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Antagonist antibody to NPR1, REGN-NPR1^{7544} (baloncibart), increases blood pressure and plasma volume

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Abstract

Hypotension and hypovolemia cause morbidity across many clinical disorders, yet pharmacologic options are limited. REGN-NPR1^{7544} (REGN7544/baloncibart), a first-in-class monoclonal antibody, allosterically antagonizes natriuretic peptide receptor 1 (NPR1), a membrane guanylyl cyclase activated by atrial natriuretic peptide (ANP) and brain natriuretic peptide to promote vasorelaxation and natriuresis. REGN-NPR1^{7544} bound human and cynomolgus monkey NPR1 with nanomolar affinity, increased systolic blood pressure (SBP) in NPR1$^{Hu/Hu}$ mice by 10–15 mmHg for up to 28 days, corrected severe hypotension in ANP-overexpressing mice, and inhibited ANP-induced mesenteric vessel relaxation. In monkeys, SBP increased by 15–20 mmHg, with biomarkers consistent with plasma volume expansion. In a randomized, placebo-controlled, phase 1 single-ascending-dose study in healthy adults (N=80), doses >100 mg were generally well-tolerated, producing sustained 5–10 mmHg SBP increases and biomarker signals consistent with volume expansion. These data implicate tonic NPR1 signaling in human blood pressure and volume regulation, supporting REGN-NPR1^{7544} as a candidate for hypotension and hypovolemia.

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Introduction

There is a diverse group of medical disorders which are characterized by insufficient vascular tone and/or intravascular volume resulting in impaired organ perfusion and/or hypotension when compensatory physiologic changes are inadequate. Examples include orthostatic hypotension, postural orthostatic tachycardia syndrome (POTS),[1] and sepsis-induced hypotension. Currently available therapeutics include vasopressors (eg, norepinephrine and midodrine) and volume expanders (eg, fludrocortisone and vasopressin). However, these agents have significant and varied shortcomings, including the need for central venous access, short duration of action, limited efficacy, and/or side effects such as electrolyte disturbances and tissue ischemia. There is a critical need for novel therapies that can augment vascular tone and/or intravascular volume, thereby improving tissue perfusion and deliver clinical benefits.

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are ligands for the natriuretic peptide receptor 1 (NPR1), a membrane-spanning guanylate cyclase. Agonism of this receptor by exogenous delivery of peptides or agonist antibodies has been demonstrated to elicit hypotensive effects.[2-4] Mechanistically, the reduction in blood pressure (BP) may arise from direct vasodilatory effects. Additionally, ANP and BNP promote natriuresis, or urinary excretion of sodium, and thus reduce intravascular volume. The potential for NPR1 antagonism to increase BP and blood volume by relieving the vasodilatory and natriuretic effects of tonic NPR1 signaling has not been studied, owing to the lack of robust pharmacological antagonists. Thus, the physiologic role of basal NPR1 signaling in healthy normotensive, normovolemic individuals is unknown,[5] despite decades of research on the natriuretic peptide system.

Genetic knockout in rodents,[6, 7] and genetic variation in *NPR1* in humans, suggest that pharmacologic blockade of NPR1 may lead to increased BP associated with increased plasma volume. NPR1 deficiency in mice that survive to adulthood is characterized by chronically elevated BP of ~16 mmHg higher compared with wild-type mice.[8] In addition to hypertension, NPR1-deficient mice have also been described as hypervolemic with reduced sodium excretion upon volume loading.[9-11] Predicted loss-of-function (LoF) mutations in *NPR1* have been characterized in humans. Common variants in *NPR1* have been associated with elevated BP in heterozygotes.[2, 12-14] Although homozygotes of common variants have been identified, it is unknown whether these mutations are partial or complete LoF.

These data suggest that pharmacologic inhibition of NPR1 may augment intravascular volume and vascular tone and thereby increase BP. Such a drug would be expected to have application in the treatment of disorders characterized by hypotension and/or hypovolemia, such as sepsis-induced hypotension, orthostatic hypotension, and POTS.

Here we describe the generation, preclinical development, and phase 1 clinical evaluation of REGN-NPR1^{7544} (also known as REGN7544 or baloncibart), a novel first-in-class NPR1-blocking monoclonal antibody (mAb). We show that in both preclinical animal models and healthy human volunteers REGN-NPR1^{7544} leads to sustained increases in BP. Additional data in humans, including biomarkers, weight changes, and analyses of urinary sodium, suggest that REGN-NPR1^{7544} promotes intravascular volume expansion.

Results

Generation of REGN-NPR1^{7544}

Fully human anti-NPR1 mAbs were generated using VelocImmune® technology (Regeneron Pharmaceuticals, Inc, Tarrytown, NY, USA.).[2, 15, 16] VelocImmune® mice were immunized by co-injection of recombinant human NPR1 and a DNA plasmid encoding mouse ANP, followed by booster immunizations with an ANP–human NPR1 protein complex. Splenocytes from high-titer animals were harvested, and antibody-producing clones with high-affinity binders to NPR1 that block NPR1 in a variety of assays were identified, cloned, and expressed as recombinant antibodies. REGN-NPR1^{7544} was chosen based on its affinity, potency, development potential, and pharmacokinetic and pharmacodynamic properties.

Preclinical characterization of REGN-NPR1^{7544}

REGN-NPR1^{7544} binds human and monkey NPR1 independent of ANP or BNP

As assessed by flow cytometry, REGN-NPR1^{7544} bound to cell surface human and cynomolgus monkey NPR1 in a concentration-dependent manner, with half maximal effective concentration (EC_{50}) values of 17.2 nM and 11.2 nM, respectively (Fig. 1A). REGN-NPR1^{7544} bound human NPR1 in the presence or absence of a saturating concentration (100 nM) of human ANP or BNP with EC_{50} values differing by no more than 1.4-fold and maximal binding values differing by no more than 1.1-fold. Likewise, REGN-NPR1^{7544} bound cell-surface cynomolgus monkey NPR1 in the absence and presence of 100 nM of human ANP or BNP with similar EC_{50} values and maximal binding values. No detectable cell-surface binding was observed for the isotype control

antibody under any of the conditions. REGN-NPR1^{7544} did not bind negative control HEK293 cells. REGN-NPR1^{7544} showed no detectable binding to rat or mouse NPR1 in HEK293 cells engineered to stably express the respective receptors (data not shown). Surface plasmon resonance (SPR) experiments were performed on a Biacore T200 instrument (Cytiva, Marlborough, MA, USA) to determine the binding kinetics and affinity of REGN-NPR1^{7544} for recombinant human and cynomolgus monkey NPR1. In these experiments, REGN-NPR1^{7544} bound to recombinant human NPR1 protein with subnanomolar affinity in the presence and absence of 10-fold excess ANP, and with nanomolar affinity in the presence of 10-fold excess BNP (Fig. 1B). Additionally, REGN-NPR1^{7544} bound with subnanomolar affinity to recombinant cynomolgus monkey NPR1.

REGN-NPR1^{7544} noncompetitively inhibits ligand-induced activation of human and cynomolgus monkey NPR1

NPR1 is a membrane guanylate cyclase that generates cyclic guanosine monophosphate (cGMP) upon ligand stimulation. We used a cGMP accumulation assay to assess the effects of REGN-NPR1^{7544} on NPR1 activity. REGN-NPR1^{7544} inhibited NPR1 activation in HEK293 cells engineered to express NPR1 from humans or cynomolgus monkeys in a concentration-dependent manner (Fig. 1C). REGN-NPR1^{7544} fully suppressed cGMP generation from both human and cynomolgus monkey NPR1 in the presence of ANP or BNP. The isotype control antibody did not show any significant inhibition of NPR1 activation by either ligand. Using the cGMP accumulation bioassay described in the Methods, a Schild analysis was performed in which concentration-response curves for ligand-induced NPR1 activation were generated in the absence of REGN-NPR1^{7544} and in the presence of 3 separate constant concentrations of REGN-

NPR1^{7544} (50 nM, 150 nM, and 450 nM) to determine the mode of antagonism (Supplementary Fig. 1A–D). A rightward parallel shift of dose-response curves of ligand by antagonist without alteration of the maximum response is indicative of surmountable and competitive inhibition where the Schild slope is ~1.[17] REGN-NPR1^{7544} did not exhibit competitive inhibition of NPR1, since a non-parallel, rightward shift of ANP and BNP dose-response curves with decreased maximal responses with increasing concentration of REGN-NPR1^{7544} was observed. The Schild slope values were 2.6 and 2.3 for ANP and BNP, respectively. The isotype control antibody had no concentration-dependent effect on maximal response of ligand-induced NPR1 activation.

REGN-NPR1^{7544} blocks NPR1 in an allosteric manner

To define the structural basis of REGN-NPR1^{7544}-mediated inhibition, its interaction with NPR1 was examined by cryogenic-electron microscopy (cryo-EM). ANP activates NPR1 by binding at a site between 2 monomer ectodomains, causing a twisting motion of one NPR1 monomer relative to the other.[18] This twist of the extracellular domains is transmitted across the membrane and leads to activation of the intracellular guanylyl cyclase.[19] Cryo-EM structures of the REGN-NPR1^{7544} fragment antigen-binding (Fab) bound to the NPR1 extracellular domain in the presence and absence of ANP reveal that the antibody binds to a site on the C terminal lobe of the extracellular domain, well away from the location of bound ANP (Fig. 1D-E, Fig. 1F (top), Supplementary Fig. 2A). The twisting motion of the ectodomains upon ANP binding is unchanged with REGN-NPR1^{7544} bound (Supplementary Fig. 2A). However, the antibody-bound conformation observed with isolated NPR1 ectodomains would not be possible for full-length NPR1 in a living cell, due to the proximity of the cell membrane to the REGN-NPR1^{7544} binding

site. Structural modeling based on a published structure of full length NPR1 dimer[19] shows that REGN-NPR1^{7544} bound to an active-state NPR1 dimer would dig deeply into the lipid bilayer, which would be severely energetically unfavorable (Supplementary Fig. 2B). REGN-NPR1^{7544} binding may twist NPR1 away from the active state conformation to relieve steric interference with the membrane; a theoretical model of this state is shown Fig. 1F (bottom). In this open state, NPR1 would not be able to generate the cGMP downstream signal or bind ANP cooperatively and tightly. Thus, REGN-NPR1^{7544} would allosterically inhibit ANP binding and NPR1 signaling, even though the antibody is not a direct blocker of ANP binding.

To confirm REGN-NPR1^{7544} effectively blocks ANP binding to NPR1, on the cell surface, we preincubated HEK293 cells expressing human NPR1 with REGN-NPR1^{7544}, followed by the addition of fluorescently tagged ANP, and performed flow cytometry to assess ANP binding (Supplementary Fig. 1E). The results demonstrated that REGN-NPR1^{7544} inhibited ANP binding to NPR1 on the cell membrane and, together with the cryo-EM data and the cGMP assay data showing non-competitive inhibition, indicate that REGN-NPR1^{7544} is an allosteric blocker of NPR1 activity.

REGN-NPR1^{7544} treatment led to a sustained dose-dependent increase in SBP in NPR1$^{Hu/Hu}$ mice

The effect of REGN-NPR1^{7544} on BP was examined in telemetered normotensive NPR1$^{Hu/Hu}$ mice. Administration of a single subcutaneous (SC) dose of REGN-NPR1^{7544} induced significant increases in systolic BP (SBP) relative to baseline, with dose-dependent effects on the magnitude and duration (Fig. 2A). REGN-NPR1^{7544} increased SBP by 8–9 mmHg for 11 days at 1 mg/kg and 10–15 mmHg for 27 days at 25 mg/kg.

Serum antibody concentrations were measured in individual NPR1$^{Hu/Hu}$ mice at Days 14 and 29 post-dose. At 25 mg/kg, REGN-NPR1^{7544} was quantifiable at both Day 14 (163.9 ± 6.7 µg/mL, mean ± standard error of the mean [SEM]) and Day 29 (87.5 ± 20.5 µg/mL, mean ± SEM), consistent with the period of statistically significant BP elevation. At 1 mg/kg, serum concentrations were detectable at Day 14 (0.7 ± 0.3 µg/mL, mean ± SEM), coinciding with observable BP effects, but fell below the lower limit of quantification (LLOQ) by Day 29, when the effect on BP was no longer evident. Together, these findings support an exposure-response relationship between REGN-NPR1^{7544} serum concentrations and pharmacodynamic activity. REGN-NPR1^{7544} also reduced urinary cGMP by more than 50% by Day 7 post-dose (Fig. 2A). Repeat dosing of REGN-NPR1^{7544} (25 mg/kg SC, twice weekly) did not further increase SBP, consistent with a ceiling effect (Supplementary Fig. 3A). Moreover, a similar rise in BP was observed with intravenous (IV) administration of REGN-NPR1^{7544} (Supplementary Fig. 3B), with the increase in SBP detectable as early as 4 hours post-dose in normotensive NPR1$^{Hu/Hu}$ mice (Supplementary Fig. 3C).

REGN-NPR1^{7544} reversed hypotension in NPR1$^{Hu/Hu}$ mice

Whether REGN-NPR1^{7544} could reverse reduced BP in hypotensive mice overexpressing ANP was then examined (Fig. 2B). A single IV dose of 50 µg/kg ANP via hydrodynamic DNA delivery (HDD) significantly reduced normalized SBP by ~30–40 mmHg by Day 1, persisting through Day 28. A single IV dose of 25 mg/kg REGN-NPR1^{7544} administered on Day 7 completely reversed the effects of ANP overexpression on normalized SBP within hours and persisted until Day 28. The isotype control antibody induced no statistically significant change in normalized SBP in mice

administered either control HDD or ANP HDD. Mice receiving both ANP HDD and REGN-NPR1^{7544} showed a significant reduction in normalized HR starting at Day 11 and persisting until Day 27. Mice administered either control or ANP HDD and isotype control antibody showed no significant change in normalized HR. Reversal of BP decrease with REGN-NPR1^{7544} was confirmed in a second model of hypotension induced by prazosin, an alpha-1-adrenergic blocker (Supplementary. Fig. 3D).

REGN-NPR1^{7544} blocks vasodilation by ANP

REGN-NPR1^{7544} may increase BP by blocking the vasodilatory effects of NPR1 activation in vascular tissue. To test this hypothesis, we performed ex vivo vessel reactivity assays using wire myography. REGN-NPR1^{7544} significantly inhibited ANP-induced relaxation of precontracted mesenteric arteries and veins isolated from NPR1$^{Hu/Hu}$ mice, supporting the hypothesis that REGN-NPR1^{7544} may augment vascular tone by blocking tonic NPR1 signaling.

