1.85±8.06
LPS	PBS		-0.90±1.2	0.26±1.06	-0.37±1.15	-1.13±0.19	4.89±7.91
LPS	*Preventative* -REGN7544	25	9.07±1.10****	3.49±0.96***	6.65±0.99****	5.60±0.32****	-2.57±5.84
LPS	*Preventative* -REGN7548		7.24±1.18****	1.93±1.08	4.93±1.09****	5.30±0.24****	-2.93±5.31
LPS	REGN7544		-0.04±1.36	2.46±1.28	1.08±1.31	-2.55±0.20**	-0.57±8.27
LPS	REGN7548		-0.44±1.26	-0.49±1.11	-0.41±1.18	0.05±0.18****	2.85±8.05

*Telemetered NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 5 mg/kg intraperitoneal injection of LPS or saline and a single intravenous injection of an NPR1 antagonist mAb or PBS ~24hours pre- or 8 hours post-LPS dose as described in Table 35. All values are mean ± SEM, n=7-10 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. LPS + PBS.*

Table 37. 8- to 48-hour Post LPS Dose Mean Change from Baseline Blood Pressures and Heart Rates

Challenge	Dose Group:	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean Arterial (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
Saline	PBS	0	0.66±0.68****	0.10±0.69****	-0.15±0.71****	0.56±0.13****	-41.12±6.54****
LPS	PBS		-7.52±1.75	-3.33±1.34	-5.77±1.48	-4.19±0.54	-10.32±5.50
LPS	*Preventative* -REGN7544	25	-3.32±2.28**	-3.41±1.69	-3.88±1.93	0.24±0.85****	-39.73±3.84****
LPS	*Preventative* -REGN7548		-6.13±2.19	-5.10±1.48	-5.84±1.76	-1.03±0.95****	-4.37±5.63
LPS	REGN7544		-1.49±2.46****	-1.92±1.71	-1.57±2.03***	-0.40±0.91****	-66.59±9.70****
LPS	REGN7548		-1.49±2.24****	-1.50±1.60	-1.63±1.85**	0.27±0.93****	-69.46±8.02****

*Telemetered NPR1^{hu/hu} mice were randomized into groups based on body weight. Animals were given a single 5 mg/kg intraperitoneal injection of LPS or saline and a single intravenous injection of an NPR1 antagonist mAb or PBS ~24 hours pre- or 8 hours post-LPS dose as described in Table 35. All values are mean ± SEM, n=7-10 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. LPS + PBS.*

[0260] NPR1 antagonist antibodies REGN7544 and REGN7548 significantly and persistently increased systemic blood pressures when administered preventatively or therapeutically to mice that developed LPS-induced hypotension following a single intraperitoneal dose of LPS.

Example 12: Each NPR1 monomer binds one REGN7544 Fab

Experimental Procedure

Complex formation of NPR1 + REGN7544 Fab + ANP

[0261] Human NPR1 extracellular domain with a C-terminal myc-myc-6xHis tag (hNPR1-mmh; SEQ ID NO: 74) was mixed with Atrial Natriuretic factor (ANP, Tocris) and REGN7544 Fab in a molar ratio of 1 hNPR1-mmh + 2 ANP + 1 REGN7544 Fab. The complex was incubated overnight at 4°C then purified over a Superdex 200 increase 10/300 GL gel filtration column equilibrated with 50 mM Tris pH 7.5, 150 mM NaCl. Peak fractions were collected and concentrated and concentrated using a 10kDa MWCO centrifugal concentrator (Amicon).

