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ORIGINAL RESEARCH ARTICLE

Pharmacokinetics and Safety of Single Intravenous Doses of JNJ-54452840, an Anti-β1-Adrenergic Receptor Antibody Cyclopeptide, in Healthy Male Japanese and Caucasian Participants

Ivo P. Nnane[1] · Alexei H. Plotnikov[2] · Gary Peters[3] · Maureen Johnson[2] · Clare Kojak[2] · Apinya Vutikullird[4] · Jay Ariyawansa[2] · Ronald De Vries[5] · Brian E. Davies[1]

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Abstract

Aim To evaluate the pharmacokinetics and safety of single intravenous doses of JNJ-54452840 infused over 1 minute in healthy male Japanese and Caucasian participants. JNJ-54452840 is a novel peptide for the treatment of chronic heart failure, with a proposed mechanism of action of binding interference and decreased production of anti-β1-adrenergic receptor (anti-β1-AR) antibodies, which stimulate the cardiac β1-AR.

Methods In this randomized, single-centre, double-blind, placebo-controlled, four-way crossover study, 32 male Japanese and Caucasian participants (16 in each group) received single intravenous doses of JNJ-54452840 20, 80 and 240 mg, and placebo, each separated by a ≥7-day washout period. Pharmacokinetics and safety were assessed predose and at specified timepoints for 24 h. Anti-β1-AR antibodies were monitored.

Results The mean JNJ-54452840 maximum observed plasma concentration (C_{max}) and area under the concentration–time curve from time zero to infinity with extrapolation of the terminal phase (AUC_{inf}) values increased linearly with dose, with rapid elimination in both groups. Dose proportionality criteria were not met between the 20 and 240 mg doses for both study cohorts. The median time to reach C_{max} (T_{max}) ranged from 1 to 5 minutes. The mean total systemic clearance after intravenous administration (CL), volume of distribution at steady state (V_{ss}), mean residence time (MRT) and terminal half-life ($T_{½}$) values were similar for both groups. The mean $T_{½}$ values ranged from 5.9 to 26.1 min in a dose-dependent manner. The overall prevalence of antibodies was 9.4 % at baseline; antibodies not present at baseline developed in five Caucasians (15.6 %) but not in Japanese participants. One participant in each group experienced a serious thromboembolic event (pulmonary embolism, ischaemic stroke).

Conclusion JNJ-54452840 demonstrated similar pharmacokinetics in both groups. JNJ-54452840 was possibly immunogenic, and two participants reported thromboembolic serious adverse events. The relationship between these events and antibody formation is not known.

✉ Brian E. Davies
bdavies5@its.jnj.com

1 Biologics Clinical Pharmacology, Janssen Research & Development, LLC, 1400 McKean Road, PO Box 776, Spring House, PA 19477, USA

2 Janssen Research & Development, LLC, Raritan, NJ, USA

3 Cardiovascular, Metabolic, Medical Office, Janssen Research & Development, LLC, 1400 McKean Road, PO Box 776, Spring House, PA 19477, USA

4 WCCT Global, LLC, Cypress, CA, USA

5 Janssen Research & Development, Beerse, Belgium

Key Points

Overall, the mean JNJ-54452840 maximum observed plasma concentration (C_{max}), area under the concentration–time curve (AUC) from time zero to the time of the last quantifiable concentration during a 24-h dosing interval (AUC_{last}) and AUC from time zero to infinity with extrapolation of the terminal phase (AUC_{inf}) values after single intravenous infusions of 20, 80 and 240 mg of JNJ-54452840 increased linearly with dose, although the dose proportionality criteria were not met between the 20 and 240 mg dose levels in healthy male Japanese and Caucasian participants.

Rapid elimination was observed for both Japanese and Caucasian male participants.

Two serious thromboembolic adverse events occurred: pulmonary embolism in a Caucasian participant and ischaemic stroke in a Japanese participant.

The development of circulating anti-β1-adrenergic receptor antibodies in five Caucasian participants after drug exposure (not present at baseline), which also persisted in some of them at follow-up evaluations, may suggest an immune response to JNJ-54452840, although the qualitative nature of the assay and its sensitivity limit the ability to reliably interpret these results. Also, there was no clear relationship between the presence of antibodies and the incidence of adverse events.

1 Introduction

Heart failure is a major and growing population health risk in Western societies, with substantial associated morbidity and mortality [1, 2]. An autoimmune response against myocardial cells may contribute to the pathogenesis of cardiac dilatation and failure in a subset of human heart failure [3]. Specifically, stimulatory anti-β1-adrenergic receptor (anti-β1-AR) autoantibodies against the second extracellular loop of the cardiac β1-AR may contribute to the pathogenesis of chronic heart failure [4–6]. The clinical relevance of anti-β1-AR autoantibodies in the pathogenesis of dilated cardiomyopathy is supported by clinical studies suggesting therapeutic benefits of antibody removal by immunoadsorption [7].

JNJ-54452840 is a novel cyclic peptide (18 amino acids), which mimics the conformation of the extracellular loop 2 of the human β1-AR. One proposed mechanism of action of JNJ-54452840 is binding to and scavenging of circulating anti-β1-AR autoantibodies, inhibiting their activity at the receptor (in vitro and ex vivo), without interference with any physiologically occurring target or structure [8]. Another proposed mechanism of JNJ-54452840 is reduction of production of anti-β1-AR autoantibodies through an effect on memory B cells (immune tolerance), without affecting overall production of other antibodies. JNJ-54452840 has no intrinsic agonist or antagonist activity for adrenergic or other receptors, and represents a potential treatment for chronic heart failure due to autoimmune mechanisms, which can be targeted to patients with detectable levels of circulating anti-β1-AR autoantibodies.

The aim of the present study was to investigate the pharmacokinetics and safety of doses of JNJ-54452840 at 20, 80 and 240 mg infused intravenously over 1 minute in both Caucasian and Japanese cohorts.

2 Methods

2.1 Study Population

Healthy male Japanese and Caucasian participants aged 20–55 years with a body weight of at least 50 kg, with a body mass index (BMI) between 18 and 30 kg/m^2, and who were considered healthy on the basis of their medical history, physical examination, laboratory assessments, vital signs measurements and a normal electrocardiogram were enrolled in this study. Japanese participants had to be born in Japan, to Japanese parents and maternal and paternal grandparents, and must not have resided outside Japan for more than 5 years, while Caucasian participants had to have Caucasian parents.