Standard vasodilators can reverse REGN-NPR1^{7544}–induced increases in BP

Given that REGN-NPR1^{7544} induces a sustained elevation in BP, albeit with a ceiling effect, there may be instances in clinical practice where reversing the elevation in BP is desirable. To address this, the efficacy of 3 orally administered vasodilatory agents approved by the U.S. Food and Drug Administration (enalapril, molsidomine, and nifedipine) targeting distinct mechanisms of action was evaluated in NPR1$^{Hu/Hu}$ mice. All 3 vasodilators successfully reversed the REGN-NPR1^{7544}–induced elevation in SBP in NPR1$^{Hu/Hu}$ mice (Fig. 2C, Supplementary Fig. 3E). These findings suggest that increases in BP induced by REGN-NPR1^{7544} can be reversed by standard

antihypertensive medications, potentially eliminating the need for separate antidote development.

REGN-NPR1^{7544} caused a dose-dependent, sustained increase in BP in normotensive monkeys

To determine whether the effect of REGN-NPR1^{7544} on BP in normotensive NPR1$^{Hu/Hu}$ mice could be replicated in large animals, we administered REGN-NPR1^{7544} to telemetered normotensive male cynomolgus monkeys. A single IV dose of 1 mg/kg or 25 mg/kg REGN-NPR1^{7544} persistently increased normalized SBP levels by 10–17 mmHg for at least 14 and 28 days, respectively. SBP in cynomolgus monkeys receiving 1 mg/kg REGN-NPR1^{7544} returned to baseline levels between Day 14 and Day 21, whereas SBP was elevated throughout the study period (ie, 28 days) in monkeys that received 25 mg/kg. Administration of 0.1 mg/kg REGN-NPR1^{7544} transiently increased SBP (Fig. 3A). The dose-dependent increase in SBP was observed during the 24- to 48-hour post-dose period, with a statistically significant increase in SBP for the groups administered 1 mg/kg or 25 mg/kg REGN-NPR1^{7544} compared with vehicle control; administration of 0.1 mg/kg of REGN-NPR1^{7544} increased normalized SBP compared with vehicle control (although not by a statistically significant margin). There was no evidence of a REGN-NPR1^{7544}–related effect on HR, as it decreased relative to baseline for all dosing groups including vehicle control (data not shown).

REGN-NPR1^{7544} serum concentrations were dose-dependent and consistent with the observed pharmacodynamic response. At 25 mg/kg, concentrations peaked on Day 1 (704 ± 70.0 µg/mL, mean ± standard deviation [SD]) and declined gradually through Day 30 (180 ± 22.0 µg/mL, mean ± SD), coinciding with a sustained increase in

SBP. At 1 mg/kg, concentrations peaked on Day 1 (19.6 ± 3.06 µg/mL, mean ± SD) and fell below the LLOQ by Day 21, consistent with BP returning toward baseline by Day 21. At 0.1 mg/kg, serum concentrations were quantifiable only on Days 1 and 2, consistent with the absence of meaningful effects on BP. All control samples were below the limit of detection. These data demonstrate an exposure-response relationship between REGN-NPR1^{7544} serum levels and NPR1-mediated elevation of BP.

In monkeys dosed with REGN-NPR1^{7544}, N-terminal pro–atrial natriuretic peptide (NT-proANP), a stable marker of ANP synthesis from cardiomyocytes, was transiently elevated in circulation (Fig. 3B). Furthermore, a significant reduction in hematocrit was observed (Fig. 3B) 1 day after dosing with REGN-NPR1^{7544}. These observations are consistent with intravascular volume expansion, which would increase cardiac chamber stretch and thereby stimulate ANP synthesis and release. These findings support a volume-mediated component, distinct from direct effects on vascular tone, through which REGN-NPR1^{7544} may increase BP.

Clinical characterization of REGN-NPR1^{7544} in healthy volunteers

To explore the effects of REGN-NPR1^{7544} in humans, a phase 1 double-blind single-ascending-dose study was performed in which a total of 80 healthy, normotensive participants were randomized to receive either REGN-NPR1^{7544} or placebo in a 3:1 ratio (NCT05970718). Cohorts of 8 participants received single doses either IV (3 mg, 10 mg, 30 mg, 100 mg, 300 mg [given for 2 cohorts], and 1000 mg) or SC (100 mg, 300 mg, and 600 mg). See Methods and Supplementary Fig. 4 for additional details.

Safety data of REGN-NPR1^{7544} in healthy volunteers

The incidence of treatment-emergent adverse events was comparable between the placebo and REGN-NPR1^{7544}–treated groups with both IV and SC administration (Supplementary Table 1). No evidence of clinically significant changes in electrolytes or serum creatinine was observed (data not shown). There were no serious adverse events related to the study drug. There was 1 adverse event of special interest amongst the participants receiving REGN-NPR1^{7544}; this was a mild increase in SBP that occurred 37 days after administration of REGN-NPR1^{7544} 300 mg SC. The SBP normalized after transient treatment with the antihypertensive agent quinapril.

REGN-NPR1^{7544} increases BP

Oscillometric BP data were collected during each clinic visit and showed a gradual increase in SBP in the week following dosing, such that cohorts dosed with ≥100 mg REGN-NPR1^{7544} (either IV or SC) displayed 5–10 mmHg increases above baseline (Fig. 4A–B). These changes were sustained for several weeks and were not observed in the combined (IV + SC) placebo group.

As an independent assessment of BP, ambulatory BP monitoring collected BP data via a cuff every 15–30 minutes continuously during a 24-hour period in cohorts dosed with ≥100 mg REGN-NPR1^{7544}. Similar to the in-clinic BP data, elevations of BP of 5–10 mmHg above baseline were observed (Fig. 4 C–D).

Furthermore, BP was derived from a pulse wave velocity instrument (see Methods), which was collected continuously for 5 days during the inpatient period. SBP increased ~5–10 mmHg from baseline in the 100 mg and 300 mg REGN-NPR1^{7544} IV groups, and

in the 300 mg REGN-NPR1^{7544} SC group relative to the pooled placebo group (data not shown).

REGN-NPR1^{7544} decreases cGMP and increases natriuretic peptides

Serum levels of cGMP, the second messenger of NPR1, were determined in participants receiving placebo and REGN-NPR1^{7544}. REGN-NPR1^{7544} exposure was associated with a maximal decrease of ~2.0 pmol/mL; this decrease represented ~40% of baseline and was sustained for up to 2 months or more, depending on the dose (Fig. 5A–B).

Additionally, the concentrations of ANP and BNP (Fig. 5C–F), as well as the long-lived byproducts of natriuretic peptide biosynthesis, NT-proANP and N-terminal pro–B-type natriuretic peptide (NT-proBNP; Supplementary Fig. 5), were determined. REGN-NPR1^{7544} exposure, but not placebo, was associated with large increases in ANP, BNP, NT-proANP, and NT-proBNP which lasted up to 28 days.

Indirect evidence indicates that REGN-NPR1^{7544} may cause expansion of plasma volume

The increases in BP and natriuretic peptides observed with exposure to REGN-NPR1^{7544} are consistent with an expansion in plasma volume. Several other lines of evidence are consistent with this hypothesis. Hemoglobin concentrations dropped, with a peak effect around 1 week, consistent with hemodilution due to acute expansion of plasma volume (Fig. 6A–B). A similar drop in hematocrit was observed in the cynomolgus study (Fig. 3B). Slow normalization of hemoglobin after Day 8 is consistent with increased production of erythrocytes but may reflect loss of the excess plasma

volume at later timepoints (Fig. 6A–B). The weight of participants in cohorts exposed to REGN-NPR1^{7544} increased 0.5–0.75 kg relative to the placebo group during the 5-day inpatient observation period, during which participants were on a fixed sodium diet (Fig. 6C–D), suggesting an expansion of plasma volume in the range of 500–750 mL. Urinary sodium excretion was generally reduced relative to placebo for several days after dosing, both when assessed as total urinary sodium in a 24-hour collection or as urinary sodium/creatinine ratio (Fig. 6E–F and Supplementary Fig. 6, respectively). These data are consistent with sodium retention for several days after dosing until equilibrium was re-established. Analysis of plasma renin activity and aldosterone levels indicated that cohorts receiving ≥100 mg REGN-NPR1^{7544} either IV or SC generally displayed increases in both plasma renin activity and aldosterone for several days after dosing (Supplementary Fig. 7).

Discussion

Here we describe REGN-NPR1^{7544}, a novel first-in-class mAb antagonist of NPR1. This work is significant in at least 2 key regards. First, for the first time since the identification of natriuretic peptides in the 1980s, this novel agent allows for conclusive demonstration of the role of tonic NPR1 signaling to regulate BP and blood volume under normotensive, euvolemic conditions in mice, monkeys, and humans. Second, these findings support the potential of REGN-NPR1^{7544} as a novel therapeutic approach for disorders characterized by hypotension and/or hypovolemia.

REGN-NPR1^{7544} bound to NPR1 with nanomolar affinity and robustly blocked receptor activity in in vitro systems, animal models, and human studies. Mechanistic analyses indicate that REGN-NPR1^{7544} acts as a noncompetitive allosteric antagonist that is not surmounted by high concentrations of endogenous ligands. Circulating cGMP, the second messenger downstream of NPR1, was reduced by up to ~40% in both mice and humans, which is consistent with direct target engagement. The residual cGMP signal likely reflects production from other sources, including NPR2 (the C-type natriuretic peptide receptor) and soluble guanylate cyclases. A key physiologic consequence of REGN-NPR1^{7544} engagement is the elevation in BP, which was observed in mice, monkeys, and humans. This pressor response aligns with human genetic evidence linking NPR1 LoF alleles to higher SBP.[2]

Two complementary mechanisms likely contribute to the pressor effects of REGN-NPR1^{7544}: inhibition of ANP/BNP-induced vasorelaxation (resulting in increased vascular tone); and expansion in intravascular volume. Evidence for a vascular

component comes from ex vivo studies of isolated arterial and venous rings in mice, in which REGN-NPR1^{7544} blunted ANP-induced relaxation (Fig. 2D). Evidence supporting volume expansion is provided by several indirect observations in cynomolgus monkeys and in the phase 1 study. In monkeys, circulating NT-proANP increased and hematocrit decreased. In humans, increases in body weight, decreases in urinary sodium excretion, reductions in hemoglobin, and elevations in circulating natriuretic peptides are all supportive of increased intravascular volume. Of note, single-cell expression data indicate that cardiomyocytes expressing ANP and BNP do not co-express NPR1 (Supplementary Fig. 9), arguing against an autocrine effect of REGN-NPR1^{7544} on natriuretic peptide levels and suggesting that the increase in natriuretic peptides is secondary to cardiac stretch caused by expanded intravascular volume.[20]

The are several different potential mechanisms that could explain REGN-NPR1^{7544}–associated expansion of intravascular volume. First, natriuretic peptides have been reported to downregulate the renin angiotensin aldosterone system (RAAS). Release of inhibition of the RAAS cascade by REGN-NPR1^{7544} would be expected to increase sodium retention in the kidney. Increases in plasma renin activity and aldosterone concentrations were indeed observed for several days after dosing in most of the cohorts receiving ≥100 mg REGN-NPR1^{7544} (Supplementary Fig. 7), suggesting that activation of the RAAS may contribute to volume expansion. A second possibility is that REGN-NPR1^{7544} directly antagonizes NPR1 expressed in renal tubular epithelia and thereby leads to sodium retention.[21-24] Biodistribution positron emission tomography (PET) studies of radiolabeled REGN-NPR1^{7544} in NPR1$^{Hu/Hu}$ mice demonstrated uptake of REGN-NPR1^{7544} in both the adrenal gland and the kidney (see Supplementary Fig.

8), consistent with both hypotheses. Third, NPR1 has been reported to regulate the leakiness of capillary endothelia and thus may affect the distribution of extracellular fluid between the intra- and extravascular spaces. Evaluation of this hypothesis awaits further studies.[25-28]

The data presented herein support the clinical development of REGN-NPR1^{7544} for disorders characterized by hypotension (eg, sepsis, orthostatic hypotension), and/or hypovolemia (eg, POTS).[29] Several properties of REGN-NPR1^{7544} may offer advantages relative to currently used therapies such as fludrocortisone, desmopressin, and norepinephrine. In the phase 1 study, a single dose of REGN-NPR1^{7544} in healthy volunteers was generally well tolerated. Notably, the BP response exhibited an apparent ceiling effect in both preclinical models and humans, consistent with a target-saturable mechanism. Once NPR1 is maximally blocked, further increases in plasma drug concentrations are not expected to produce additional pharmacodynamic effects but only contribute to the duration of the effect. The ceiling effect on BP may enhance the safety profile of REGN-NPR1^{7544}, reduce the chances of excessive elevation of BP, and may eliminate the need for close clinical monitoring. Additionally, studies in mice indicate the pressor effects can be reversed with standard-of-care anti-hypertensive agents (Fig. 2C); no specific rescue agent may be required. A long duration of action following single doses was observed in both the preclinical and the phase 1 studies, suggesting dosing intervals of several weeks or more may be possible in chronic conditions. REGN-NPR1^{7544} is not apparently associated with electrolyte disturbances, in contrast to other volume expanders such as fludrocortisone (hyperkalemia) and desmopressin (hyponatremia). Finally, SC administration without the need for peripheral

or central IV access could enable use in outpatient or emergency settings outside the hospital. Notably, several target disorders have been reported to have normal or even elevated natriuretic peptide levels despite hypotension and/or hypovolemia (eg, sepsis,[30] orthostatic hypotension,[31-33] and POTS).[34] These data suggest the presence of ongoing natriuretic peptide signaling in disease settings where such signaling would be counter-productive and thus raising the potential for a benefit of NPR1 blockade in these disease settings.

The data in this report identify REGN-NPR1^{7544} as a potentially efficacious therapeutic for hypotensive and/or hypovolemic disorders and as a valuable reagent to further define the physiology of the natriuretic peptide system.