Cryo EM grid preparation and data collection

[0262] The NPR1-ANP-REGN7544 complex was diluted to 0.87 mg/ml, and Poly (Maleic Anhydride-alt-1-Decene) substituted with 3-(Dimethylamino) Propylamine (PMAL-C8; Anatrace Cat. #P5008) was added to 0.15% final concentration. The sample was deposited onto a freshly plasma cleaned UltrAufoil grid (Quantifoil GmbH). Excess solution was blotted away with filter paper and plunge frozen into liquid ethane using a Vitrobot Mark IV (Thermo Fisher Part #1086439). The grid was loaded into a Titan Krios G3i (Thermo Fisher, Part #1137337) equipped with a Bioquantum energy filter + K3 direct electron detector (Gatan Inc, Part #1147213). Movies were collected using EPU v 2.7 (Thermo Fisher) at 105,000x magnification, corresponding to a pixel size of 0.86 Å. A dose rate of 15 electrons per pixel per second was used, and each

movie was 2 seconds and 46 frames, corresponding to a total dose of ~40 electrons per Å².

Cryo-EM data processing

[0263] All cryo-EM data processing was carried out using cryoSPARC v3.2.0 (Structura Biotechnology Inc.). The movies were aligned using patch motion correction and patch CTF estimation. A total of 6692 movies were collected and then 6474 movies selected after motion correction and patch CTF. An initial set of particles picked using blob picker were subjected to 2D classification to generate templates for template picking. ~1.6 million particles picked by template picking were subjected to multiple rounds of 2D classification, resulting in 814,655 'good' complex particles. Ab initio reconstruction with 6 classes followed by heterogeneous refinement generated one "good" class containing 310,944 particles that corresponded to the full NPR1-ANP-REGN7544 Fab in an isotropic map. Non-uniform refinement of the particles in the "good" class was followed by local refinement resulted in a 3.15 Å resolution (FSC=0.143) map that was used for model building. Into this map, we manually placed models of the NPR1 (taken from PDB code 1T34) and the two Fabs (taken from prior Regeneron antibody structures). These models were then manually rebuilt using Coot (v 0.8.2 Medical Research Council Laboratory of Molecular Biology) and real space refined against the map using Phenix (v 1.15.2 The PHENIX Industrial Consortium).

Complex formation of NPR1 + REGN7544 Fab

[0264] Human NPR1 extracellular domain with a C-terminal myc-myc-6xHis tag (hNPR1-mmh; SEQ ID NO: 74) was mixed with the REGN7544 Fab in a molar ratio of 1 hNPR1-mmh + 1 REGN7544 Fab. The complex was incubated overnight at 4°C, then purified over a Superdex 200 increase 10/300 GL gel filtration column equilibrated with 50 mM Tris pH 7.5, 150 mM NaCl. Peak fractions were collected and concentrated and concentrated using a 10kDa MWCO centrifugal concentrator (Amicon).

Cryo EM grid preparation and data collection

[0265] The NPR1-REGN7544 complex was diluted to 0.8 mg/ml, and Poly (Maleic Anhydride-alt-1-Decene) substituted with 3-(Dimethylamino) Propylamine (PMAL-C8; Anatrace Cat. #P5008) was added to 0.15% final concentration. The sample was deposited onto a freshly plasma cleaned UltraAufoil grid (Quantifoil GmbH). Excess solution was blotted away with filter paper and plunge frozen into liquid ethane using a Vitrobot Mark IV (Thermo Fisher Part #1086439). The grid was loaded into a Titan Krios G3i (Thermo Fisher, Part #1137337) equipped with a Bioquantum energy filter + K3 direct electron detector (Gatan Inc, Part #1147213). Movies were collected using

EPU v 2.7 (Thermo Fisher) at 105,000x magnification, corresponding to a pixel size of 0.86 Å. A dose rate of 15 electrons per pixel per second was used, and each movie was 2 seconds and 46 frames, corresponding to a total dose of ~40 electrons per Å².