The exclusion criteria included a positive test for human immunodeficiency virus (HIV) 1, hepatitis B surface antigen (HBsAg) antibodies or hepatitis C antibodies; and use of any prescription or nonprescription medication (including vitamins and herbal supplements), except for acetaminophen, within 14 days before the first dose of the study drug. Participants were also excluded if they had a history of drug or alcohol abuse or a known allergy to the study drug. Participants refrained from alcohol intake and from taking any methylxanthine-containing products (e.g. coffee, tea, cola, chocolate bars or energy drinks) for 48 h before each study drug infusion and throughout the inpatient periods of the study.

The study protocol was approved by an institutional review board at the study site, and the study was conducted

in accordance with the ethical principles originating in the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice guidelines and applicable regulatory requirements; and in compliance with the protocol. All participants provided written informed consent to participate in the study.

2.2 Study Design

This randomized, single-centre, double-blind, placebo-controlled, four-way crossover study (ClinicalTrials.gov study ID: NCT01809353) consisted of three phases: a screening phase of up to 28 days (days −28 to −1); a double-blind, placebo-controlled treatment phase consisting of four treatment periods, each 3 days in duration (day −1 to day 2), separated by 7- to 14-day washout periods; and a follow-up phase with visits occurring 7–10 days and 21–28 days after the last dose of study medication and completion of the final pharmacokinetic sample collection. The total duration of the study was up to 12 weeks, except for participants who developed anti-β1-AR antibodies, who had repeat antibody testing every 3 months until the antibodies resolved or until 1 year.

In the double-blind treatment phase, 16 Japanese (group A) and 16 Caucasian (group B) male participants were randomized into four treatment sequences (1:1:1:1, $n = 4$) based on a computer-generated randomization schedule. Participants fasted overnight for at least 10 h before laboratory sample collection (i.e. haematology, chemistry, urinalysis) on day −1 of each treatment period. On day 1 of each treatment period, a single intravenous infusion of JNJ-54452840 20 mg (treatment A), 80 mg (treatment B), 240 mg (treatment C) or placebo (sterile normal saline, treatment D) was administered over 1 minute, using an infusion pump. The four treatment sequence codes were ADBC, BACD, CBDA and DCAB.

2.3 Pharmacokinetic and Immunogenicity (Anti-β1-AR Antibodies) Evaluations

2.3.1 Sample Collection

Venous blood samples (4 ml each) for determination of JNJ-54452840 serum concentrations were collected at the following timepoints relative to the start of the infusion: predose; at 1 min (end of infusion); at 5, 7, 10, 15, 20, 30 and 45 min; and at 1, 2, 4, 8 and 24 h postdose. Venous blood samples (4 ml) for determination of anti-β1-AR antibody levels were collected before the first administration of the study drug and at the follow-up visits, which occurred approximately 7–10 days and 21–28 days after the last study procedure in the last treatment period. If circulating anti-β1-AR antibodies developed after drug exposure, then additional testing was performed every 3 months until antibody levels fell below the level of detection of the assay, or until 1 year, whichever occurred earlier.

2.3.2 Bioanalytical Procedures

Serum concentrations of JNJ-54452840 were analysed using a validated high-performance liquid chromatography–mass spectrometry/mass spectrometry (HPLC–MS/MS) assay method with a quantification range of 2–2000 ng/ml. The serum samples were extracted using mixed-mode cation exchange solid-phase extraction.

The extracts were analysed using an HPLC–MS/MS system consisting of an API-5500 tandem mass spectrometer operating in the TurboIonspray™ positive-ion multiple reaction monitoring (MRM) mode (Applied Biosystems, Foster City, CA, USA) and a Shimadzu LC30AD HPLC system (Shimadzu Benelux, Antwerp, Belgium). A Waters Acquity UPLC BEH300 C18 column (1.7 μm, 2.1 × 50 mm) was used at 40 °C, applying a linear gradient using a mobile phase consisting of 0.01 M ammonium carbonate in water (A) and methanol (B). JNJ-54452840 and its internal standard were monitored at mass transitions m/z 699.9 → 936.4 and 707.9 → 945.0, respectively.

A cell-based competitive enzyme-linked immunosorbent assay (ELISA) method was used for the detection of anti-β1-AR antibodies in human samples [9]. As the structure of JNJ-54452840 is similar to the extracellular loop 2 of the β1-AR, this assay was used to assess potential immunogenicity to JNJ-54452840. This method reports results as percentage inhibition with an adjustment factor for the binding of the mouse 23-6-7 monoclonal antibody to β1-ARs expressed in SF9 insect cells. A positive signal due to the specific binding of an anti-β1-AR (23-6-7) mouse antibody to coated SF9 cells, overexpressing β1-AR, can be detected by adding anti-mouse antibody peroxidase (POD) to the assay. The lowest concentration detected of a control antibody was approximately 15 μg/ml in the cell-based antibody screening assay. After accounting for assay dilution, this corresponded to a concentration of approximately 145 μg/ml of the control antibody in serum.

2.4 Pharmacokinetic Analyses

Pharmacokinetic parameters were determined for each dose of JNJ-54452840 on the basis of the individual participant plasma concentration–time data via noncompartmental methods using validated Phoenix WinNonlin® software version 6.2.1 (Pharsight Corporation, Mountain View, CA, USA). For any participant who had anti-β1-AR

antibodies present at baseline or during the treatment periods, pharmacokinetic parameters were analysed both separately and together with all others.

Pharmacokinetic parameters included the maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the concentration–time curve (AUC) from time zero to the time of the last quantifiable concentration during a 24-h dosing interval (AUC_{last}), AUC from time zero to infinity with extrapolation of the terminal phase (AUC_{inf}), terminal half-life ($T_{½}$), mean residence time (MRT), total systemic clearance after intravenous administration (CL) and volume of distribution at steady state (V_{ss}). Descriptive statistics, including the arithmetic mean, standard deviation (SD), median, minimum and maximum, were calculated for serum concentrations of JNJ-54452840 at each sampling time and for all derived pharmacokinetic parameters of JNJ-54452840.