Methods

In vitro characterization of REGN-NPR1^{7544}

Flow cytometry was used to evaluate REGN-NPR1^{7544} binding to HEK293 cells expressing human, cynomolgus monkey, or rabbit NPR1 alone or in the presence of ANP or BNP. Parental HEK293 cells were used as a negative control cell line. Cells were incubated with REGN-NPR1^{7544} or isotype control antibody at concentrations of 25 pM to 500 nM with or without 100 nM of human ANP or BNP for 30 minutes on ice. Cell-surface binding was detected by the addition of 1 µg/mL allophycocyanin-conjugated goat anti-human immunoglobulin G (IgG) antibody and incubation on ice for 30 minutes. Control samples without the primary antibody were included to determine background signal in the presence of the secondary antibody alone. Cell viability was confirmed with eBioscience™ Fixable Viability Dye eFluor™ 450 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Following the staining procedure, cells were analyzed on a CytoFLEX (Beckman Coulter Life Sciences, Brea, CA, USA) flow cytometer.

Mean fluorescence intensity (MFI) values were obtained using FlowJo software (FlowJo LLC, Ashland, OR, USA) and analyzed with GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) using a 4-parameter logistic equation over a 10-point dose-response curve to obtain EC_{50} values. Relative maximal binding was calculated as the change in MFI observed at the highest tested antibody concentration (500 nM) relative to the background signal in MFI observed with the secondary antibody alone.

NPR1 antagonism was assessed using a cGMP accumulation assay. HEK293 cells expressing human and cynomolgus monkey NPR1 were detached from the plates with an enzyme-free dissociation buffer, and the cell suspension was replated in low-volume 96-well plates at 1000 cells/well in OptiMEM (Gibco, Thermo Fisher Scientific) containing 0.1% fetal bovine serum. Cells were stimulated with ANP or BNP across a concentration range (ANP: 0.0002–10 nM for both species; BNP: 0.0005–30 nM for human NPR1, and 0.0051–300 nM for cynomolgus monkey NPR1), plus a no-ligand control, to generate ligand-dependent dose–response curves.

To measure REGN-NPR1^{7544}–mediated inhibition of ligand-dependent NPR1 activation, cells were pretreated for 15 minutes at 37°C with REGN-NPR1^{7544} or isotype control (REGN1945) antibodies across 0.017 nM to 1 μM, plus a no-antibody control. After pretreatment, cells were stimulated with a fixed concentration of ANP or BNP and incubated for 10 minutes at room temperature. A ligand-free control was included to assess whether the antibody activated NPR1 in the absence of ligands. The homogeneous time-resolved fluorescence (HTRF) assay was performed using a cGMP HTRF kit according to the manufacturer's instructions (Cisbio cGMP kit, 62GM2PEH; PerkinElmer, Waltham, MA, USA). The fluorescence intensity was detected using an EnVision® multilabel plate reader (Revvity), and the fluorescence resonance energy transfer (FRET) ratio was calculated according to the 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 11-point concentration-response curve to obtain the half maximal inhibitory concentration values for the anti-NPR1 antibody using GraphPad Prism 8. The EC_{50}

values of ANP or BNP were also calculated using a 4-parameter logistic equation over an 11-point concentration-response curve.

The mode of REGN-NPR1^{7544} antagonism of ligand-mediated NPR1 activation was evaluated by a Schild analysis[17] using a cGMP accumulation assay. Human NPR1 HEK293 cells were suspended as described above and pretreated with REGN-NPR1^{7544} at 0, 50, 150, or 450 nM for 15 minutes at room temperature. Cells were then stimulated with ANP (1 pM to 1 µM) or BNP (3 pM to 3 µM) for 5 minutes. A no-ligand negative control was included. The HTRF assay, detection of fluorescence intensity, calculation of cGMP concentration, and analysis using GraphPad Prism 8 (GraphPad Software) were all performed as described above, and the decrease of ANP or BNP maximal response by anti-NPR1 antibody was calculated. The Schild slope was determined by a 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.

Kinetic binding interactions of REGN-NPR1^{7544} with NPR1 proteins

SPR experiments were performed on a Biacore T200 instrument (Cytiva) to determine the binding affinity of REGN-NPR1^{7544} for recombinant human and cynomolgus monkey NPR1. Mouse anti-human Fc gamma monoclonal antibody (anti-hFcγ) was immobilized on the surface of a sensor chip using standard amine-coupling chemistry. REGN-NPR1^{7544} was then captured on anti-hFcγ surfaces until a signal of 40–63 response units was reached.

Human (mmH-tagged) NPR1 (hNPR1.mmH) was preincubated with a 10-fold molar excess of human ANP or BNP for at least 2 hours in advance of injections.

Recombinant NPR1 proteins were individually injected over the captured REGN-NPR1^{7544} surfaces in 2-fold serial dilutions at concentrations of 1.25–40 nM at a flow rate of 50 µL/min for 2.5 minutes followed by a dissociation phase of 30 minutes or, for hNPR1.mmH in the presence of 10-fold excess of BNP at 25°C and in the presence of 10-fold excess of ANP at 37°C, 3 minutes. Each concentration was tested in duplicate.

Specific binding signals were obtained by a double referencing procedure and plotted as SPR sensorgrams. The double referencing was performed by first subtracting the signal of each injection over a reference surface (anti-hFcγ) from the signal over the experimental surface (anti-hFcγ–captured REGN-NPR1^{7544}), thereby removing contributions from refractive index changes. In addition, running buffer injections were performed to allow subtraction of the signal changes resulting from the dissociation of captured REGN-NPR1^{7544} from the coupled anti-hFcγ surface. The kinetic parameters were obtained by globally fitting these specific binding signals to a 1:1 binding model with mass transport limitation using Biacore evaluation software (Cytiva). The equilibrium dissociation constant (KD) was calculated from the ratio of the dissociation rate constant (kd) to the association rate constant (ka) (KD = kd/ka). The dissociative half-life was calculated by dividing 0.693 (natural logarithm of 2) by the experimentally determined kd.

Structural characterization of NPR1/ REGN-NPR1^{7544} complexes with and without bound ANP via cryo-EM analysis

For cryo-EM studies, complexes of human NPR1 ectodomain bound by enzymatically digested Fab regions of REGN-NPR1^{7544} were purified by gel filtration. ANPs were

added in 2-fold molar excess over NPR1 ectodomain and incubated for at least 30 min at room temperature to produce complexes with bound ANP. These complexes were then concentrated to ~1 mg/ml and applied to UltrAuFoil 0.6/1 grids (Quantifoil GmbH, Jena, Germany) immediately after the addition of 0.15% of PMAL-C8 amphipol to the samples, and vitrified for cryo-EM using a Vitrobot Mark IV (Thermo Fisher Scientific). Cryo-EM data were collected using a Titan Krios G3i microscope (Thermo Fisher Scientific) equipped with a K3 Bioquantum GIF imaging system (Gatan, Pleasanton, CA, USA). Image processing was carried out using cryoSPARC (Structura Biotechnology Inc, Toronto, ON, Canada). Our previously reported structure of NPR1 bound by REGN5381 was used as an initial model for the NPR1 dimer, after removing the REGN5381 Fab chains.[2] The previous REGN5381 Fab model was adjusted to have the sequence of REGN-NPR1^{7544} and placed in the correct location to serve as the initial model for these complexes. Structures were additionally rebuilt and refined using Coot (MRC Laboratory of Molecular Biology, Cambridge, UK) and PHENIX (Lawrence Berkeley National Laboratory, Berkeley, CA, USA).

Preclinical mouse studies

Assessment of the effect of a single dose of REGN-NPR1^{7544} on BP

NPR1$^{hu/hu}$ mice were generated on the C57BL/6NTac (75%)/129S6SvEvTac (25%) background using the VelociGene® platform (Regeneron Pharmaceuticals, Inc.).[35] The hemodynamic effects of REGN-NPR1^{7544} in NPR1$^{Hu/Hu}$ mice[2] were evaluated in telemetered mice. Briefly, male *NPR1*$^{Hu/Hu}$ mice were implanted with PA-C10 telemeters and allowed to recover for at least 7 days. Mice aged 22–25 weeks were randomized into 4 dose groups and administered a single SC injection on study Day 1. Groups

received phosphate-buffered saline (PBS; 5 mL/kg, n=5), an IgG4P isotype control antibody (25 mg/kg, n=5), or REGN-NPR1^{7544} at either 1 mg/kg (n=5) or 25 mg/kg (n=6). Animals were monitored for 28 days following administration. Serum samples were collected on Days 14 and 28 to measure antibody levels.

In a separate experiment, mice received a single SC injection of either isotype control antibody (25 mg/kg, n=6) or REGN-NPR1^{7544} (25 mg/kg, n=5). Urine was collected using diuresis cages (Tecniplast USA, West Chester, PA, USA), and cGMP was measured according to the manufacturer's protocol (Cisbio cGMP kit, 62GM2PEH; Revvity).

To examine the effects of repeat dosing, telemetered NPR1$^{Hu/Hu}$ male mice (16–18 weeks old) received either isotype control (25 mg/kg, SC, twice weekly; n=5) or REGN-NPR1^{7544} (25 mg/kg, SC) administered as either a single dose or twice weekly (n=5 per group) and were monitored for 21 days.

To evaluate the effect of REGN-NPR1^{7544} on BP following IV dosing, telemetered *NPR1*$^{Hu/Hu}$ mice administered a single IV injection of either PBS (5 mL/kg, n=6) or REGN-NPR1^{7544} (25 mg/kg, n=6) and were monitored for 7 days following administration.

REGN-NPR1^{7544} in telemetered NPR1$^{Hu/Hu}$ mice with ANP overexpression–induced hypotension

The hemodynamic effects of a single IV dose of 25 mg/kg of REGN-NPR1^{7544} were evaluated in NPR1$^{Hu/Hu}$ mice. A heterozygous humanized mouse strain was generated

using VelociGene® technology at Regeneron Pharmaceuticals[35, 36] and was inbred to generate homozygous NPR1$^{Hu/Hu}$ mice. Male mice (13–15 weeks old) were implanted SC with a PA-C10 Pressure Transmitter (Data Sciences International, St. Paul, MN, USA) under the scapula or along the lateral flank between the forelimb and hindlimb. The tip of the pressure-sensing catheter of the telemetry device was positioned in the free-flowing blood of the aortic arch via the left carotid artery. Mice were allowed to recover for at least 7 days. On Day 0, overexpression of ANP was induced in 2 groups of mice using hydrodynamic delivery of 50 µg/kg plasmic DNA encoding full-length mouse ANP (pRG977mmANP), while a third (control) group received 50 µg/kg of the same plasmid vector without the coding sequence (pRG977). On Day 7, 25 mg/kg IV isotype control was administered to the control group, while the ANP overexpression mice received 25 mg/kg IV isotype control or REGN-NPR1^{7544}. The 3 groups contained 5, 6, and 8 mice, respectively.

SBP, diastolic BP (DBP), and HR were continuously recorded through Day 28. Measurements recorded 2 days prior to dosing served as the pre-study baseline for each mouse. SBP and HR values were normalized to pre-study baseline measurements and graphed as daily binned data from Day 7 through Day 27 (ie, the last full 24-hour dataset available).

Effect of REGN-NPR1^{7544} on BP and reversibility of REGN-NPR1^{7544} effects by vasodilators in telemetered NPR1$^{Hu/Hu}$ mice

Telemetered NPR1$^{Hu/Hu}$ mice (prepared as described above) received a single SC dose of either 5 mL/kg PBS (control group) or 25 mg/kg REGN-NPR1^{7544} (4 groups) on Day 0 followed by daily oral dosing from Days 7 to 13 with water 10 mL/kg (control) or water

10 mL/kg, nifedipine 20 mg/kg, enalapril 25 mg/kg, or molsidomine 10 mg/kg (REGN-NPR1^{7544} groups). Group sizes ranged from 5 to 7 animals, and all cohorts were followed for a total of 13 days. BP and HR were measured as above.

REGN-NPR1^{7544} in vivo biodistribution study

Generation of antibody radioconjugates for ^{89}Zr-immunoPET

Deferoxamine (DFO) conjugates of REGN-NPR1^{7544} and isotype control antibody REGN1945 were generated using an amine reactive, heterobifunctional chelator, p-isothiocyanatobenzyl-deferoxamine (p-SCN-Bn-DFO; B-705, Macrocyclics, Plano, TX, USA). Antibodies were prepared to a concentration of 10 mg/mL in PBS and the pH was raised to 9.0 using 1 M sodium carbonate, followed by the dropwise addition of p-SCN-Bn-DFO in 2% dimethyl sulfoxide (v/v) to a final reaction ratio of 4-fold molar excess over antibody. After a 30-minute incubation at 37°C with gentle shaking, reaction mixtures were buffer-exchanged into 10 mmol/L histidine, pH 5.5, using PD-10 columns (GE Healthcare, Chicago, IL, USA), which also removed unreacted chelator. Monomeric protein purity was assessed by size-exclusion high-performance liquid chromatography (Superdex 200 Increase 10/300 GL column [Cytvia], PBS pH 7.4 mobile phase, 0.75 mL/min flow rate, absorption monitored at 280 nm). Purity was calculated by comparing the integrated areas of the high-molecular-weight fraction and the main monomeric peak at 280 nm. The DFO-to-antibody ratio and protein concentration were determined by UV absorption spectroscopy (Nanodrop 2000c; Thermo Fisher Scientific) using the extinction coefficient of DFO.

For radiolabeling, 250 µg of each DFO conjugate was resuspended in 200 µL 1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), pH 7.4. Approximately 5 mCi of ^{89}Zr-oxalate was neutralized with HEPES and combined with the conjugates, followed by a 45-minute room-temperature incubation. Reaction mixtures were then buffer-exchanged into 250 mmol/L sodium acetate, pH 5.5, on a PD-10 column, eluted in 1.8 mL, and sterile-filtered (0.2 µm filter). Specific activity of the elution product was calculated from measured radioactivity and protein mass was determined by UV spectroscopy, using the DFO-corrected antibody extinction coefficient.

PET imaging and ex vivo biodistribution

For longitudinal in vivo PET imaging and terminal ex vivo biodistribution, adult NPR1$^{Hu/Hu}$ mice were injected IV with 0.2 mg/kg of the anti-NPR1 antibody-radioconjugate or the isotype control antibody-radioconjugate via the lateral tail vein. Mice were imaged longitudinally at 1 hour post-injection on Day 0, and at 24 hours (Day 1), 48 hours (Day 2), 72 hours (Day 3), and 144 hours (Day 6). An ex vivo biodistribution analysis was performed on tissues of interest harvested from mice upon euthanasia following the last image acquisition on Day 6. Example PET imaging of the uptake of REGN-NPR1^{7544} in key blood-regulating organs can be seen in Supplementary Fig. 8.