Cryo-EM data processing

[0266] All cryo-EM data processing was carried out using cryoSPARC v3.2.0 (Structura Biotechnology Inc.). The movies were aligned using patch motion correction and patch CTF estimation. A total of 10,378 movies were collected and then 9660 movies selected after motion correction and patch CTF. An initial set of particles picked using blob picker were subjected to 2D classification to generate templates for template picking. ~1.7 million particles picked by template picking were subjected to multiple rounds of 2D classification, resulting in 762,681 'good' complex particles. Ab initio reconstruction with 6 classes followed by heterogeneous refinement generated one "good" class containing 256,375 particles that corresponded to the full NPR1-REGN7544 Fab in an isotropic map. Non-uniform refinement of the particles in the "good" class was followed by local refinement resulted in a 3.42 Å resolution (FSC=0.143) map that was used for model building. Into this map, models of the NPR1 (taken from PDB code 1DP4) and the two Fabs (taken from prior Regeneron antibody structures) were manually placed. These models were then manually rebuilt using Coot (v 0.8.2 Medical Research Council Laboratory of Molecular Biology) and real space refined against the map using Phenix (v 1.15.2 The PHENIX Industrial Consortium).

Results

[0267] The structures of hNPR1 + ANP + REGN7544 Fab and hNPR1 + REGN7544 Fab showed that each NPR1 monomer binds one REGN7544 Fab. The Fab binds to residues in the lower (C-terminal, closer to the cell membrane) lobe of the extracellular domain. With REGN7544 bound, the NPR1 dimer adopts an inactive conformation in the absence of ANP, and an active conformation in the presence of ANP. These conformations are very similar to the observed states of NPR1 with no antibody bound in the presence or absence of ANP; however, these studies were done with soluble extracellular domains, not the full membrane-bound form of NPR1. Both heavy and light chain of REGN7544 interact with the NPR1 extracellular domain. Contact residues in the NPR1 extracellular domain remain the same either in the presence or absence of ANP. There are 15 contact residues through the heavy chain and 7 contact residues through the light chain. The residues in the NPR1 extracellular domain directly interacting with the REGN7544 Fab are Arg143, Leu144, Glu384, Leu401, Val402, Ala103, Ser405, Gly406, Arg407, Lys408, Trp411, Leu413, Gly414, Tyr415, and Pro416.

Example 13: Assessment of the Ability of Vasodilators to Reverse REGN7544-Induced Changes in Blood Pressure

[0268] The ability of three different vasodilatory agents (Nifedipine (Sigma-Aldrich), Enalapril (Sigma-Aldrich), and Molsidomine (Sigma-Aldrich)) with different mechanistic targets to reverse REGN7544-induced increase in blood pressure was evaluated in NPR1-humanized mice. Enalapril, Molsidomine, and Nifedipine, the three vasodilatory agents used, were administered orally. Enalapril lowers blood pressure by inhibition of angiotensin-converting enzyme (ACE), which lowers blood pressure by decreasing the production of angiotensin II, a vasoconstrictor, and increasing levels of bradykinin, a peptide that increases the diameter of blood vessels. Molsidomine, a nitric oxide donor, and nifedipine, a calcium channel blocker, also lower blood pressure.

[0269] The dosing scheme for evaluating the reversal of REGN7544-induced effects by vasodilators in telemetered NPR1-humanized mice is summarized in the following table:

Table 38

Dose Groups				Mice Used		Duration of Study
Dosing on Day 0		Daily Dosing Days 7-13		n	Age (Weeks)	
Test Article	Dose Administered (SC)	Test Article	Dose Administered (Oral Gavage)			
PBS	5 mL/kg	Water	10 mL/kg	6	13 to 15	13 days
REGN7544	25 mg/kg	Water	10 mL/kg	6		
		Nifedipine	20 mg/kg	6		
		Enalapril	25 mg/kg	7		
		Molsidomine	10 mg/kg	7		

PBS, phosphate-buffered saline; SC, subcutaneous

[0270] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*, PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7 (169 hours post SC dosing), mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Diastolic and systolic blood pressure was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment, and these measurements were used to calculate pulse pressure. Mean change in pulse pressure normalized to baseline for each treatment group was measured i) between 149 and 221 hours after administration of REGN7544

or vehicle control and ii) between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment.