2.5 Safety Evaluations

Safety was assessed throughout the study by monitoring of treatment-emergent adverse events (TEAEs; present after the first study drug dose and either absent before or worsened relative to the pretreatment state), serious TEAEs, TEAEs of interest (bradyarrhythmia and immunogenicity), treatment discontinuation, vital signs, laboratory abnormalities, cardiac telemetry monitoring of rhythm, electrocardiographic parameters and circulating anti-β1-AR antibodies.

2.6 Statistical Analysis

There was no statistical hypothesis testing in this study, as the purpose of the study was to describe the pharmacokinetics and safety of JNJ-54452840.

2.6.1 Sample Size

Assuming an intersubject coefficient of variation (CV) of 35 % for the AUC and C_{max} values of JNJ-54452840 for healthy Japanese and Caucasian males, a sample size of 12 participants completing the study per group at each dose level would be sufficient to estimate the mean AUC and C_{max} values of JNJ-54452840 for each group at each dose level to fall within 80–125 % of their true value with 95 % confidence. Sixteen participants were to be randomized into each group to ensure that at least 12 participants completed all assigned treatments in each group.

2.6.2 Dose Proportionality of JNJ-54452840 Exposure

Exploratory analysis to evaluate the dose proportionality of C_{max} and AUC_{inf} following intravenous administration of JNJ-54452840 in healthy male Japanese and Caucasian participants was performed as proposed by Smith et al. [9]. The criterion for concluding dose proportionality was based on the 90 % confidence interval (CI) for the slope of the linear regression model with (a) the logarithm of the dose-normalized pharmacokinetic parameters (C_{max} and AUC_{inf}) as the dependent variable; (b) the logarithm of the dose as a predictor; (c) period and sequence as fixed effects; and (d) participant as a random effect. Dose proportionality would be concluded if the 90 % CI for the slope of the regression line fell entirely within [ln(0.80)/ln(r), ln(1.25)/ln(r)], where r is the ratio of the highest dose (240 mg) to the lowest dose (20 mg) and ln denotes the natural logarithm, i.e. dose proportionality would be concluded if the 90 % CI for the slope of the regression line fell within (−0.0898 to 0.0898). If deviation from dose proportionality was observed, further evaluation of the data to identify the dose(s) where deviation occurred was performed by constructing 90 % CIs for the ratio of geometric means for each pair of doses. Mixed-effect modelling of the logarithm of the dose-normalized pharmacokinetic parameter with dose, period and sequence as fixed effects and subject as a random effect were utilized to obtain least squares means and intersubject variance to construct the CIs.

2.6.3 Comparison of JNJ-54452840 Exposure in Japanese and Caucasian Participants

Mixed-effects models were used to fit the log-transformed, dose-normalized pharmacokinetic parameters with dose, race, period and dose-by-race interaction as fixed effects and subject as a random effect, and to compare the pharmacokinetic parameters (C_{max} and AUC_{inf}) of JNJ-54452840 in Japanese and Caucasian participants. Using the estimated least squares means and intrasubject variances, 90 % CIs for the ratios of the mean pharmacokinetic parameters were obtained for each dose for the assessment of pharmacokinetic comparability between Japanese and Caucasian participants. Pharmacokinetic comparability of JNJ-54452840 in Japanese and Caucasian participants was concluded if the 90 % CI fell within the 80–125 % limits for C_{max} and AUC_{inf}. If the dose-by-race interaction a priori was not statistically or clinically significant, then a reduced model without an interaction term was fitted to the comparison of the two races.

3 Results

3.1 Participant Disposition and Demographic Characteristics

A total of 32 participants (group A: 16 Japanese; group B: 16 Caucasians) were enrolled in the study and randomized

Table 1 Demographic and baseline characteristics

Parameter	Japanese (group A)	Caucasians (group B)
N	16	16
Sex, *n* (%)		
Male	16 (100)	16 (100)
Race, *n* (%)		
Asian	16 (100)	0
White	0	16 (100)
Ethnicity, *n* (%)		
Not Hispanic or Latino	16 (100)	16 (100)
Age (years)		
Mean (SD)	35.1 (9.1)	38.4 (10.1)
Median	36.0	41.5
Range	23–52	23–53
Weight (kg)		
Mean (SD)	69.4 (9.6)	75.6 (5.5)
Median	67.20	76.25
Range	54.9–86.5	68.5–85.7
Height (cm)		
Mean (SD)	173.6 (4.4)	175.7 (5.6)
Median	174.5	175.6
Range	162.8–179.1	167.5–185.7
Body mass index (kg/m^2)		
Mean (SD)	22.9 (2.5)	24.5 (1.7)
Median	22.5	24.0
Range	20–27	21–28

SD standard deviation

to one of the four treatment sequences. In general, at baseline, Caucasian participants in group B were older, were taller, weighed more, and had a greater BMI than Japanese participants in group A (Table 1).

A total of 29 participants (90.6 %), with 13 Japanese participants (81.3 %) in group A and 16 Caucasian participants (100 %) in group B, completed the study through the days 21–28 visit. Three participants in group A discontinued the study prematurely; one withdrew consent (after receiving one placebo and one JNJ-54452840 240 mg dose), one discontinued because of a TEAE of viral upper respiratory tract infection (after receiving only one dose of JNJ-54452840 240 mg) and one completed all assigned treatments (received all four treatment doses) but discontinued from the study after the days 7–10 follow-up visit because of a serious adverse event (SAE) of ischaemic cerebral infarction.

3.2 Pharmacokinetics

The serum concentrations of JNJ-54452840 were below the quantification limit (2.0 ng/ml) at predose in all participants in group A and group B, except at predose (period 2, day 1) for one participant in group B, where a measurable plasma concentration of JNJ-54452840 (148 ng/ml) was determined.