Molecubes Benchtop β-cube and X-cube scanners (MOLECUBES NV, Ghent, Belgium) were used to acquire the sequential PET and computed tomography (CT) images. Mice underwent induction anesthesia using isoflurane and were kept under a continuous flow of isoflurane during imaging. A low-dose micro-CT image was acquired

followed by a 10-minute PET acquisition. PET images were reconstructed up to 15 iterations and CT images were reconstructed using the Molecubes Reconstruction Engine for Molecular Imaging (MOLECUBES NV) to an isometric voxel size of 200 μm with little resolution loss using the preconfigured settings. The PET and CT images were co-registered and processed using VivoQuant (InviCRO, Boston, MA, USA) to visualize PET-CT images as maximum intensity projections on a color scale depicting the percentage injected dose per gram.

For ex vivo biodistribution analysis, blood was collected from each mouse via cardiac puncture, and multiple tissues of interest (including blood, heart, aorta, inferior vena cava, lungs, liver, spleen, adrenal glands, kidneys, femur, and quadriceps muscle) were harvested and placed into 12 x 75 mm polystyrene disposable culture tubes to measure the uptake of radioactivity using a gamma counter (Hidex Automatic Gamma Counter; Hidex Oy, Turku, Finland) with an inbuilt scale to record the weight of each tissue. The percentage of injected dose of radioactivity in each tissue was calculated and divided by the weight of that tissue to obtain a concentration value indicative of normalized uptake per organ. Data are reported as mean ± SD.

Gene expression analysis

Single-nucleus transcriptional profiling data from the human heart were obtained from https://singlecell.broadinstitute.org/single_cell/study/SCP1303/.[20] The combined h5ad object for these data was converted to a Seurat object,[37] and author-derived cell types were used in generating Supplementary Fig. 9.

Preclinical non-human primate studies

REGN-NPR1^{7544} in telemetered male cynomolgus monkeys

Male cynomolgus monkeys (4–6 years old) had a radiotelemetry transmitter (PhysioTel Digital Model L11 [Data Sciences International]) surgically implanted intra-abdominally along the right side of the abdominal wall between the pelvis and the last rib (approximately), with the attached BP catheter located in the internal iliac artery and the tip of the catheter located in the descending aorta. Monkeys were allowed to recover for at least 5 weeks and then assigned to groups of 5, which received PBS (1 mL/kg) or REGN-NPR1^{7544} at 0.1, 1, or 25 mg/kg by IV injection. All animals were followed for 28 days for telemetry assessments and 30 days for NT-proANP measurements.

SBP, DBP, and HR were continuously recorded through the end of the experiment. Measurements recorded 3 days prior to the study were averaged to serve as the pre-study baseline for each animal. SBP and HR values were normalized to pre-study baseline measurements and graphed as daily binned data, and, for SBP, as hourly binned data through 48 hours post-dose. Non-normalized SBP and DBP values, as well as normalized DBP values, were graphed as daily binned data.

Serum samples were collected from each animal and used to determine the levels of NT-proANP using a commercially available (Abcam, Cambridge, UK) enzyme-linked immunosorbent assay (ELISA) assay. For the analysis, sample and sheep anti-proANP raised against the N-terminus of human proANP and conjugated to a horseradish peroxidase (HRP) conjugate were added to wells that were pre-coated with polyclonal sheep anti-proANP antibody and incubated at room temperature for 3 hours in the dark.

The proANP present in the sample binds to the pre-coated antibody in the well and to the anti-human proANP–HRP conjugate. A tetramethylbenzidine detection substrate was then added to each well, and samples were incubated at room temperature for an additional 30 minutes in the dark. A stop solution was added to each well, and absorbance was measured at 450 nm. The enzyme-catalyzed color change of the substrate is directly proportional to the amount of proANP present in the sample.

Measurement of serum antibody levels

Serum concentrations of REGN-NPR1^{7544} and isotype control were quantified using a Gyrolab xPlore immunoassay platform, which uses centrifugal microfluidics and laser-induced fluorescence detection on a streptavidin-coated Bioaffy 200 CD (Gyros Protein Technologies AB, Uppsala, Sweden). A biotinylated anti-human kappa light chain monoclonal antibody (20 µg/mL) captured antibodies from singlet serum samples and duplicate standards (0.488–2000 ng/mL) diluted in PBS + 0.5% bovine serum albumin/5% normal mouse serum, and detection was performed with an Alexa Fluor 647-conjugated anti-human IgG1/IgG4 monoclonal antibody (0.5 µg/mL). Concentrations were determined by a 4-parameter logistic curve fit in Gyrolab Evaluator software (Gyros Protein Technologies AB) and reported as the mean of 2 replicates, with an LLOQ of 0.01 µg/mL. Serum REGN-NPR1^{7544} levels in monkey serum were measured by ELISA. Microtiter plates were coated with an anti-human IgG Fc capture antibody, and captured antibody was detected using a biotinylated anti-human IgG kappa light chain antibody followed by NeutrAvidin-HRP and a luminol-based peroxidase substrate, generating a signal proportional to total human IgG concentration.

Phase 1 clinical study

This was a phase 1, first-in-human, double-blind, randomized, placebo-controlled study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of single ascending doses of REGN-NPR1^{7544} in healthy adults (NCT05970718).

The study consisted of a screening period (Days –33 to 1), an 8 day in-clinic period consisting of pre-dose (Day –2 to –1) and post-dose portions (Days 1 to 6), and a 155-day outpatient monitoring period (Days 7 to 162) (Supplementary Fig. 4). During the inpatient period, participants were maintained on a fixed sodium diet of 3–3.5 g/day starting 2 days before dosing and were observed until discharge on Day 6 post-dose.

Inclusion/exclusion criteria

Participants aged 18–55 years with a body mass index of 18–32 kg/m^2, who were judged by the investigator to be in good health based on medical history, physical examination, vital sign measurements, electrocardiograms, and laboratory safety testing performed at screening and/or prior to administration of initial dose of study drug, were eligible for inclusion. Normal BP and HR readings were also required.

Key exclusion criteria included a history of clinically significant cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, psychiatric, or neurological disease, as assessed by the investigator, that may confound the results of the study or pose an additional risk to the participant by study participation; and history in the past 2 years of a diagnosis of hypertension, symptomatic hypotension (BP <90/50 mmHg with associated symptoms), symptomatic orthostatic hypotension, postural orthostatic tachycardia syndrome, syncope, or clinically significant cardiac arrhythmia,

or history of Raynaud's or inadequate arterial pulses in the bilateral digits or any prior finger injury that would preclude wearing signal monitors for a non-invasive pulse wave analysis device.

Study procedures

Eligible participants were enrolled into cohorts of 8, with each cohort randomized 3:1 to receive a single dose of REGN-NPR1^{7544} or placebo, either IV (6 ascending dose cohorts: 3 mg; 10 mg; 30 mg; 100 mg; 300 mg [repeated twice], and 1000 mg or SC (3 ascending dose cohorts: 100 mg; 300 mg; and 600 mg). Escalation to the next dose level or dosing of SC cohorts occurred after review of data collected through Day 8 for at least 6 participants in the previous cohort, with initiation of SC dosing only after review of data from the equivalent or higher IV dose level cohort. All dose escalation decisions were made jointly by the principal investigator and the sponsor.

BP was monitored by 3 different techniques. During the in-clinic period, a continuous pulse wave instrument (Edwards Lifesciences, Irvine, CA, USA) was used to collect continuous hemodynamic data over 3 separate ~2-hour periods during the daytime and for ~7 hours overnight for each 24-hour period. Additionally, all participants underwent oscillometric BP measurements, including at least every 8 hours during the inpatient period and once at each outpatient study visit. Finally, for those cohorts dosed with ≥100 mg REGN-NPR1^{7544} (either IV or SC), 24-hour ambulatory BP monitoring (ABPM) was performed on 5–11 occasions during the outpatient follow-up period depending on the dose given. Baseline ABPM was also performed during the screening period.

Safety assessments included monitoring of body weight, vital signs (oscillometric and orthostatic BP and HR, temperature, and respiratory rate), physical examination, electrocardiogram findings, laboratory testing, monitoring for anti-drug antibodies, and monitoring of adverse events.

Serum samples were also collected for drug concentration as well as exploratory biomarker measurements and assessments including ANP, NT-proANP, BNP, NT-proBNP, and urinary and plasma cGMP.

Rescue treatment

Per protocol, rescue treatment was instituted when certain BP criteria were met or at the discretion of the investigator. Rescue therapies included labetalol 100 mg orally three times daily, then twice daily, which could be titrated to 800 mg total per day, and the ACE inhibitor quinapril 10 mg orally once daily that could be used and titrated as needed. Per discretion of the investigator, other approved antihypertensive agents could be used. The goal of rescue treatment was to lower SBP to ≤140 mmHg or to ≤20 mmHg above the pre-dose maximum. Once rescue treatment was initiated, participants were re-evaluated at each subsequent visit. Additional visits for BP monitoring could be scheduled at the discretion of the investigator.

Statistical analysis

Preclinical assessments

For all parameters measured, data are presented as mean ± SEM. To compare SBP change at 0–24 hours and 24–48 hours against control, an ordinary 1-way analysis of

variance (ANOVA) with Dunnett's multiple comparison test was performed. A mixed-effects 2-way ANOVA with Dunnett's multiple comparison test was performed to examine the effect of treatment on SBP and HR compared to baseline, and on NT-proANP levels in serum. *P* values <0.05 were considered statistically significant. Statistical analyses were performed with GraphPad Prism 8 software (GraphPad Software).

Clinical assessments

No formal statistical hypothesis was carried out in this phase 1 study. The overall enrolment target was ~88 participants, with a total of 8 participants (6 randomized to REGN-NPR1^{7544} and 2 randomized to placebo) enrolled in each cohort. The sample size was considered appropriate to assess safety and tolerability. For continuous variables, descriptive statistics included mean, SD, 95% confidence intervals, first quartile, median, third quartile, minimum, and maximum. Plots of values over time, as well as the absolute change over time, were evaluated. For categorical and ordinal data, frequencies and percentages were determined. Data for participants on placebo were pooled across cohorts.

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Disclosures/conflicts of interest

KBD-N, EM, JHK, MED, MCF, ARH, AJ-HH, EG, AR, PK, AS, HAI, XJ, DZ, JT, XM, SKS, KG, MA, KT, MX, XC, ER, RS, HP, DP, MT, MW, TH, AJM, WO, BOA, GDY, GAH, and LGM are stockholders and employees of Regeneron. CW has no competing interests to declare.

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Author contributions

KD, LM, MD, EM, KT, MA, BAO, and GH contributed to conceptualization. KD, LM, MD, JK, AS, EG, PK, EM, KT, MA, BAO, GH, DP, and HP contributed to methodology. JT, MX, ER, and RS contributed to validation. KD, MF, MX, ER, RS, MT, and MW

contributed to formal analysis. MF, ARH, AJH, AR, AS, HA, XJ, DZ, JT, XM, SKS, KG, AS, CW, and XC contributed to investigation. JT, MF, MX, ER, and RS contributed to data curation. LM, KD, EM, and GDY contributed to writing the original draft. LM, KD, EM, and GDY contributed to review and editing the manuscript. KD, LM, TH, AJM, WO, GH, GDY, and BAO contributed to supervision.

Data sharing

Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, and statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing: 1) once the product and indication has been approved by major health authorities (eg, FDA, EMA, PMDA, etc.) or development of the product has been discontinued globally for all indications on or after April 2020 and there are no plans for future development; 2) if there is legal authority to share the data; and 3) there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/.

References

1. Vernino, S., et al., *Postural orthostatic tachycardia syndrome (POTS): State of the science and clinical care from a 2019 National Institutes of Health expert consensus meeting - part 1.* Auton Neurosci, 2021. **235**: p. 102828.

2. Dunn, M.E., et al., *Agonist antibody to guanylate cyclase receptor NPR1 regulates vascular tone.* Nature, 2024. **633**(8030): p. 654-661.

3. Tokudome, T., et al., *Endothelial natriuretic peptide receptor 1 play crucial role for acute and chronic blood pressure regulation by atrial natriuretic peptide.* Hypertension, 2022. **79**(7): p. 1409-1422.

4. Saito, Y., et al., *Clinical application of atrial natriuretic polypeptide in patients with congestive heart failure: Beneficial effects on left ventricular function.* Circulation, 1987. **76**(1): p. 115-24.

5. Bie, P., *Natriuretic peptides and normal body fluid regulation.* Compr Physiol, 2018. **8**(3): p. 1211-1249.

6. Lopez, M.J., et al., *Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide.* Nature, 1995. **378**(6552): p. 65-8.

7. Oliver, P.M., et al., *Natriuretic peptide receptor 1 expression influences blood pressures of mice in a dose-dependent manner.* Proc Natl Acad Sci U S A, 1998. **95**(5): p. 2547-51.

8. Oliver, P.M., et al., *Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A.* Proc Natl Acad Sci U S A, 1997. **94**(26): p. 14730-5.

9. Skryabin, B.V., et al., *Hypervolemic hypertension in mice with systemic inactivation of the (floxed) guanylyl cyclase-A gene by alphaMHC-Cre-mediated recombination.* Genesis, 2004. **39**(4): p. 288-98.

10. Shi, S.J., et al., *Natriuretic peptide receptor A mediates renal sodium excretory responses to blood volume expansion.* Am J Physiol Renal Physiol, 2003. **285**(4): p. F694-702.

11. Sabrane, K., et al., *Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide.* J Clin Invest, 2005. **115**(6): p. 1666-74.

12. Vandenwijngaert, S., et al., *Blood pressure-associated genetic variants in the natriuretic peptide receptor 1 gene modulate guanylate cyclase activity.* Circ Genom Precis Med, 2019. **12**(8): p. e002472.

13. Surendran, P., et al., *Discovery of rare variants associated with blood pressure regulation through meta-analysis of 1.3 million individuals.* Nat Genet, 2020. **52**(12): p. 1314-1332.

14. Liu, C., et al., *Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci.* Nat Genet, 2016. **48**(10): p. 1162-70.

15. Macdonald, L.E., et al., *Precise and in situ genetic humanization of 6 Mb of mouse immunoglobulin genes.* Proc Natl Acad Sci U S A, 2014. **111**(14): p. 5147-52.

16. Murphy, A.J., et al., *Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice.* Proc Natl Acad Sci U S A, 2014. **111**(14): p. 5153-8.

17. Kenakin, T., *Competitive antagonism. Pharmacological analysis of drug-receptor interaction*. Second ed ed. 1993, New York: Raven Press.