[0271] Each vasodilator tested lowered pulse pressure (PP) in NPR1-humanized mice pretreated with REGN7544 (Figure 11). Indeed, the administration of vasodilators was shown to reverse pulse pressure-increasing effects of REGN7544.

[0272] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*, PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7, mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Pulse pressure was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment. Mean change in systolic blood pressure normalized to baseline for each treatment group was measured between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment.

[0273] The vasodilators decreased systolic blood pressure (SBP) in NPR1-humanized mice pretreated with REGN7544 (Figure 12). Indeed, the administration of vasodilators was found to reverse REGN7544-induced increase in systolic blood pressure.

[0274] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*, PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7, mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Pulse pressure was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment. Mean change in heart rate normalized to baseline for each treatment group was measured between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment.

[0275] No observable effect on heart rate following administration of enalapril, molsidomine, and nifedipine was recorded (data not shown). Indeed, the administration of vasodilators in the presence of REGN7544 was shown to have no effect on heart rate.

[0276] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*,

PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7, mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Systolic and diastolic blood pressure was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment, and these measurements were used to calculate pulse pressure. Mean pulse pressure for each treatment group was measured between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment. The administration of vasodilators reversed pulse pressure-increasing effects of a single SC dose of REGN7544 (data not shown).

[0277] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*, PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7, mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Systolic blood pressure was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment. Mean systolic blood pressure for each treatment group was measured between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment. The administration of vasodilators reverses systolic blood pressure-increasing effects of a single SC dose of REGN7544 (data not shown).

[0278] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*, PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7, mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Heart rate was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment. Mean heart rate for each treatment group was measured between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment. The administration of vasodilators following a single dose of REGN7544 had no compensatory effect on heart rate (data not shown).

[0279] Male telemetered NPR1-humanized mice ages 13 to 15 weeks were administered a single dose of 25 mg/kg REGN7544 (n=28) or vehicle control (*i.e.*,

PBS) (n=7) via subcutaneous (SC) injection on Day 0. Beginning on Day 7, mice pretreated with REGN7544 began receiving daily doses of nifedipine (20 mg/kg), enalapril (25 mg/kg), molsidomine (10 mg/kg), or water (n=7/group) via oral gavage (PO), while all mice pretreated with vehicle control received water. Diastolic blood pressure was recorded for all animals continuously 72 hours prior to administration of REGN7544 or vehicle control through the end of the experiment. Mean diastolic blood pressure (i) normalized to baseline and (ii) non-normalized for each treatment group was measured between 3 days prior to administration of REGN7544 or vehicle control through the end of the experiment. The administration of enalapril reversed diastolic blood pressure-increasing effects of a single SC dose of REGN7544 (data not shown).

[0280] Thus, the reversibility of hemodynamic effects following SC dosing of 25 mg/kg REGN7544 was tested by oral gavage of 3 clinical vasodilators. The three different vasodilatory agents, nifedipine, enalapril, and molsidomine, each reversed the REGN7544-induced increase in PP while having no observable significant effect on heart rate. REGN7544-mediated increases in PP were separately reversed by any one of three clinical vasodilators (nifedipine, enalapril, and molsidomine) administered by oral gavage.

[0281] The present disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the disclosure 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.