The mean serum concentration–time profiles of JNJ-54452840 in group A and group B are shown in Fig. 1 on linear and semi-logarithm scales. In each treatment group, the JNJ-54452840 serum concentration peaked at 1 minute after the intravenous infusion and then declined bi-exponentially until the serum concentrations were below the quantification limit in most participants by 45 min, 2 h and 4 h through 24 h for the 20, 80 and 240 mg treatments, respectively (Fig. 1). One participant in group A at 20 min following the 20 mg treatment and one participant in group B at 1 h following the 240 mg treatment exhibited unusually high serum concentrations of JNJ-54452840 (13.9- and 13.1-fold greater than the mean, respectively). These two incongruous serum concentration values were included in the summary statistics of serum concentrations of JNJ-54452840 and had an impact on the mean profiles (Fig. 1). However, the incongruous serum concentration values were excluded from the calculation of pharmacokinetic parameters. Overall, the mean JNJ-54452840 serum concentration–time profiles were similar in group A

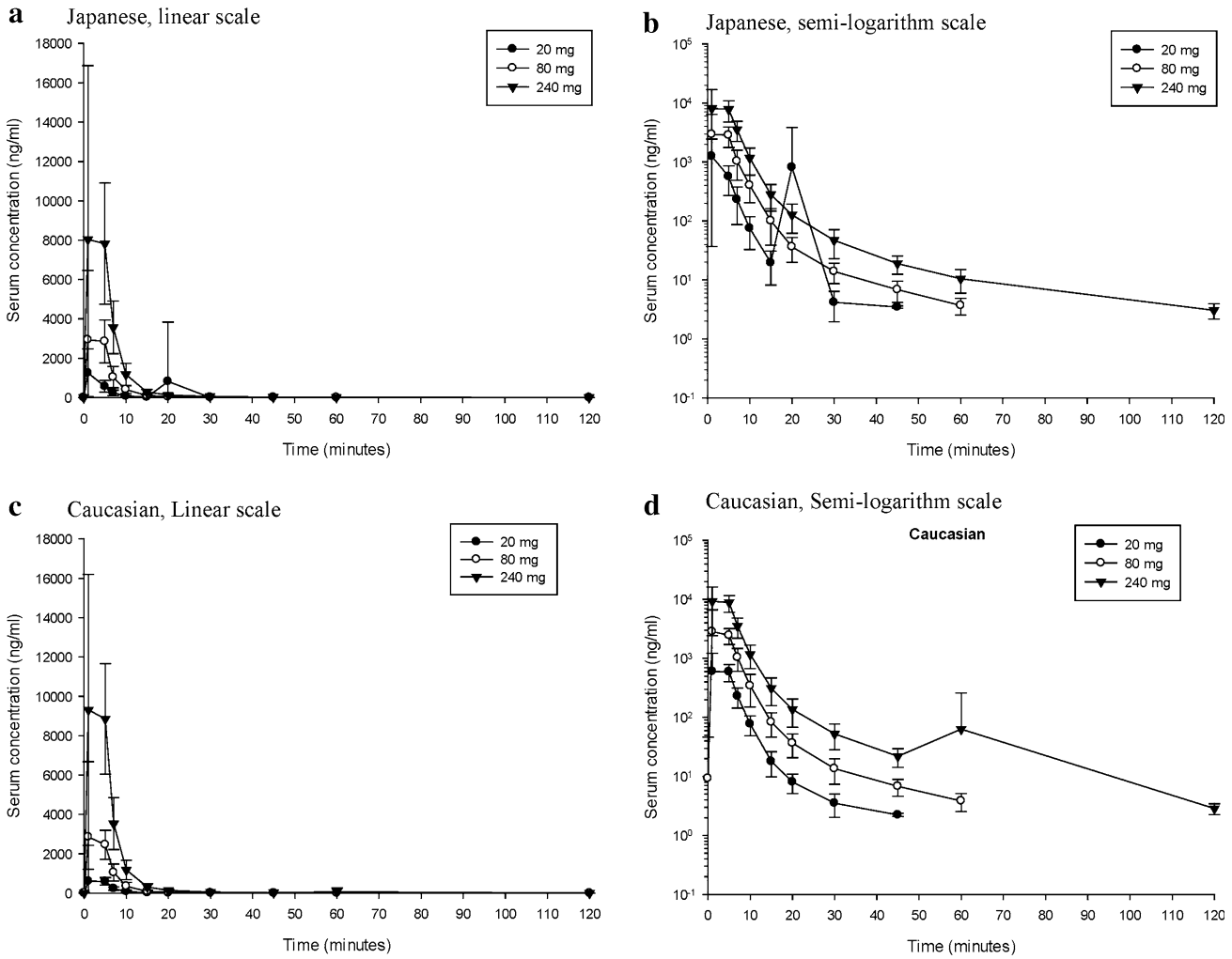

Fig. 1 Mean (standard deviation) serum JNJ-54452840 concentration–time profiles, on linear and semi-logarithm scales, following single intravenous doses of 20, 80 and 240 mg in **a**, **b** healthy Japanese and **c**, **d** healthy Caucasians

and group B for the same dose levels, except at 1 min after the intravenous infusion of the 20 mg dose, where the mean serum concentration for group A was 2.1-fold greater (but within 1 SD) in comparison with group B.

Summary statistics for the pharmacokinetic parameters for all study participants are given in Table 2. Across all treatment groups, the median T_{max} values in group A and group B ranged from 1 minute to 5 minutes following the 20, 80 and 240 mg intravenous infusion doses. The mean C_{max} values following 20, 80 and 240 mg of JNJ-54452840 in group A were 1429.4, 4392.9 and 11,211.9 ng/ml, respectively, and the corresponding C_{max} values in group B were 857.9, 4019.4 and 11,872.5 ng/ml, respectively. The mean AUC_{inf} values after single intravenous infusion of 20, 80 and 240 mg of JNJ-54452840 in group A were 5286.5, 19,493.4 and 57,087.0 ng·min/ml, respectively, and the corresponding AUC_{inf} values in group B were 4126.6, 17,824.5 and 64,054.4 ng·min/ml, respectively.

Overall, the mean C_{max}, AUC_{last} and AUC_{inf} values increased linearly with dose, as shown in Fig. 2, although the dose proportionality criteria were not met between the 20 mg and 240 mg dose levels for both group A and group B. The 90 % CIs were not contained in the pre-specified interval of (−0.0898 to 0.0898) for C_{max} and AUC_{inf} between 20 and 240 mg doses for both group A and group B, indicating that C_{max} and AUC_{inf} did not increase in a dose-proportional manner in both groups. However, dose proportionality was achieved for AUC_{inf} between 20 and 80 mg in group B participants only. There was no dose proportionality for C_{max} in both groups.