18. Ogawa, H., et al., *Crystal structure of hormone-bound atrial natriuretic peptide receptor extracellular domain: Rotation mechanism for transmembrane signal transduction.* J Biol Chem, 2004. **279**(27): p. 28625-31.

19. Liu, S., et al., *Architecture and activation of single-pass transmembrane receptor guanylyl cyclase.* Nature Structural & Molecular Biology, 2025. **32**(3): p. 469-478.

20. Chaffin, M., et al., *Single-nucleus profiling of human dilated and hypertrophic cardiomyopathy.* Nature, 2022. **608**(7921): p. 174-180.

21. Wang, W., et al., *Biphasic effects of ANP infusion in conscious, euvolumic rats: Roles of AQP2 and ENaC trafficking.* Am J Physiol Renal Physiol, 2006. **290**(2): p. F530-41.

22. Nonoguchi, H., K. Tomita, and F. Marumo, *Effects of atrial natriuretic peptide and vasopressin on chloride transport in long- and short-looped medullary thick ascending limbs.* J Clin Invest, 1992. **90**(2): p. 349-57.

23. Pandey, K.N., *Genetic and epigenetic mechanisms regulating blood pressure and kidney dysfunction.* Hypertension, 2024. **81**(7): p. 1424-1437.

24. Neelamegam, K., et al., *Renal tubule cell-specific Npr1 is essential to regulate blood pressure and kidney dysfunction.* Hypertension, 2026. **83**(7): p. e25890.

25. Zimmerman, R.S., et al., *High-dose atrial natriuretic factor enhances albumin escape from the systemic but not the pulmonary circulation.* Circ Res, 1990. **67**(2): p. 461-8.

26. Huxley, V.H., et al., *Increased capillary hydraulic conductivity induced by atrial natriuretic peptide.* Circ Res, 1987. **60**(2): p. 304-7.

27. Groban, L., A.W. Cowley, and T.J. Ebert, *Atrial natriuretic peptide augments forearm capillary filtration in humans.* Am J Physiol, 1990. **259**(1): p. H258-H263.

28. Ando, S., et al., *Atrial natriuretic peptide increases human capillary filtration and venous distensibility.* J Hypertens, 1992. **10**(5): p. 451-7.

29. Kulapatana, S., et al., *Blood volume deficit in postural orthostatic tachycardia syndrome assessed by semiautomated carbon monoxide rebreathing.* Clin Auton Res, 2025. **35**(2): p. 267-276.

30. Cheng, H., et al., *N-terminal pro-brain natriuretic peptide and cardiac troponin I for the prognostic utility in elderly patients with severe sepsis or septic shock in intensive care unit: A retrospective study.* J Crit Care, 2015. **30**(3): p. 654.e9-14.

31. Axelrod, F.B., et al., *Atrial natriuretic peptide response to postural change and medication in familial dysautonomia.* Clin Auton Res, 1994. **4**(6): p. 311-8.

32. Richards, A.M. and I.G. Crozier, *Elevated atrial natriuretic factor in the plasma: cause or effect in "idiopathic orthostatic hypotension"?* Int J Cardiol, 1990. **29**(2): p. 252-4.

33. Tunny, T.J., et al., *INAPPROPRIATELY ELEVATED LEVELS OF ATRIAL NATRIURETIC PEPTIDE MAY CONTRIBUTE TO THE PATHOPHYSIOLOGY OF ORTHOSTATIC HYPOTENSION.* Clinical and Experimental Pharmacology and Physiology, 1992. **19**(5): p. 283-286.

34. Hamrefors, V., et al., *Syndromes of orthostatic intolerance and syncope in young adults.* Open Heart, 2017. **4**(1): p. e000585.

35. Valenzuela, D.M., et al., *High-throughput engineering of the mouse genome coupled with high-resolution expression analysis.* Nat Biotechnol, 2003. **21**(6): p. 652-9.

36. Poueymirou, W.T., et al., *F0 generation mice fully derived from gene-targeted embryonic stem cells allowing immediate phenotypic analyses.* Nat Biotechnol, 2007. **25**(1): p. 91-9.

37. Hao, Y., et al., *Dictionary learning for integrative, multimodal and scalable single-cell analysis.* Nature Biotechnology, 2024. **42**(2): p. 293-304.

Figure 1. In vitro characterization of REGN-NPR1^{7544}. A. Flow cytometry shows REGN-NPR1^{7544} binds human and monkey NPR1 independent of ANPs or BNPs. B. SPR experiments performed on a Biacore T200 instrument determined the binding affinity of REGN-NPR1^{7544} for recombinant human and cynomolgus monkey NPR1 C. REGN-NPR1^{7544} blocks ANP and BNP-induced NPR1 activation in HEK cells expressing either human or cynomolgus monkey NPR1. D. A 3.15 Å cryo-EM structure of the human NPR1 ectodomain + ANP + REGN-NPR1^{7544} Fab is shown in surface representation, with the NPR1 ectodomains colored dark gray and white, and ANP colored blue. The 2 REGN-NPR1^{7544} Fabs are colored dark green (heavy chain) and light green (light chain). A side view is depicted on the left, and a top-down (looking at the cell membrane) view is depicted on the right. E. Same views as in panel D, with the addition of a model of the remainder of the two REGN-NPR1^{7544} IgG molecules. The modeled Fc domains are colored yellow, and the modeled second Fab domains are colored pale green. F. The side view from panel E is shown along with a schematic of the lipid bilayer, positioned and scaled to match the scale of the rest of the depiction. See Supplementary Figure 2B for details. Regions of the REGN-NPR1^{7544} IgG molecules that would clash with the membrane are shaded red. The angle (in this view orientation) between the two NPR1 ectodomain monomers is indicated. The bottom of panel F shows a depiction equivalent to the top of panel F, showing a theoretical model in which the NPR1 ectodomain monomers, and their bound REGN-NPR1^{7544} IgGs, have been adjusted to relieve the clash with the membrane.

$^{**}P$=0.005.

cGMP is a downstream second messenger of NPR1 activation. Blockade of NPR1 is expected to decrease cGMP generation.

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; cryo-EM, cryogenic -electron microscopy; Fab, fragment antigen-binding; hNPR1.mmH, human (mmH-tagged) NPR1; K_D, equilibrium dissociation constant; MFI, mean fluorescence intensity; MfNPR1.mmH, monkey (mmH-tagged) NPR1; NPR1, natriuretic peptide receptor 1; SPR, surface plasmon resonance; t½, dissociative half-life.

Figure 2. A. REGN-NPR1^{7544} showed a dose-dependent, sustained increase in blood pressure and a decrease in cGMP in normotensive NPR1$^{Hu/Hu}$ mice. B. REGN-NPR1^{7544} reverses ANP overexpression-induced hypotension in NPR1$^{Hu/Hu}$ mice. C. Standard vasodilators reversed the REGN-NPR1^{7544} –induced blood pressure increase in NPR1$^{Hu/Hu}$ mice. D. REGN-NPR1^{7544} significantly inhibited ANP-induced relaxation of precontracted mesenteric vessels isolated from NPR1$^{Hu/Hu}$ mice, confirming direct modulation of vascular tone.

***P*=0.005. ANP, atrial natriuretic peptide; cGMP, cyclic guanosine monophosphate; HDD, hydrodynamic DNA delivery; IV, intravenous; NPR1, natriuretic peptide receptor 1; PO, oral; SC, subcutaneous.

Figure 3. A. REGN-NPR1^{7544} showed a dose-dependent, sustained increase in blood pressure in normotensive monkeys. B. REGN-NPR1^{7544} showed a transient increase in NT-proANP and a decrease in hematocrit in monkeys, suggestive of increased intravascular volume.

***P*=0.0049.

ANP, atrial natriuretic peptide; IV, intravenous; NT-proANP, N-terminal pro-atrial natriuretic peptide.

Figure 4. A and B. Blood pressure as assessed by in-clinic oscillometric measurements displays increases over baseline of 5–10 mmHg in participants receiving REGN-NPR1^{7544}. C and D. Daytime blood pressure as assessed by ambulatory 24-hour monitoring displays increases of 5–10 mmHg over baseline lasting >40 days in participants receiving REGN-NPR1^{7544}.

IV, intravenous; SC, subcutaneous; SD, standard deviation; SE, standard error.

Figure 5. A and B. REGN-NPR1^{7544} displays reductions in plasma cGMP, the NPR1 second messenger, consistent with target engagement. The duration of action is dose dependent and >29 days for doses ≥100 mg. C and D. Treatment with REGN-NPR1^{7544} is associated with a 4–10-fold increase in BNP which reaches peak at 3–4 days post-dose. E and F. Treatment with REGN-NPR1^{7544} is associated with a 2–5-fold increase in ANP which reaches peak at 4–8 days post-dose.

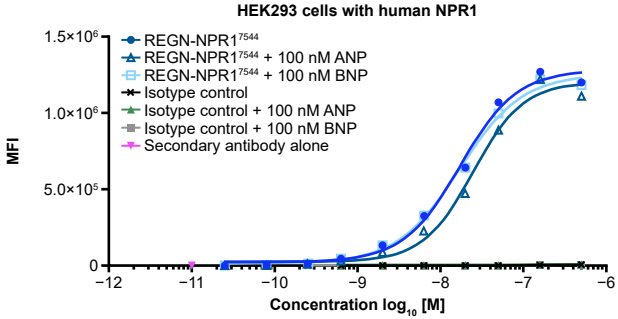
ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; IV, intravenous; NPR1, natriuretic peptide receptor 1; SC, subcutaneous; SD, standard deviation; SE, standard error.

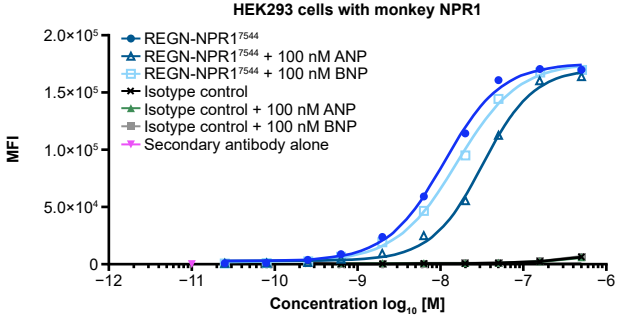
Figure 6. A and B. Analysis of hemoglobin concentration, weight, and urinary sodium excretion are consistent with plasma volume expansion. REGN-NPR1^{7544} is associated with decreases in hemoglobin of 10 g/L, consistent with hemodilution due to plasma volume expansion. C and D. Increases in body weight of approximately 0.5–0.75 kg occurred within days after REGN-NPR1^{7544} dosing, consistent with fluid retention. E and F. Sodium excretion in 24-hour urine collections is reduced following REGN-NPR1^{7544} exposure.

IV, intravenous; SC, subcutaneous; SD, standard deviation; SE, standard error.

A.

Cell binding: Human and monkey NPR1 overexpressing HEK293 cells



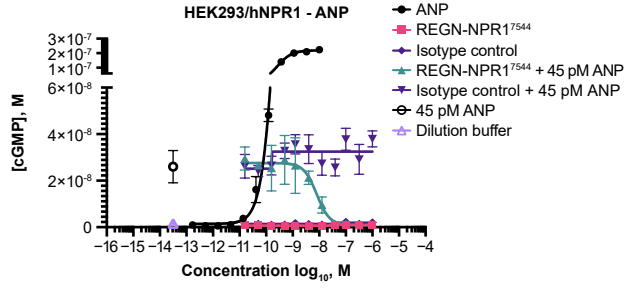


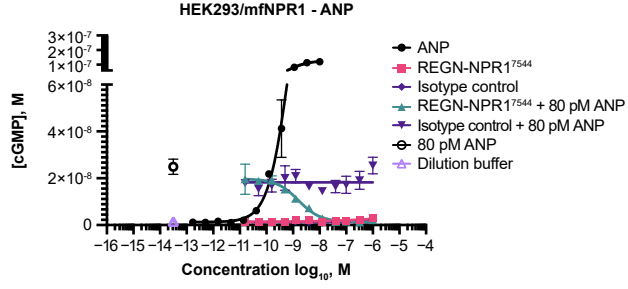
B.

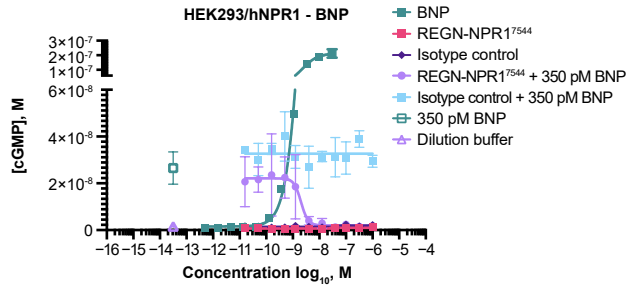
Biacore binding kinetics

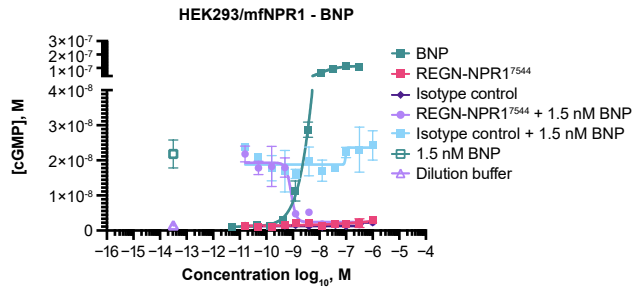
Protein injected over surface-captured REGN-NPR1^{7544}	Kinetic binding parameters	
	K_D (M)	$t_{½}$ (minutes)
hNPR1.mmH	3.97 × 10^{-11}	815.1
hNPR1.mmH + 10 x ANP	1.45 × 10^{-10}	253.3
hNPR1.mmH + 10 x BNP	2.51 × 10^{-9}	14.3
MfNPR1.mmH	3.14 × 10^{-11}	650.3

