nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Automated data-collection was carried out using EPU (Thermo Fisher Scientific) at a nominal magnification of 105,000x, corresponding to a pixel size of 0.86 Å
Data analysis	Preclinical analysis was performed using standard statistical software (GraphPad Prism 7 and GraphPAD Prism 9.0.2). Genetic analysis was performed using REGENIE version 2+.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing

once the product and indication has been approved by major health authorities (e.g. 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/.

PDB codes: 1T34, 1DP4

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	The findings are applicable to all sexes and genders. No sex- or gender-based analyses have been performed.
Reporting on race, ethnicity, or other socially relevant groupings	No race, ethnicity, or other socially relevant groupings based analyses have been performed.
Population characteristics	Healthy male or female adults 18 to 55 years of age (inclusive) were included in the clinical study. Participants were screened for systolic BP 100–140 mmHg (inclusive) and diastolic BP 60–90 mmHg (inclusive). If participants in a dosing cohort experienced clinically significant hypotension or tachycardia, the BP inclusion criteria could be adapted to allow for increases in the range (systolic BP, 130–165 mmHg; diastolic BP, 60–100 mmHg), though adaptation of the inclusion criteria was not necessary. After initial screening, participants attended a 2-day inpatient treatment/observation period prior to dosing and were placed on a fixed-sodium diet to ensure consistency in sodium intake and to reduce the variability in BP due to dietary sodium. Noninvasive hemodynamics were measured using pulse wave analysis technology (ClearSight, Edwards Lifesciences) beginning 1 day prior to randomization and continued through to the end of the inpatient monitoring period.
Recruitment	Participants were recruited at a single site (Belgium). There was no selection bias in recruitment of patients in the trial.
Ethics oversight	The study protocol was approved by institutional review boards or relevant ethics committee, and the research ethics committee of the University Hospitals of Leuven. The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki, and was consistent with the International Conference on Harmonization/Good Clinical Practices and applicable regulatory requirements.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	A total of 48 healthy volunteers were randomized to receive a single intravenous injection of REGN5381 (0.3, 1, 3, 10, 30, or 100 mg) or placebo: 5–6 patients per active dose group and 16 patients treated with placebo. If four cohorts were enrolled, a standard deviation change in blood pressure of 8 mmHg would enable a minimum detectable difference of 7.7 mmHg (based on a 1-sided t-test at a significance level of 0.05).
Data exclusions	There were no data exclusions
Replication	Not applicable for clinical data.
Randomization	Participants were randomized 6:2 to receive single-dose intravenous REGN5381 (0.3, 1, 3, 10, 30, or 100 mg) or intravenous placebo.
Blinding	This is a double-blinded study. Study subjects, the investigators, and study site personnel were blinded to all randomization assignments throughout the study. The Sponsor Medical/Study Director, Study Monitor, and any other Regeneron personnel who were in regular contact with the study site will remained blinded to all subject randomization assignments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

REGN5381 (Regeneron Pharmaceuticals, Inc.)
REGN2567 (Regeneron Pharmaceuticals, Inc.)
Mouse anti-his monoclonal antibody (Cytiva)

Validation

REGN5381 is a human immunoglobulin G4-based monoclonal antibody (developed by Regeneron Pharmaceuticals, Inc.) that binds and activates NPR1 in both the presence and absence of the endogenous ligands ANP and BNP. A high-throughput screen, VelocImmune® technology, was used to identify REGN5381. For additional information please visit: https://clinicaltrials.gov/ct2/show/record/NCT04506645

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293 from Human Embryonic Kidney

Authentication

Human 16-Marker STR Profile, Interspecies Contamination Test by IDEXX BioAnalytics (completed 11/9/2021). Identity match was shown to be >80%.