The mean C_{max} and AUC_{inf} values for group A were generally similar to those for group B in the 80 and 240 mg treatment groups. The dose-by-race interaction was not a significant term in the model, and therefore the interaction term was dropped from the model and the reduced model was used for fitting. The reduced mixed-effects modelling

Table 2 Summary statistics of individual pharmacokinetic parameters of JNJ-54452840 following intravenous treatment doses of 20, 80 and 240 mg in Japanese and Caucasian healthy male participants

Pharmacokinetic parameter	Japanese (group A)			Caucasians (group B)		
	20 mg ($N = 14$)	80 mg ($N = 14$)	240 mg ($N = 16$)	20 mg ($N = 16$)	80 mg ($N = 16$)	240 mg ($N = 16$)
AUC_{inf} (ng·min/ml)						
Mean	5286.5	19,493.4	57,087.0	4126.6	17,824.5	64,054.4
SD	2649.95	7740.56	21,111.70	1068.53	7159.42	19,789.09
Range	1750–9604	9867–35,549	28,961–92,763	2928–6987	8482–35,793	31,936–98,585
AUC_{last} (ng·min/ml)						
Mean	5257.5	19,417.6	56,948.1	4098.4	17,743.3	63,947.3
SD	2643.19	7743.06	21,116.90	1067.09	7149.00	19,780.55
Range	1728–9547	9761–35,472	28,856–92,635	2899–6973	8429–35,710	31,856–98,430
CL (L/min)						
Mean	5.18	4.74	4.74	5.12	5.18	4.13
SD	3.25	1.85	1.65	1.18	1.99	1.41
Range	2.08–11.43	2.25–8.11	2.59–8.29	2.86–6.83	2.24–9.43	2.43–7.52
C_{max} (ng/ml)						
Mean	1429.4	4392.9	11,211.9	857.9	4019.4	11,872.5
SD	1062.93	2677.14	7347.46	501.05	3242.31	4787.91
Range	343–3610	1980–12,200	5520–31,200	446–2500	1650–14,800	5060–20,300
MRT (min)						
Mean	4.5	5.6	6.2	5.1	5.7	5.9
SD	1.61	1.52	1.86	1.28	1.41	1.45
Range	2–7	3–8	3–9	3–7	3–7	4–9
$T_{½}$ (min)						
Mean	5.9	13.7	23.5	6.9	14.4	26.1
SD	2.22	3.96	8.52	3.39	2.60	2.84
Range	3–11	6–21	10–37	4–17	10–19	20–30
T_{max} (min)						
Median	1.0	4.2	4.9	5.0	5.0	3.0
Range	1–5	1–5	1–6	1–5	1–5	1–5
V_{ss} (L)						
Mean	25.76	27.716	31.10	26.45	30.51	25.19
SD	21.71	15.63	17.63	9.58	15.23	12.80
Range	5.55–65.16	6.67–64.48	7.10–67.64	7.75–43.82	6.88–57.39	11.10–56.73

AUC area under the concentration–time curve, AUC_{inf} AUC from time zero to infinity with extrapolation of the terminal phase, AUC_{last} AUC from time zero to the time of the last quantifiable concentration during a 24-h dosing interval, *CL* total systemic clearance after intravenous administration, C_{max} maximum observed plasma concentration, *MRT* mean residence time, *SD* standard deviation, $T_{½}$ terminal half-life, T_{max} time to reach C_{max}, V_{ss} volume of distribution at steady state

and statistical analysis results are shown in Table 3 and support the conclusion that there were no significant differences in JNJ-54452840 exposure in group A and group B participants, since the 90 % CI for AUC_{inf} was contained within the 80–125 % bioequivalence limits. However, pairwise comparisons based on mixed-model analyses showed that C_{max} for the lowest dose (20 mg) deviated from the values for the other doses between the two groups; the mean C_{max} and AUC_{inf} values for the 20 mg treatment group were 66.6 and 28.1 % greater, respectively, for group A (but within 1 SD) in comparison with those for group B.

The mean CL values ranged from 4.13 to 5.18 L/min, the mean V_{ss} values ranged from 25.18 to 31.1 L and the mean MRT values ranged from 4.5 to 6.2 min after intravenous infusion of 20, 80 and 240 mg of JNJ-54452840 for both group A and group B. The mean CL, V_{ss} and MRT values were similar for group A and group B.

Across the 20, 80 and 240 mg JNJ-54452840 doses, the mean $T_{½}$ values for each dose were similar for group A and