CLAIMS

What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof that binds specifically to natriuretic peptide receptor 1 (NPR1) protein, wherein the antibody or antigen-binding fragment thereof binds to NPR1 and blocks NPR1.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody is a fully human monoclonal antibody.
3. The antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the antibody has one or more properties selected from the group consisting of: (a) is a fully human monoclonal antibody; (b) binds to human NPR1 at 25°C and at 37°C with a dissociation constant (K_D) of less than 1.7nM, as measured in a surface plasmon resonance assay; (c) binds to monkey NPR1 at 25°C and 37°C with a K_D of less than 1.99nM, as measured in a surface plasmon resonance assay; (d) binds to human NPR1 in the presence of ANP at 25°C and at 37°C with a K_D of less than 1.52nM, as measured in a surface plasmon resonance assay; (e) inhibits ligand-induced NPR1 activation, as measured by cGMP accumulation assay; (f) binds to human NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 2.9nM, as measured by electrochemiluminescence-based immunoassay; (g) binds to monkey NPR1 in the presence or absence of ANP or BNP with an EC_{50} of less than 4.2nM, as measured by electrochemiluminescence-based immunoassay; (h) increases the systemic blood pressures when administered to normotensive and hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose; (i) increases the systemic blood pressures when administered to ANP overexpression-induced hypotensive mice, wherein the increase in systemic blood pressures lasts for up to about 28 days upon administration of a single dose; and (j) increases systemic blood pressures in LPS-induced hypotensive mice.
4. The antibody or antigen-binding fragment thereof of any one of claims 1-3, 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); and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR), wherein the HCVR comprises:
 - (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55;

- (ii) an amino acid sequence having at least 90% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55;
- (iii) an amino acid sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55; or
- (iv) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55, said amino acid sequence having no more than 12 amino acid substitutions,

and the LCVR comprises:

- (a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63;
- (b) an amino acid sequence having at least 90% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63;
- (c) an amino acid sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63; or
- (d) an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63, said amino acid sequence having no more than 10 amino acid substitutions.

5. The antibody or antigen-binding fragment thereof of any one of claims 1-4 comprising a heavy chain variability region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55.

6. The antibody or antigen-binding fragment thereof of any one of claims 1-5 comprising a light chain variability region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63.

7. The antibody or antigen-binding fragment thereof of any one of claims 1-4 comprising three complementarity determining regions (CDRs) contained within a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 2, 22, 38, and 55; and three CDRs contained within a light chain variable region (LCVR) selected from the group consisting of SEQ ID NOs: 10, 30, 46, and 63.

8. The antibody or antigen-binding fragment of any one of claims 1-7 comprising a heavy chain variable region (HCVR)/light chain variable region (LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 38/46, and 55/63.

9. The antibody or antigen-binding fragment thereof of any one of claims 1-7 comprising:

- (a) a heavy chain determining region (HCDR)1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 24, 40, and 57;
- (b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 26, 42, and 59;
- (c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 28, 44, and 61;
- (d) a light chain determining region (LCDR)1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 48, and 65;
- (e) a LCDR2 domain having an amino acid sequence selected from the group consisting of AAS and GAS; and
- (f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, and 69.

10. The antibody or antigen-binding fragment thereof of any one of claims 1-9 comprising complementarity determining regions (CDRs) selected from the group consisting of: (a) SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, AAS, and SEQ ID NO: 16; (b) SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 12, AAS, and SEQ ID NO: 32; (c) SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 48, AAS and SEQ ID NO: 16; and (d) SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 65, GAS, and SEQ ID NO: 69.

11. The antibody or antigen-binding fragment thereof of any one of claims 1-10 comprising a heavy chain variable region (HCVR)/light chain variable region (LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 38/46, and 55/63.

12. The antibody or antigen-binding fragment thereof of any one of claims 1-11 comprising a heavy chain and a light chain, wherein the heavy chain is selected from the group consisting of SEQ ID NOs: 18, 34, 51 and 71.

13. The antibody or antigen-binding fragment thereof of any one of claims 1-11 comprising a heavy chain and light chain, wherein the light chain is selected from the group consisting of SEQ ID NOs: 20, 36, 53, and 73.

14. The antibody or antigen-binding fragment thereof of any one of claims 1-11 comprising a heavy chain and a light chain, wherein the heavy chain is selected from the group consisting of SEQ ID NOs: 18, 34, 51 and 71; and the light chain is selected from the group consisting of SEQ ID NOs: 20, 36, 53, and 73.