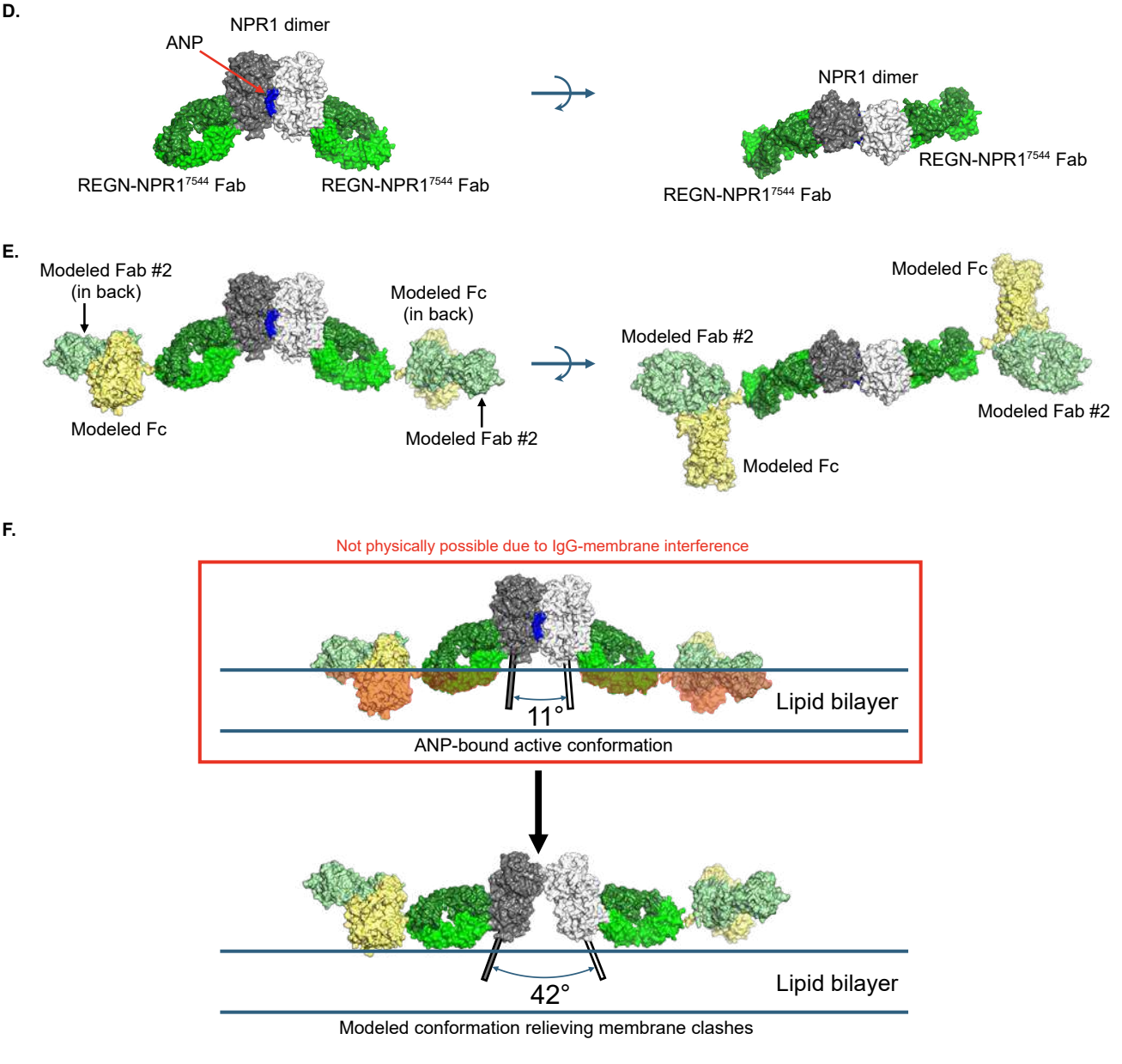
C.

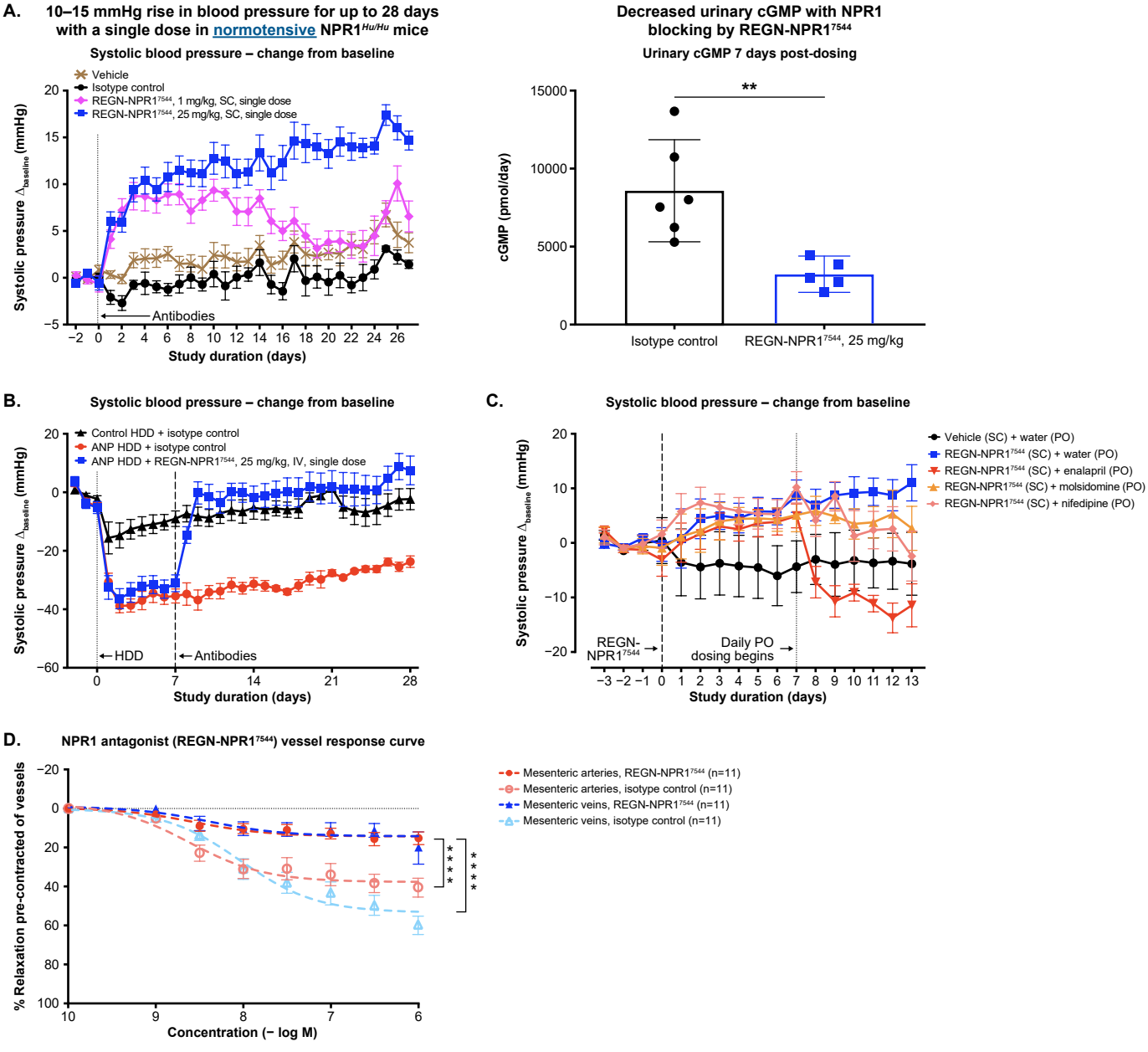




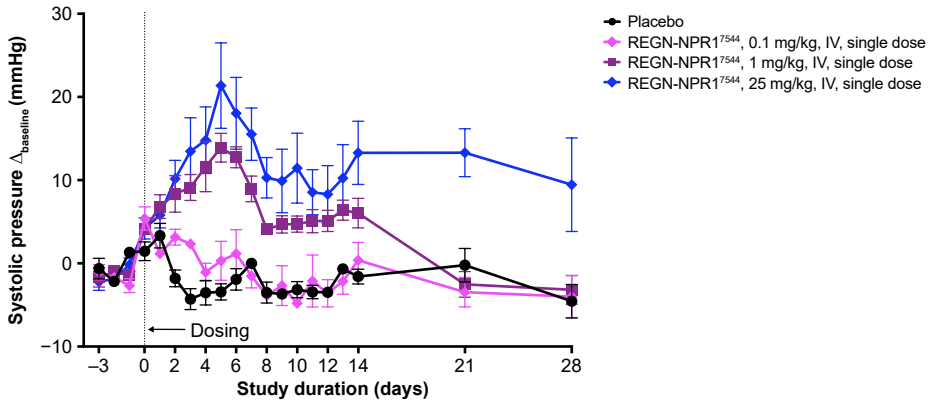




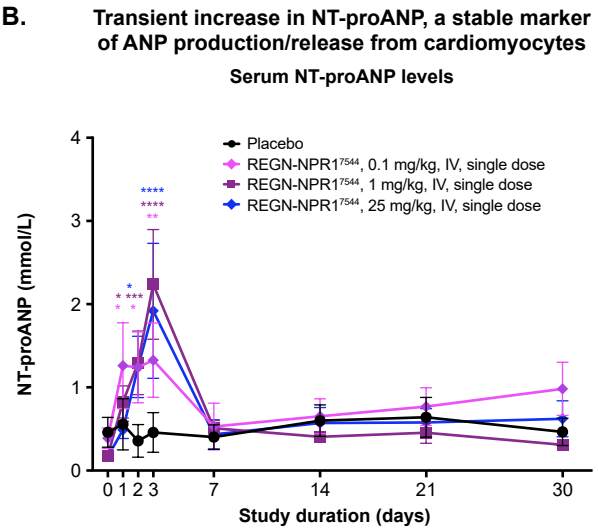

D.
NPR1 dimer
ANP
REGN-NPR1^{7544} Fab
REGN-NPR1^{7544} Fab
NPR1 dimer
REGN-NPR1^{7544} Fab
REGN-NPR1^{7544} Fab
E.
Modeled Fab #2
(in back)
Modeled Fc
(in back)
Modeled Fc
Modeled Fab #2
Modeled Fab #2
Modeled Fc
Modeled Fc
Modeled Fab #2
F.
Not physically possible due to IgG-membrane interference
11°
Lipid bilayer
ANP-bound active conformation
42°
Lipid bilayer
Modeled conformation relieving membrane clashes

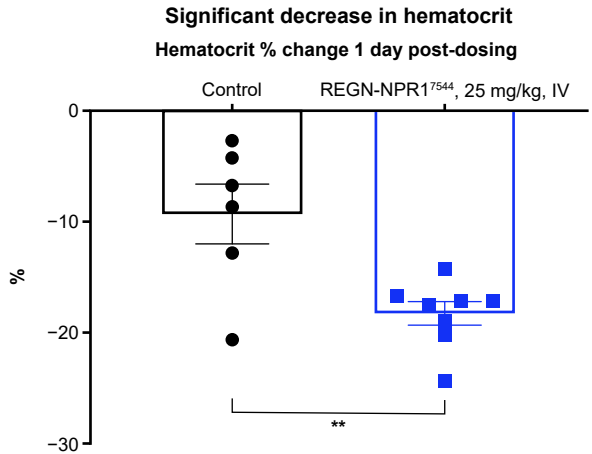

A.
10–15 mmHg rise in blood pressure for up to 28 days with a single dose in normotensive NPR1Hu/Hu mice
Systolic blood pressure – change from baseline
Vehicle
Isotype control
REGN-NPR17544, 1 mg/kg, SC, single dose
REGN-NPR17544, 25 mg/kg, SC, single dose
Systolic pressure Δbaseline (mmHg)
Antibodies
Study duration (days)
Decreased urinary cGMP with NPR1 blocking by REGN-NPR17544
Urinary cGMP 7 days post-dosing
cGMP (pmol/day)
**
Isotype control
REGN-NPR17544, 25 mg/kg
B.
Systolic blood pressure – change from baseline
Control HDD + isotype control
ANP HDD + isotype control
ANP HDD + REGN-NPR17544, 25 mg/kg, IV, single dose
HDD
Antibodies
Study duration (days)
C.
Systolic blood pressure – change from baseline
Vehicle (SC) + water (PO)
REGN-NPR17544 (SC) + water (PO)
REGN-NPR17544 (SC) + enalapril (PO)
REGN-NPR17544 (SC) + molsidomine (PO)
REGN-NPR17544 (SC) + nifedipine (PO)
REGN-NPR17544
Daily PO dosing begins
Study duration (days)
D.
NPR1 antagonist (REGN-NPR17544) vessel response curve
Mesenteric arteries, REGN-NPR17544 (n=11)
Mesenteric arteries, isotype control (n=11)
Mesenteric veins, REGN-NPR17544 (n=11)
Mesenteric veins, isotype control (n=11)
% Relaxation pre-contracted of vessels
****
****
Concentration (− log M)

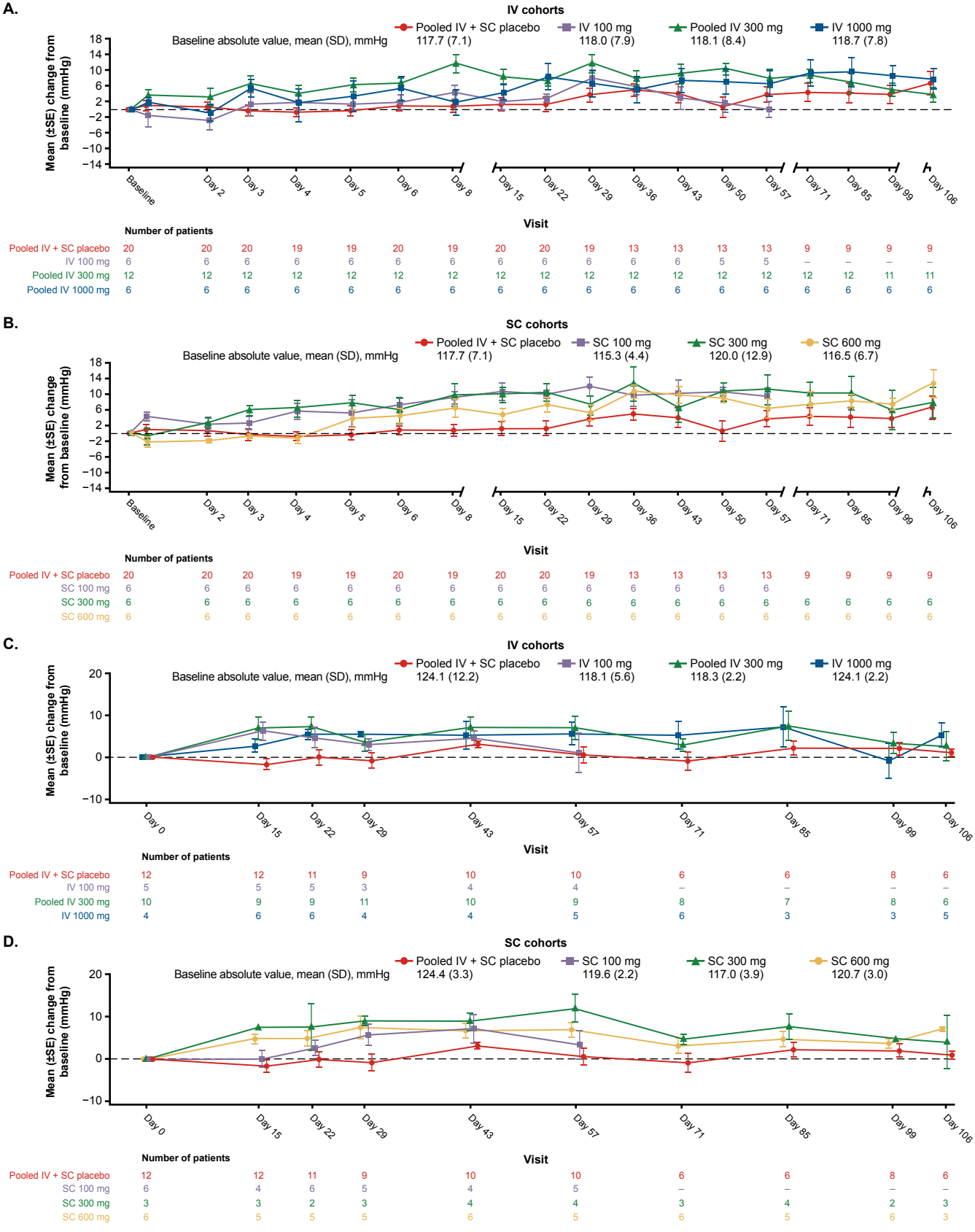
A.