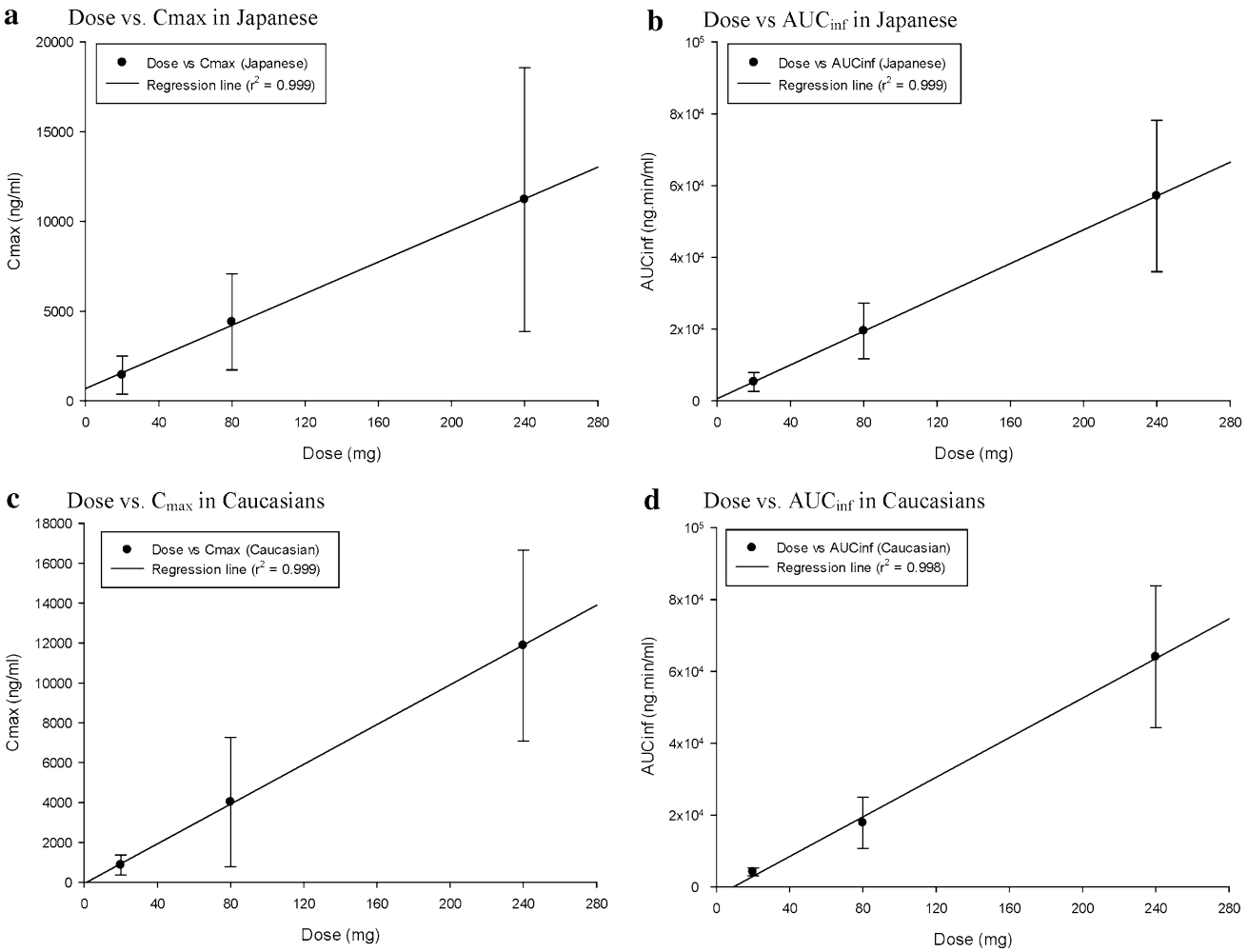

Fig. 2 Relationships between JNJ-54452840 dose and mean (standard error) maximum observed serum concentration (C_{max}) and mean (standard error) area under the concentration–time curve from time zero to infinity with extrapolation of the terminal phase (AUC_{inf}) following single intravenous doses of 20, 80 and 240 mg in **a**, **b** healthy Japanese and **c**, **d** healthy Caucasians

Table 3 Estimated difference in JNJ-54452840 maximum observed serum concentration (C_{max}) and area under the concentration–time curve from time zero to infinity with extrapolation of the terminal phase (AUC_{inf}) least squares means and confidence intervals (CIs) between Japanese and Caucasian healthy male participants

Pharmacokinetic parameter	Intersubject CV (%)	Geometric LSM for Japanese	Geometric LSM for Caucasians	Ratio of geometric LSM (%)	90 % CI lower limit	90 % CI upper limit
AUC_{inf} (μg·h/ml)	37.04	4552.52	4390.50	103.69	88.31	121.75
C_{max} (μg/ml)	60.05	949.92	839.24	113.19	92.84	138.00

Comparability of pharmacokinetic parameters (C_{max} and AUC_{inf}) of JNJ-54452840 in Japanese and Caucasian participants was obtained by fitting a reduced mixed-effects model to the log-transformed, dose-normalized pharmacokinetic parameters, with dose, race and period as fixed effects and subject as a random effect

CV coefficient of variation, *LSM* least squares mean

group B, ranged from 5.9 to 26.1 min and appeared to be dose dependent for both groups. Of note, the serum concentrations of JNJ-54452840 were below the quantification limit (2.0 ng/ml) in most participants in group A and group B by 45 min, 2 h and 4 h through 24 h after the intravenous infusion of JNJ-54452840 for the 20, 80 and 240 mg treatment groups, respectively, and this may possibly explain the dose-dependent increase in $T_{½}$ values. However, the mean $T_{½}$ values for each treatment group were similar for group A and group B participants.

3.3 β1-AR Antibody Incidence and Effect on JNJ-54452840 Levels

The overall incidence of anti-β1-AR antibodies was 25 % (8/32 participants) at any time during the study. Two participants (12.5 %) in group A and six participants (37.5 %) in group B tested positive for circulating anti-β1-AR antibodies at some timepoints during the course of the study. Of these, both participants in group A and one in group B tested positive at predose (period 1, day 1). The serum concentrations of JNJ-54452840 for the two participants in group A with positive antibodies were generally lower in comparison with others in group A who tested negative across all doses, while the serum concentrations were generally similar for participants in group B, irrespective of the presence or absence of circulating antibodies. However, it is difficult to draw definitive conclusions about the impact of anti-β1-AR autoantibodies on JNJ-54452840 pharmacokinetics, since the number of participants who tested positive for anti-β1-AR autoantibodies was small.

For the three participants who were positive at baseline, one remained positive at both the days 7–10 and days 21–28 follow-up posttreatment visits, one was still positive only at the days 7–10 visit and one was negative at both follow-up visits (Table 4). Five participants in group B developed antibodies after drug exposure (one was positive at the days 7–10 visit only, three were positive at the days 21–28 visit only and one was positive at both visits). With extended follow-up testing, all participants became negative by the 6-month visit, except for one who remained positive for anti-β1-AR antibodies as of the last (1-year) visit (Table 4). Overall, 24 out of 32 participants (75 %; 14 [87.5 %] in group A and 10 [62.5 %] in group B) were negative for circulating anti-β1-AR antibodies for the duration of the study.

3.4 Safety

No deaths were reported during the study. Overall, TEAEs were reported by 50 % (8/16) of the participants in group A and group B. The most common TEAEs in the combined treatment groups were antibody test positive (15.6 %), diarrhoea (6.3 %) and headache (6.3 %). Most of the TEAEs were mild in intensity and were considered by the investigator to be unrelated to the administration of the study drug.

TEAEs of antibody test positive were reported in five participants in group B, who developed circulating anti-β1-AR antibodies during the course of the study. None of these participants showed any symptoms or signs indicating cardiac dysfunction at any time during the study.