15. The antibody or antigen-binding fragment thereof of any one of claims 1-14, wherein the antibody or antigen-binding fragment thereof binds to residues in the lower lobe of the extracellular domain of NPR1.

16. The antibody or antigen-binding fragment thereof of any one of claims 1-15, wherein the antibody or antigen-binding fragment thereof interacts with at least one of the NPR1 residues selected from the group consisting of Arg143, Leu144, Glu384, Leu401, Val402, Ala103, Ser405, Gly406, Arg407, Lys408, Trp411, Leu413, Gly414, Tyr415, and Pro416.

17. The antibody or antigen-binding fragment thereof of any one of claims 1-16 comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 18; and the light chain comprises an amino acid sequence of SEQ ID NO: 20.

18. The antibody or antigen-binding fragment thereof of any one of claims 1-16 comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 34; and the light chain comprises an amino acid sequence of SEQ ID NO: 36.

19. The antibody or antigen-binding fragment thereof of any one of claims 1-16 comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 51; and the light chain comprises an amino acid sequence of SEQ ID NO: 53.

20. The antibody or antigen-binding fragment thereof of any one of claims 1-16 comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 71; and the light chain comprises an amino acid sequence of SEQ ID NO: 73.

21. An antibody or antigen-binding fragment thereof that competes for binding to natriuretic peptide receptor 1 (NPR1) protein with the antibody or antigen-binding fragment thereof of any one of claims 1-20.

22. An antibody or antigen-binding fragment thereof that binds to the same epitope as the antibody or antigen-binding fragment thereof of any one of claims 1-20.

23. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-20 and a pharmaceutically acceptable carrier or diluent.

24. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a heavy chain variable region (HCVR) of an antibody as set forth in any one of claims 1-20.

25. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a light chain variable region (LCVR) of an antibody as set forth in any one of claims 1-20.

26. A vector comprising the polynucleotide molecule of claim 24.

27. A vector comprising the polynucleotide molecule of claim 25.

28. A host cell expressing the vector of claim 26 and/or 27.

29. A method of producing an anti-NPR1 antibody or antigen-binding fragment thereof, comprising growing the host cell of claim 28 under conditions permitting production of the antibody or fragment, and recovering the antibody or fragment so produced.

30. The method of claim 29, further comprising formulating the antibody or antigen-binding fragment thereof as a pharmaceutical composition comprising an acceptable carrier.

31. A method of treating, preventing, or ameliorating at least one symptom or indication of a NPR1-associated disease or disorder, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-20 to a subject in need thereof.

32. The method of claim 31, wherein the NPR1-associated disease or disorder is selected from the group consisting of hypotension, circulatory shock, septic shock, neurogenic orthostatic hypotension, postural orthostatic tachycardia syndrome (POTS), heart failure, cardiogenic shock, obesity, renal failure, chronic kidney disease,

macular edema, glaucoma, stroke, lung disorders, pulmonary fibrosis, inflammation, asthma, skeletal growth disorders, bone fractures, diabetes, hypoglycemia, and cancer.

33. The method of claim 31 or 32, wherein the pharmaceutical composition is administered prophylactically or therapeutically to the subject in need thereof.

34. The method of any one of claims 31-33, wherein the pharmaceutical composition is administered in combination with a second therapeutic agent or therapy.

35. The method of claim 34, wherein the second therapeutic agent or therapy is selected from the group consisting of an angiogenesis inhibitor, a vasoconstrictor/vasopressor, an immunosuppressant, ascorbic acid, a calcineurin inhibitor, a corticosteroid, a VEGF inhibitor, a decongestant, an antidepressant, hormonal birth control, a stimulant (including cardiac stimulant), caffeine, extracorporeal membrane oxygenation, ventricular assist device, intra-aortic balloon pump, a lifestyle modification, a dietary supplement, an anti-microbial drug, insulin, and an anti-inflammatory drug.

36. The method of any one of claims 31-35, wherein the pharmaceutical composition is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.