KIREPORTS
KIReports.org
CLINICAL RESEARCH

Sotatercept Safety and Effects on Hemoglobin, Bone, and Vascular Calcification

Daniel W. Coyne[1], Hem N. Singh[2], William T. Smith[2,9], Ana Carolina Giuseppi[2], Jamie N. Connarn[2], Matthew L. Sherman[3], Frank Dellanna[4], Hartmut H. Malluche[5,8] and Keith A. Hruska[1,6,7,8]

[1]Department of Medicine, Division of Nephrology, Washington University School of Medicine, St. Louis, Missouri, USA; [2]Celgene Corporation, Summit, New Jersey, USA; [3]Acceleron Pharma Inc., Cambridge, Massachusetts, USA; [4]MVZ Davita Rhein-Ruhr, Düsseldorf, Germany; [5]Division of Nephrology, Bone and Mineral Metabolism, University of Kentucky, Lexington, Kentucky, USA; [6]Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA; and [7]Department of Cell Biology, Washington University School of Medicine, St. Louis, Missouri, USA

Introduction: Patients with end-stage kidney disease (ESKD) exhibit anemia, chronic kidney disease–mineral bone disorder (CKD–MBD), and cardiovascular disease. The REN-001 and REN-002 phase II, multicenter, randomized studies examined safety, tolerability, and effects of sotatercept, an ActRIIA-IgG1 fusion protein trap, on hemoglobin concentration; REN-001 also explored effects on bone mineral density (BMD) and abdominal aortic vascular calcification.

Methods: Forty-three patients were treated in REN-001 (dose range: sotatercept 0.3–0.7 mg/kg or placebo subcutaneously [s.c.] for 200 days) and 50 in REN-002 (dose range: 0.1–0.4 mg/kg i.v. and 0.13–0.5 mg/kg s.c. for 99 days).

Results: In REN-001, frequency of achieving target hemoglobin response (>10 g/dl [6.21 mmol/l]) with sotatercept was dose-related and greater than placebo (0.3 mg/kg: 33.3%; 0.5 mg/kg: 62.5%; 0.7 mg/kg: 77.8%; 0.7 mg/kg [doses 1 and 2]/0.4 mg/kg [doses 3–15]: 33.3%; placebo: 27.3%). REN-002 hemoglobin findings were similar (i.v.: 16.7%−57.1%; s.c.: 11.1%–42.9%). Dose-related achievement of ≥2% increase in femoral neck cortical BMD was seen among only REN-001 patients receiving sotatercept (0.3–0.7 mg/kg: 20.0%–57.1%; placebo: 0.0%). Abdominal aortic vascular calcification was slowed in a dose-related manner, with a ≤15% increase in Agatston score achieved by more REN-001 sotatercept versus placebo patients (60%–100% vs. 16.7%). The most common adverse events during treatment were hypertension, muscle spasm, headache, arteriovenous fistula site complication, and influenza observed in both treatment and placebo groups.

Conclusion: In patients with ESKD, sotatercept exhibited a favorable safety profile and was associated with trends in dose-related slowing of vascular calcification. Less-consistent trends in improved hemoglobin concentration and BMD were observed.

Kidney Int Rep (2019) **4,** 1585–1597; https://doi.org/10.1016/j.ekir.2019.08.001
KEYWORDS: bone mineral density; end-stage kidney disease; hemoglobin; pharmacodynamics; sotatercept; vascular calcification

Patients with ESKD experience anemia associated with decreased renal biosynthesis of erythropoietin, iron deficiency, and elevated hepcidin.[1–4] Several erythropoiesis-stimulating agents (ESAs) increase hemoglobin (Hb) levels in ESKD patients yet pose safety challenges, without modifying cardiovascular risk.[5–9] Sotatercept (ACE-011), an activin receptor type IIA (ActRIIA)-IgG1 fusion protein trap, binds with high affinity to activin A and other members of the transforming growth factor-β (TGF-β) superfamily[10] and acts on late-stage erythropoiesis to increase production of mature erythrocytes.[11,12] Dose-dependent effects on Hb were seen with sotatercept in healthy volunteers.[13,14]

In addition, ESKD is associated with CKD–MBD, which is associated with cardiovascular disease, renal

Correspondence: Keith A. Hruska, Departments of Pediatrics, Medicine, and Cell Biology, Rm 5109, MPRB Building, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, Missouri 63110, USA. E-mail: hruska_k@kids.wustl.edu

[8]Equal contributors.

[9]Formerly of Celgene Corporation.