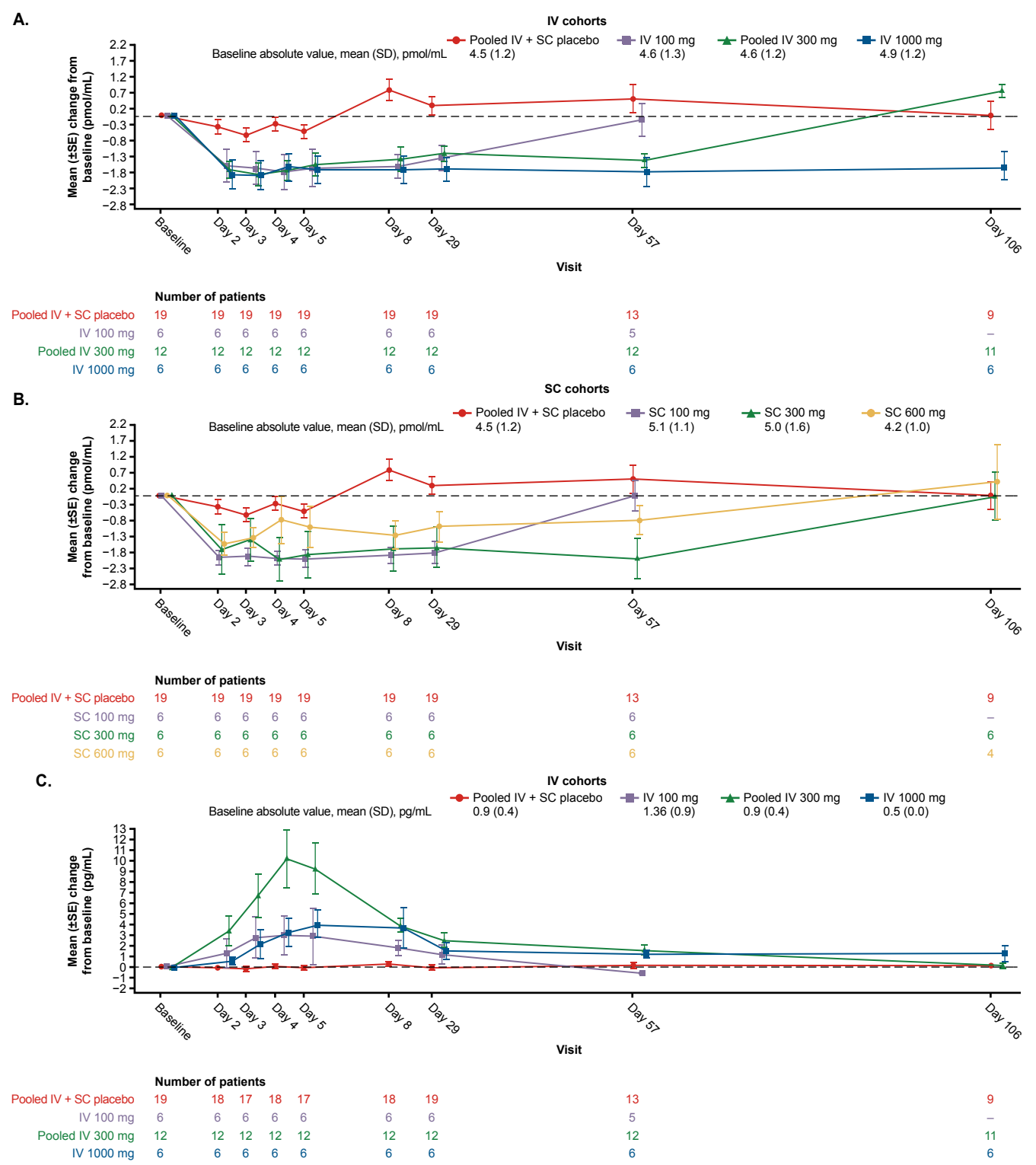

Systolic blood pressure – change from baseline
Systolic pressure Δbaseline (mmHg)
30
20
10
0
−10
Dosing
−3 0 2 4 6 8 10 12 14 21 28
Study duration (days)
Placebo
REGN-NPR17544, 0.1 mg/kg, IV, single dose
REGN-NPR17544, 1 mg/kg, IV, single dose
REGN-NPR17544, 25 mg/kg, IV, single dose

B.

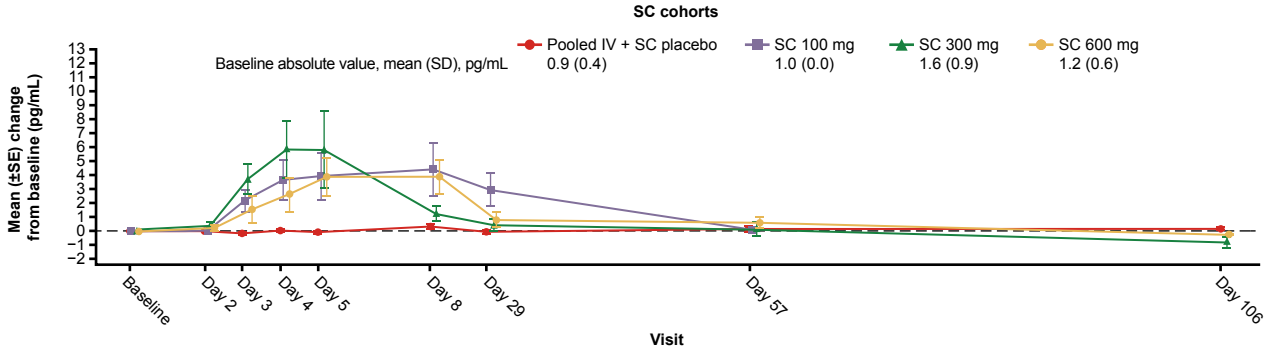

Transient increase in NT-proANP, a stable marker of ANP production/release from cardiomyocytes
Serum NT-proANP levels
NT-proANP (mmol/L)
4
3
2
1
0
0 1 2 3 7 14 21 30
Study duration (days)
Placebo
REGN-NPR17544, 0.1 mg/kg, IV, single dose
REGN-NPR17544, 1 mg/kg, IV, single dose
REGN-NPR17544, 25 mg/kg, IV, single dose


Significant decrease in hematocrit
Hematocrit % change 1 day post-dosing
Control
REGN-NPR17544, 25 mg/kg, IV
%
0
−10
−20
−30
**


A.
IV cohorts
Pooled IV + SC placebo 117.7 (7.1)
IV 100 mg 118.0 (7.9)
Pooled IV 300 mg 118.1 (8.4)
IV 1000 mg 118.7 (7.8)
Baseline absolute value, mean (SD), mmHg
Mean (±SE) change from baseline (mmHg)
Baseline
Day 2
Day 3
Day 4
Day 5
Day 6
Day 8
Day 15
Day 22
Day 29
Day 36
Day 43
Day 50
Day 57
Day 71
Day 85
Day 99
Day 106
Visit
Number of patients
Pooled IV + SC placebo 20 20 20 19 19 20 19 20 20 19 13 13 13 13 9 9 9 9
IV 100 mg 6 6 6 6 6 6 6 6 6 6 6 6 5 5 – – – –
Pooled IV 300 mg 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 11 11
Pooled IV 1000 mg 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
B.
SC cohorts
Pooled IV + SC placebo 117.7 (7.1)
SC 100 mg 115.3 (4.4)
SC 300 mg 120.0 (12.9)
SC 600 mg 116.5 (6.7)
Baseline absolute value, mean (SD), mmHg
Mean (±SE) change from baseline (mmHg)
Visit
Number of patients
Pooled IV + SC placebo 20 20 20 19 19 20 19 20 20 19 13 13 13 13 9 9 9 9
SC 100 mg 6 6 6 6 6 6 6 6 6 6 6 6 6 6
SC 300 mg 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
SC 600 mg 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
C.
IV cohorts
Pooled IV + SC placebo 124.1 (12.2)
IV 100 mg 118.1 (5.6)
Pooled IV 300 mg 118.3 (2.2)
IV 1000 mg 124.1 (2.2)
Baseline absolute value, mean (SD), mmHg
Mean (±SE) change from baseline (mmHg)
Day 0
Day 15
Day 22
Day 29
Day 43
Day 57
Day 71
Day 85
Day 99
Day 106
Visit
Number of patients
Pooled IV + SC placebo 12 12 11 9 10 10 6 6 8 6
IV 100 mg 5 5 5 3 4 4 – – – –
Pooled IV 300 mg 10 9 9 11 10 9 8 7 8 6
IV 1000 mg 4 6 6 4 4 5 6 3 3 5
D.
SC cohorts
Pooled IV + SC placebo 124.4 (3.3)
SC 100 mg 119.6 (2.2)
SC 300 mg 117.0 (3.9)
SC 600 mg 120.7 (3.0)
Baseline absolute value, mean (SD), mmHg
Mean (±SE) change from baseline (mmHg)
Visit
Number of patients
Pooled IV + SC placebo 12 12 11 9 10 10 6 6 8 6
SC 100 mg 6 4 6 5 4 5 – – – –
SC 300 mg 3 3 2 3 4 4 3 4 2 3
SC 600 mg 6 5 5 5 6 5 6 5 6 3


A.
IV cohorts
Pooled IV + SC placebo 4.5 (1.2)
IV 100 mg 4.6 (1.3)
Pooled IV 300 mg 4.6 (1.2)
IV 1000 mg 4.9 (1.2)
Baseline absolute value, mean (SD), pmol/mL
Mean (±SE) change from baseline (pmol/mL)
2.2
1.7
1.2
0.7
0.2
−0.3
−0.8
−1.3
−1.8
−2.3
−2.8
Baseline
Day 2
Day 3
Day 4
Day 5
Day 8
Day 29
Day 57
Day 106
Visit
Number of patients
Pooled IV + SC placebo 19 19 19 19 19 19 19 13 9
IV 100 mg 6 6 6 6 6 6 6 5 –
Pooled IV 300 mg 12 12 12 12 12 12 12 12 11
IV 1000 mg 6 6 6 6 6 6 6 6 6
B.
SC cohorts
Pooled IV + SC placebo 4.5 (1.2)
SC 100 mg 5.1 (1.1)
SC 300 mg 5.0 (1.6)
SC 600 mg 4.2 (1.0)
Baseline absolute value, mean (SD), pmol/mL
Mean (±SE) change from baseline (pmol/mL)
2.2
1.7
1.2
0.7
0.2
−0.3
−0.8
−1.3
−1.8
−2.3
−2.8
Baseline
Day 2
Day 3
Day 4
Day 5
Day 8
Day 29
Day 57
Day 106
Visit
Number of patients
Pooled IV + SC placebo 19 19 19 19 19 19 19 13 9
SC 100 mg 6 6 6 6 6 6 6 6 –
SC 300 mg 6 6 6 6 6 6 6 6 6
SC 600 mg 6 6 6 6 6 6 6 6 4
C.
IV cohorts
Pooled IV + SC placebo 0.9 (0.4)
IV 100 mg 1.36 (0.9)
Pooled IV 300 mg 0.9 (0.4)
IV 1000 mg 0.5 (0.0)
Baseline absolute value, mean (SD), pg/mL
Mean (±SE) change from baseline (pg/mL)
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
Baseline
Day 2
Day 3
Day 4
Day 5
Day 8
Day 29
Day 57
Day 106
Visit
Number of patients
Pooled IV + SC placebo 19 18 17 18 17 18 19 13 9
IV 100 mg 6 6 6 6 6 6 6 5 –
Pooled IV 300 mg 12 12 12 12 12 12 12 12 11
IV 1000 mg 6 6 6 6 6 6 6 6 6

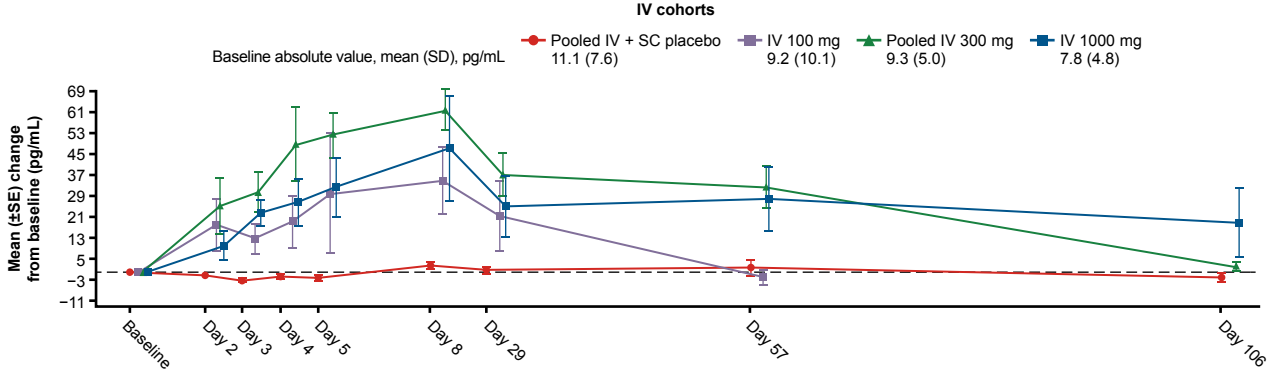
D.