A total of two participants, one in group A and the other in group B, reported SAEs whose severity was considered by the investigator to be severe and related to the administration of study drug. A 40-year-old Japanese participant experienced a left middle cerebral artery distribution ischaemic cerebral infarction, confirmed by computed tomography (CT) and magnetic resonance imaging (MRI) scans, approximately 16 days after the final administration of JNJ-54452840 (20 mg in treatment period 4). The predominant clinical manifestation was aphasia, which persisted at the last follow-up approximately 6 months after onset. Risk factors for stroke included smoking, borderline hypertension and hypercholesterolaemia. The investigations completed for hypercoagulability and atrial fibrillation, including a carotid ultrasound to evaluate dissection, were negative. The work-up was not complete, as no carotid angiography (a more accurate test for dissection) or transoesophageal echocardiography (testing for patent foramen ovale) were performed to detect heart and blood vessel diseases and conditions.

A 46-year-old Caucasian participant was reported to have a pulmonary embolism approximately 16 days after the final administration of JNJ-54452840 (240 mg), confirmed by CT angiography. He was anticoagulated with enoxaparin followed by warfarin and was considered to have recovered after 6 months, at which time warfarin was discontinued. Investigations revealed that the participant was heterozygous for factor V Leiden, without other thrombophilias. A history of bed rest over the few days before the event and some history of dental infection may have contributed to the event.

No laboratory abnormalities were reported, and no clinically important mean changes over time or between treatment groups were observed for laboratory analytes, vital signs measurements, electrocardiogram parameters or physical examination findings.

4 Discussion

By presenting a homologous structure for the binding of anti-β1-AR antibodies, JNJ-54452840 is a novel modality being investigated for the treatment of heart failure due to autoimmune mechanisms [4, 7, 10]. Preclinical safety pharmacology assessments did not suggest thrombogenicity or immunogenicity risks. JNJ-54452840 was formerly known as COR-1, from Corimmun GmbH, Germany, and was licensed by the sponsor of this study. Münch et al. [8] reported on one placebo-controlled phase 1 clinical study of JNJ-54452840 (ClinicalTrials.gov study ID: NCT01043146) in 50 healthy male Caucasian participants, which investigated its pharmacokinetics, pharmacodynamics (ex vivo assay using rat plasma containing stimulating antibodies) and safety following escalating single intravenous doses of 10, 40, 80, 160 and 240 mg infused

Table 4 Summary of participants with positive antibodies to JNJ-5445280: anti-β1-adrenergic receptor (anti-β1-AR) autoantibodies mean (standard deviation [SD]) percentage inhibition cut-off adjusted values by study visit

Study cohort and JNJ-5445280 treatment period	Serum sample collection by visit	Anti-β1-AR antibody mean (SD) inhibition % cut-off adjusted values
Group A: Japanese		
ADBC[a]	Period 1, day 1, predose	33.75 (9.58)
	Follow-up at days 7–10	Negative[b]
	Follow-up at days 21–28	Negative[b]
CBDA[a]	Period 1, day 1, predose	14.54 (5.50)
	Follow-up at days 7–10	10.07 (7.73)
	Follow-up at days 21–28	10.25 (1.26)
Group B: Caucasians		
BACD[a]	Period 1, day 1, predose	2.10 (7.78)
	Follow-up at days 7–10	9.19 (3.20)
	Follow-up at days 21–28	Negative[b]
DCAB[c]	Follow-up at days 7–10	Negative[b]
	Follow-up at days 21–28	2.72 (1.41)
	Extended follow-up at 3 months	14.91 (2.64)
	Extended follow-up at 6 months	4.89 (4.56)
	Extended follow-up at 9 months	1.21 (2.17)
	Extended follow-up at 1 year	37.50 (9.22)
CBDA[c]	Follow-up at days 7–10	Negative[b]
	Follow-up at days 21–28	0.45 (0.14)
	Extended follow-up at 3 months	1.59 (5.40)
	Extended follow-up at 6 months	Negative[b]
BACD[c,d]	Follow-up at days 7–10	41.06 (2.59)
	Follow-up at days 21–28	34.02 (1.13)
	Extended follow-up at 3 months	7.09 (6.29)
	Extended follow-up at 6 months	Negative[b]
DCAB[c]	Follow-up at days 7–10	Negative[b]
	Follow-up at days 21–28	9.08 (0.80)
	Extended follow-up at 3 months	Negative[b]
ADBC[c]	Follow-up at days 7–10	37.95 (4.57)
	Follow-up at days 21–28	Negative[b]

ADBC JNJ-5445280 20 mg/placebo/JNJ-5445280 80 mg/JNJ-5445280 240 mg, *BACD* JNJ5445280 80 mg/JNJ-5445280 20 mg/JNJ-5445280 240 mg/placebo, *CBDA* JNJ-5445280 240 mg/JNJ-5445280 80 mg/placebo/JNJ-5445280 20 mg, *DCAB* placebo/JNJ-5445280 240 mg/JNJ-5445280 20 mg/JNJ-5445280 80 mg

[a] Participants tested positive for anti-β1-AR antibody at predose

[b] Negative (≤0); anti-β1-AR antibody fell below the level of detection of the assay

[c] Participants tested negative for anti-β1-AR antibody at predose

[d] Participant had pulmonary embolism

over 5 minutes. The findings demonstrated that JNJ-54452840 was generally safe and well tolerated; it was rapidly eliminated (MRT 4.5–8.1 min), as is usually observed with peptides, with $T_{½}$ values of 6.4, 20, 29, 25 and 22 minutes for the 10 mg to 240 mg doses, respectively. In that phase 1 study [8], there was an almost linear dose relationship with C_{max} and AUC, and T_{max} did not change with escalating doses (4.3–5.6 min). The plasma CL was determined to be between 1.0 and 1.6 L/min, and V_{ss} varied between 4.6 and 14.0 L. In this study, the mean CL and V_{ss} values reported in the Caucasian cohort were between 4.1 and 5.2 L/min and between 31.1 and 25.2 L, respectively, across the 20–240 mg doses (Table 2). The significantly lower values for V_{ss} and CL in healthy participants with similar baseline characteristics described by Münch et al. [8] are likely due to differences in the assay methodology used in measurement of drug plasma concentrations. The earlier phase 1 study used a screening competitive cell-binding ELISA methodology [11], compared with a direct and validated HPLC–MS/MS assay with high sensitivity in this study. In addition, development of anti-β1-AR antibodies was not observed during an

observation period of 43 days after drug administration in the previous phase 1 study. Plasma levels of JNJ-54452840 blocked up to 76 % of anti-β1-AR antibodies in a dose-dependent manner, using an ex vivo assay procedure [8].