Mycoplasma contamination

Confirmed to be negative for mycoplasma - Tested by IDEXX BioAnalytics (completed by 9/30/2020)

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

The preclinical studies involved mice, beagle dogs and male cynomolgus monkeys (Macaca fascicularis)

Wild animals

The study did not involve wild animals

Reporting on sex

Sex-based analyses were not performed

Field-collected samples

Preclinical in vivo studies
NPR1hu/hu mice were generated on a C57BL/6NTac (75%)/129S6SvEvTac (25%) background using the Velocigene® platform. The generation of these mice was necessitated by the poor conservation of the NPR1 amino acid sequence between human and mouse, and the need to generate a human antibody. Each animal was implanted with PA-C10 (Data Sciences International, St. Paul, MN, USA) radiotelemetry devices for the recording of central arterial pressures. The transmitter was located in the carotid artery of each mouse. Mice were fed normal chow (PicoLab® Rodent Diet 20, #5001), housed under standard conditions, and allowed to acclimate for at least 7 days prior to being placed on study. REGN5381 was diluted with sterile phosphate-buffered saline for subcutaneous injection into mice.
Telemetered NPR1hu/hu mice were randomly assigned to one of five dosing groups (n = 4–7 per group) based on body weight, and received a single subcutaneous dose of saline or REGN5381 (1, 5, 25, or 50 mg/kg) on study day 0. The BP of each animal was continuously monitored for 28 days. Urine samples for urinalysis and urinary biomarkers were collected on day 28 post-dose.
Non-human primate studies
Thirty cynomolgus monkeys (Macaca fascicularis) of Mauritius origin, aged 2–4 years and weighing 3–5 kg (Charles River Laboratories, Inc., Reno, NV, USA), were enrolled for studies. For telemetry studies, each animal was implanted with PhysioTel Digital model L11 (Data Sciences International) radiotelemetry devices for recording the central arterial pressure and ECG waveforms. The transmitter was located intramuscularly in a dorsal position and lateral to the median plane below the ribs, and the attached BP catheter was located in the femoral artery with the tip of the catheter located in the descending aorta.
Animals were acclimated to laboratory housing for a minimum of 5 weeks before initiation of dosing (including recovery time after surgical implantation when performed). During treatment, non-human primates were housed individually in stainless-steel cages in a controlled environment under standard conditions. They were provided Purina Certified Primate Diet 5048 biscuits twice daily and assorted fresh fruit or vegetables. Reverse osmosis–filtered water was provided ad libitum by means of an automatic watering

system.
Dog telemetry study
Two beagle dogs (Canis lupus familiaris) aged 8-10 months and weighing 9–10.5 kg were received from Marshall BioResources (North Rose, NY) and enrolled in the study. For telemetry studies, each animal was implanted with PhysioTel Digital model D70 (Data Sciences International, St. Paul, MN) radiotelemetry devices for recording the central arterial pressure.
Animals were acclimated to laboratory housing for a minimum of 2 weeks before initiation of dosing (including recovery time after surgical implantation when performed). During treatment, dogs were housed in groups of two in a controlled environment under standard conditions. They were provided PMI Nutrition international, LLC Certified Canine LabDiet 5007 daily.
Acute anesthetized dog study
Twelve beagle dogs (Canis lupus familiaris) aged 11–13 months and weighing 9–15 kg were received from Covance (Cumberland, VA, USA) and enrolled in the study. General procedures for animal care and housing met current American Association for Accreditation of Laboratory Animal Care International recommendations, Guide for the Care and Use of Laboratory Animals and the U.S. Department of Agriculture through the Animal Welfare Act (as amended, and conformed to testing facility SOP)47-49. Dogs were housed in runs (up to two dogs per run). The temperature and humidity ranges of the study room were set to maintain 74 ± 10°F and 50 ± 20%, respectively. The light cycle was set to maintain 12 hours on/12 hours off. Dogs were offered Certified Canine Diet (LabDiet 5007) once daily except on the day of that dog's scheduled experiment; each dog was kept on an overnight fast before the day of its scheduled experiment. Dogs were provided fresh water ad libitum using an automatic watering system except when removed from the run. Anesthetized dogs were instrumented with venous and arterial catheters. Each dog received a single intravenous bolus dose of saline (control, n = 6) or 25 mg/kg REGN5381 (n = 6). Following dose administration, dogs were monitored for acute hemodynamic changes.