Received 23 May 2019; revised 29 July 2019; accepted 3 August 2019; published online 13 August 2019

osteodystrophy with low bone volume, and disordered mineral metabolism.[15–18] In CKD, activin receptors are stimulated in skeletal, vascular, and heart tissues, implicating a possible role for sotatercept in ameliorating CKD-related cardiovascular disease and osteodystrophy.[19] A murine analog of sotatercept increased bone formation, leading to increased bone density and strength and decreased atherosclerotic vascular calcification in a preclinical CKD model.[10,20,21]

We report findings from 2 phase II, multicenter, randomized studies (REN-001 and REN-002) of ESKD patients undergoing hemodialysis and with anemia. Both studies evaluated the multiple-dose safety, pharmacokinetic, and pharmacodynamic effects of sotatercept on serum Hb concentration. REN-001 also explored effects on BMD and vascular calcification. REN-001 was placebo-controlled and evaluated sotatercept for the correction of anemia after ESA washout; REN-002 was an open-label study that evaluated Hb maintenance in ESKD patients after switching from a prior ESA. These studies shared an objective of exploring dosing strategies that would safely impact pharmacodynamic parameters, primarily Hb, and might translate into clinical benefit in larger clinical trials.

METHODS

Patients

For either study, patients had to be aged ≥18 years with ESKD, on hemodialysis (≥3 hours of high-flux hemodialysis at each session for ≥12 weeks before screening), and on a stable ESA dose for ≥6 weeks before screening. Proven ESA responsiveness was required (mean Hb values: 10–12 g/dl [6.21–7.45 mmol/l]). Patients were excluded if they had anemia due to non-renal causes, were receiving peritoneal dialysis, had systemic hematologic disease, were medically unstable, or had uncontrolled diabetes mellitus (HbA1c >9%), hypertension (home systolic blood pressure [BP] >160 mm Hg, or home diastolic BP >90 mm Hg), or heart failure (New York Heart Association classification ≥3).

Study Design

ACE-011-REN-001 (REN-001; NCT01146574) Part 1 was a double-blind, placebo-controlled study evaluating pharmacokinetics, dialysis clearance, and safety of sotatercept following a single s.c. dose (0.1 mg/kg). REN-001 Part 2 was a randomized, single-blind, placebo-controlled study of s.c. sotatercept administration in doses ranging from 0.3 to 0.7 mg/kg, evaluating for the correction of anemia after ESA washout. Patients could receive treatment over 200 days if they did not require rescue or if the Hb threshold was not exceeded; the sotatercept 0.3, 0.5, and 0.7 mg/kg dose groups received up to 8 doses, given every 28 days, and the 0.7/0.4 mg/kg dose group received up to 15 doses (doses 1 and 2: 0.7 mg/kg; doses 3–15: 0.4 mg/kg), given every 14 days.

ACE-011 REN-002 (REN-002; NCT01999582) was an open-label, randomized study evaluating i.v. and s.c. sotatercept administration routes in doses ranging from 0.1 to 0.4 mg/kg (i.v.) and 0.13 to 0.5 mg/kg (s.c.) evaluating Hb maintenance after switching from a prior ESA. Patients could receive up to 8 doses given every 14 days over 99 days.

Study designs for REN-001 and REN-002 are illustrated in Figure 1. In both studies, if Hb fell below 9 g/dl (5.59 mmol/l), patients received rescue ESA treatment and discontinued sotatercept dosing. In REN-002, dose escalation in increments of 0.1 mg/kg was permitted in dose group 3 if an Hb reduction ≥1 g/dl (0.62 mmol/l) occurred or absolute Hb was <10 g/dl (6.21 mmol/l). Both studies followed patients for 112 days after their last sotatercept dose.

Dosing Rules Based on Hb Concentration

Sotatercept doses were delayed if absolute Hb concentration exceeded defined thresholds (REN-001: ≥11 g/dl [6.83–8.07 mmol/l]; failed to decrease to <11 g/dl [6.83 mmol/l] by day 36 [day 29 + 7 days = window for groups 1–3] or by day 19 [day 15 + 4 days for group 3a]; REN-002: >12 to <13 g/dl [7.45–8.07 mmol/l]). Doses were also delayed if the Hb rate of rise was ≥1 g/dl (0.62 mmol/l) at any time since the last dose (REN-002: over 14 days). In REN-001, a half-dose reduction of sotatercept occurred when Hb was <11 g/dl (6.83 mmol/l), but the rate of rise was >2 g/dl (1.24 mmol/l) (over 28 days, groups 1–3) or >1 g/dl (0.62 mmol/l) (over 14 days, group 3a). Sotatercept was redosed when the Hb concentration returned to <11 g/dl (6.83 mmol/l) (REN-001: resumed at half the previous dose; REN-002: resumed at the full assigned dose). Sotatercept was discontinued if the absolute Hb was ≥13 g/dl (8.07 mmol/l) (REN-002 only; if Hb was <14 g/dl [8.69 mmol/l], it could be reconfirmed within 7 days) or Hb was >12 g/dl (7.45 mmol/l) to <13 g/dl (8.07 mmol/l) for 2 consecutive weeks (REN-001 only).