Number of patients

Pooled IV + SC placebo	19	18	17	18	17	18	19	13	9
SC 100 mg	6	6	6	6	6	6	6	6	–
SC 300 mg	6	6	6	6	6	6	6	6	6
SC 600 mg	6	6	6	6	6	5	6	6	4

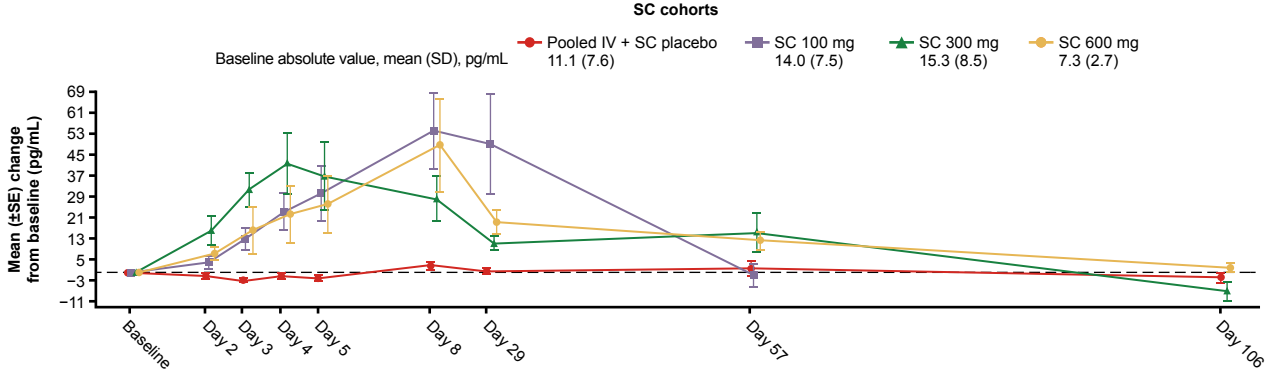
E.



Number of patients

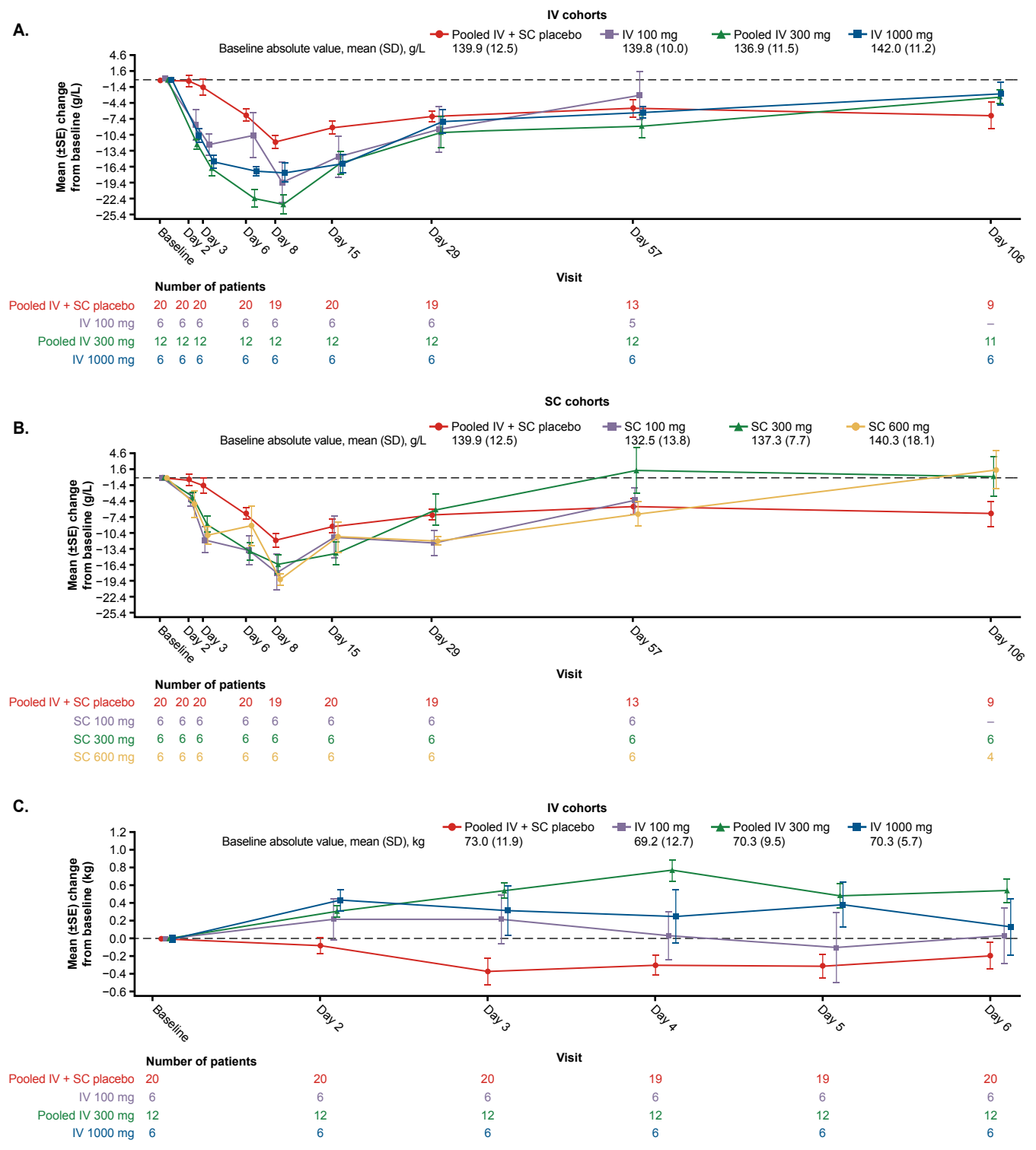
Pooled IV + SC placebo	19	18	17	18	17	18	19	13	9
IV 100 mg	6	6	6	6	6	6	6	5	–
Pooled IV 300 mg	12	12	12	12	12	12	12	12	11
IV 1000 mg	6	6	6	6	6	6	6	6	6

F.

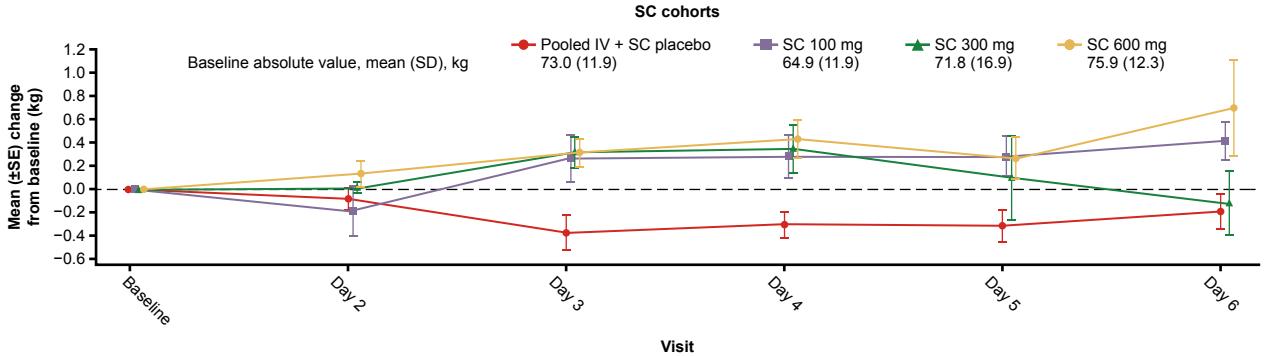


Number of patients

Pooled IV + SC placebo	19	18	17	18	17	18	19	13	9
SC 100 mg	6	6	6	6	6	6	6	6	–
SC 300 mg	6	6	6	6	6	6	6	6	6
SC 600 mg	6	6	6	6	6	5	6	6	4

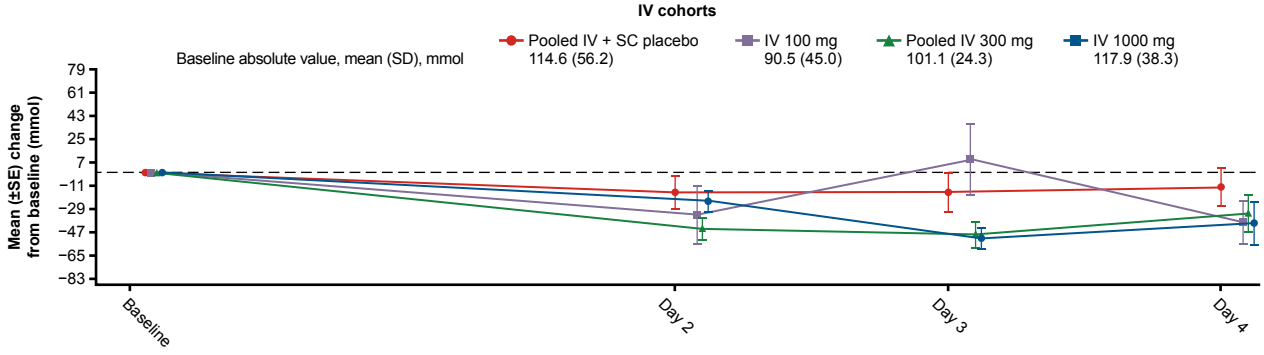

A.
IV cohorts
Pooled IV + SC placebo
IV 100 mg
Pooled IV 300 mg
IV 1000 mg
Baseline absolute value, mean (SD), g/L
139.9 (12.5)
139.8 (10.0)
136.9 (11.5)
142.0 (11.2)
Mean (±SE) change from baseline (g/L)
4.6
1.6
−1.4
−4.4
−7.4
−10.4
−13.4
−16.4
−19.4
−22.4
−25.4
Baseline
Day 2
Day 3
Day 6
Day 8
Day 15
Day 29
Day 57
Day 106
Visit
Number of patients
Pooled IV + SC placebo 20 20 20 20 19 20 19 13 9
IV 100 mg 6 6 6 6 6 6 6 5 –
Pooled IV 300 mg 12 12 12 12 12 12 12 12 11
IV 1000 mg 6 6 6 6 6 6 6 6 6
B.
SC cohorts
Pooled IV + SC placebo
SC 100 mg
SC 300 mg
SC 600 mg
Baseline absolute value, mean (SD), g/L
139.9 (12.5)
132.5 (13.8)
137.3 (7.7)
140.3 (18.1)
Mean (±SE) change from baseline (g/L)
4.6
1.6
−1.4
−4.4
−7.4
−10.4
−13.4
−16.4
−19.4
−22.4
−25.4
Baseline
Day 2
Day 3
Day 6
Day 8
Day 15
Day 29
Day 57
Day 106
Visit
Number of patients
Pooled IV + SC placebo 20 20 20 20 19 20 19 13 9
SC 100 mg 6 6 6 6 6 6 6 6 –
SC 300 mg 6 6 6 6 6 6 6 6 6
SC 600 mg 6 6 6 6 6 6 6 6 4
C.
IV cohorts
Pooled IV + SC placebo
IV 100 mg
Pooled IV 300 mg
IV 1000 mg
Baseline absolute value, mean (SD), kg
73.0 (11.9)
69.2 (12.7)
70.3 (9.5)
70.3 (5.7)
Mean (±SE) change from baseline (kg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
Baseline
Day 2
Day 3
Day 4
Day 5
Day 6
Visit
Number of patients
Pooled IV + SC placebo 20 20 20 19 19 20
IV 100 mg 6 6 6 6 6 6
Pooled IV 300 mg 12 12 12 12 12 12
IV 1000 mg 6 6 6 6 6 6

D.



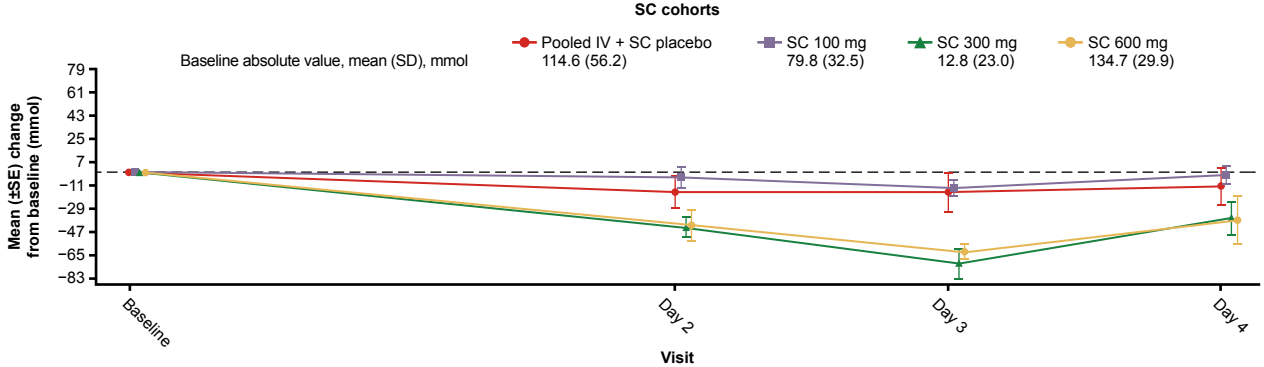
Number of patients						
Pooled IV + SC placebo	20	20	20	19	19	20
SC 100 mg	6	6	6	6	6	6
SC 300 mg	6	6	6	6	6	6
SC 600 mg	6	6	6	6	6	6

E.



Number of patients				
Pooled IV + SC placebo	20	20	20	19
IV 100 mg	6	6	6	6
Pooled IV 300 mg	12	12	12	12
IV 1000 mg	6	6	6	6

F.



Number of patients				
Pooled IV + SC placebo	20	20	20	19
SC 100 mg	6	6	6	6
SC 300 mg	6	6	6	6
SC 600 mg	6	6	6	6

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DevalarajaNPR1benchtobedsideMAN20Aug2026FINALsuppl.docx