Ethics oversight

All mouse experiments were conducted in compliance with protocols approved by the Regeneron Pharmaceuticals Institutional Animal Care and Use Committee, in accordance with state and federal guidelines. All dog experiments were conducted in compliance with protocols approved by Charles River Labs or Battelle Institutional Animal Care and Use Committees. All nonhuman primate experiments were conducted in compliance with protocols approved by the Charles River Institutional Animal Care and Use Committee, in accordance with state and federal guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

NCT04506645

Study protocol

Qualified researchers may request access to the study protocol at https://vivli.org/.

Data collection

Patient data was collated in a clinical setting, and study location including time period of recruitment and data collection is detailed on ClinicalTrials.gov.

Outcomes

Predefined Primary Endpoint
Here we present the first part study results. The primary endpoint in the study was the type, incidence, and severity of TEAEs following single IV dose administrations of REGN5381 or placebo over time in healthy normotensive and otherwise healthy hypertensive adults.

Predefined Secondary Endpoints
The secondary endpoints and assessments included:
• Change from baseline in SBP, DBP, mean arterial pressure (MAP), pulse pressure (PP), HR, and SV over time
• Maximum change from baseline in SBP, DBP, MAP, PP, HR, and SV across the first 24 hours postdose
• Change from baseline in the 24-hour mean SBP, DBP, MAP, PP, and HR measured from 0 to 24 hours, 24 to 48 hours, and 48 to 72 hours postdose
• Concentrations of REGN5381 over time
• Number and percentage of subjects who develop anti-drug antibodies (ADA) and titers over time

Predefined Exploratory Endpoints
The exploratory endpoints and assessment included:
• Change from baseline in urine cGMP and plasma cGMP over time
• Change from baseline in renin, aldosterone, N-terminal (NT)-proBNP and cardiac troponin T after dose administration over time
• Change from baseline in derivative of blood pressure over time (dP/dt)
• Change from baseline in urine output and sodium clearance over time
• Change from baseline in BP variability over time
• Change in SV, stroke volume variation (SVV), SBP, DBP, and MAP over time following a crystalloid fluid bolus

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cells were suspended with enzyme free cell dissociation solution (Specialty Media, S-004-C) after rinsed with PBS once. The suspended cells were then collected with flow buffer (PBS supplemented with 2% FBS) and centrifuged. After removing supernatant, the cell pellets were resuspended and aliquoted 0.5 million cells in flow buffer into a 96-well plate (ThermoFisher Scientific, 249946) followed with a 30-minute incubation with primary antibody on ice. Cells were then incubated with Alexa Fluor 488- or APC-conjugated goat anti-human IgG secondary antibody (Jackson ImmunoResearch, 109-547-003 or 109-136-170) for 30 minutes on ice and viability dye staining (Invitrogen, L10120, or ThermoFisher Scientific, 65-0863-18) for 15 to 30 minutes on ice. Cells were washed twice with flow buffer or once with PBS post the secondary antibody staining or viability staining. The cells were then analyzed on a flow cytometer after centrifuged through a 96-well filter plate (PALL Corporation, 8027) into an U-bottom 96-well plate (Costar, 3360).
Instrument	CytoFlex Flow Cytometer, Beckman Coulter
Software	FlowJoTM 10, LLC Ashland, OR, USA
Cell population abundance	The final cell population was 25.9% to 64.0% of the total events.
Gating strategy	For measuring specific binding of REGN5381 to human and cynomolgus monkey NPR1, the cells were sequentially gated on lymphocytes (SSC-A vs FSC-A), viable cells, and single cells (FSC-H vs FSC-A). For measuring specific binding of REGN5381 to cNPR1, the cells were sequentially gated on lymphocytes (SSC-A vs FSC-A) and viable cells.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.