On the basis of the characteristics of JNJ-54452840 (i.e. composed of natural amino acids and with rapid elimination), no differences in pharmacokinetics or safety between participants of different ethnicities are expected. This study was conducted to confirm this expectation that the pharmacokinetics and safety of single intravenous doses of JNJ-54452840 of 20, 80 and 240 mg infused over 1 minute are similar in healthy male participants of Japanese and Caucasian ethnicities.

Overall, the mean C_{max}, AUC_{last} and AUC_{inf} values of JNJ-54452840 increased linearly with dose, although dose proportionality criteria were not met between the 20 and 240 mg dose levels, and rapid elimination of JNJ-54452840 was observed for both Japanese and Caucasian male participants, consistent with the findings from the previous phase 1 study [8]. Lack of dose proportionality for C_{max} and AUC_{inf} was likely due to high estimated intrasubject variance, and the study was not sufficiently powered to show dose proportionality. The apparent volume of distribution of JNJ-5445280 for both study cohorts (approximately 25.18–31.1 L) is relatively small compared with the total body water volume (approximately 40 L) and shows that JNJ-5445280 is distributed in vascular as well as extravascular tissue spaces, and it is not indicative of distribution in total body water. The clearance of JNJ-5445280 was rapid, with mean values of 4.13–5.18 L/min across both cohorts. The clearance of JNJ-544528 was higher than the hepatic blood flow rate (1.5 L/min) or the renal blood flow filtration rate (1.2 L/min) in humans [12].

The relatively high total clearance is primarily represented by hepatic and renal elimination. In general, peptides exhibit rapid clearance and short half-lives due to degradation by proteolysis and renal elimination [13]. Exploratory metabolite identification has pointed to 11 metabolites via an initial ring-opening mechanism occurring either between aspartic acid and phenylalanine or between aspartic acid and alanine amino acid residues followed by subsequent splitting of amino acids from C-terminus and N-terminus ends of the peptide.

Pharmacokinetic parameters were generally similar in Japanese and Caucasian participants following single intravenous infusions of 80 and 240 mg, although for the 20 mg treatment dose, Japanese participants were observed to have a somewhat greater C_{max} and systemic exposure in comparison with Caucasians. Various factors, including the lower bodyweight at baseline of Japanese versus Caucasian participants (mean values of 69.4 versus 75.6 kg) and anti-β1-AR autoantibodies, may explain the observed higher exposure of JNJ-54452840 in Japanese compared with Caucasian participants in the 20 mg group. The serum JNJ-54452840 concentrations were generally higher in Japanese participants who tested negative for anti-β1-AR autoantibodies than in Japanese participants who tested positive for anti-β1-AR autoantibodies in all treatment groups. Since only 2 of 16 Japanese (12.5 %) compared with 6 of 16 Caucasians (37.5 %) tested positive for anti-β1-AR autoantibodies after treatment with JNJ-54452840, it is possible that the higher exposure of JNJ-54452840 in Japanese compared with Caucasian participants in the 20 mg group may have been due, at least in part, to the absence of anti-β1-AR autoantibodies in most Japanese participants.

Two thromboembolic SAEs (pulmonary embolism and ischaemic cerebral infarction) occurred in participants in this study. The participant with the pulmonary embolism developed anti-β1-AR antibodies, which were detected at the days 7–10 follow-up visit and declined over the course of the next two follow-up visits until they became negative at the 6-month visit of the extended follow-up period. The participant with the ischaemic stroke did not appear to develop antibodies. Both participants had some predisposing risk factors for these events, including a factor V Leiden mutation in the participant with the pulmonary embolism, and a history of smoking, possible hypertension and increased cholesterol in the participant with the ischaemic cerebral infarction.

A review of the phase 1 safety literature experience suggests that thromboembolic events rarely, if ever, occur in healthy subjects. The overall incidence of adverse events (AEs) in 1015 healthy volunteers in 54 phase 1 studies who received 23 different active drugs or placebo over 12,143 days of follow-up was 12.8 %, with no reported thromboembolic events [14]. A 5-year survey of all AEs (including clinically relevant laboratory findings) in 142 phase 1 studies in 1559 healthy participants who received 32 different active drugs or placebo over a total observation period of 29,664 follow-up days showed an overall AE incidence of 8.8 % [15]. Only six out of 2604 AEs were considered serious (none thromboembolic), and two were assessed as possibly drug related (pseudoallergic reaction, prolonged orthostatic dysregulation). In a 12-year study of 805 phase 1 studies involving 29,162 healthy prison volunteers, there were 64 medical events and 58 medically significant adverse drug reactions (none thromboembolic), which all resolved [16]. Therefore, the occurrence of the two serious thromboembolic events with similar timing in relation to study drug dosing in this study is clearly unusual and suggests a possible safety signal.

The development of circulating anti-β1-AR antibodies in five Caucasian participants after drug exposure (not present at baseline), which also persisted in some at follow-up evaluations (one participant was still positive for

antibodies at 1 year), may suggest an immune response to JNJ-54452840, although the qualitative nature of the assay and its sensitivity limit the ability to reliably interpret these results. Also, there was no clear relation between the presence of antibodies and the incidence of AEs. Of the two participants with an SAE, one tested positive and one tested negative for antibodies, while the other four participants who tested positive for antibodies did not report serious TEAEs.

5 Conclusion

Serum concentrations of JNJ-54452840 increased linearly as the intravenous dose of JNJ-54452840 was increased from 20 to 240 mg, although the dose proportionality criteria were not met between the 20 and 240 mg doses, and rapid elimination was observed in both Japanese and Caucasian participants. The pharmacokinetics of JNJ-54452840 were generally similar in Japanese and Caucasian participants after a single intravenous infusion of JNJ-54452840. The study agent was possibly immunogenic, with antibodies being detected with use of a cell-based assay in a subset of participants. Two participants reported thromboembolic SAEs in the study. The relationship between these SAEs and antibody formation is not known.

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