Dosing Rules Based on Home BP

In addition to recording predialysis and postdialysis seated BP, home BP monitoring was used to evaluate safety because of a patient who experienced progressive hypertension due to a rapid rise in her Hb level in a study of healthy postmenopausal women. Patients were assigned a home BP monitor and trained in its use. Sotatercept dosing could be modified, interrupted, or discontinued based on BP increases. Sotatercept was

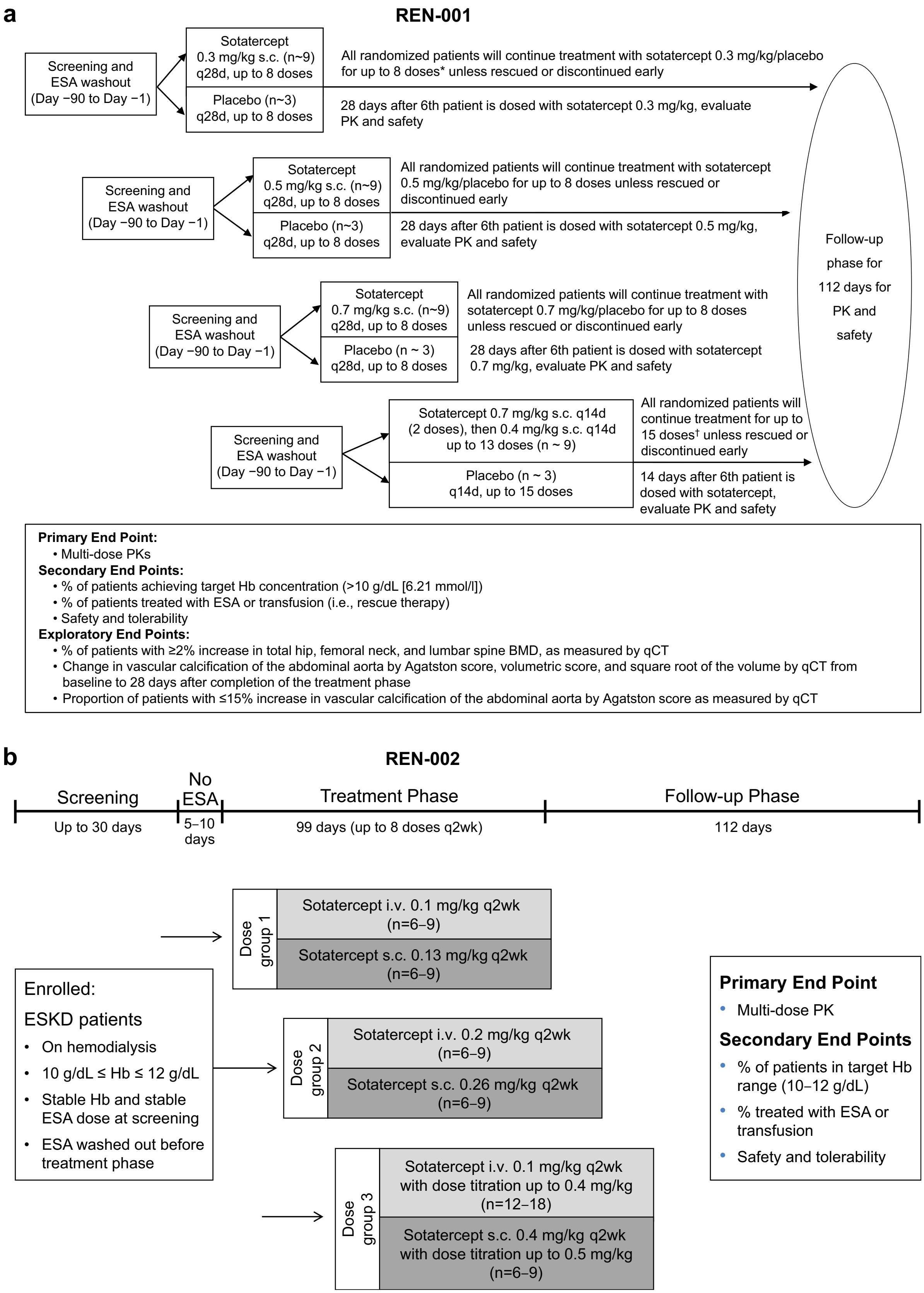

Figure 1. Study designs for REN-001 (a) and REN-002 (b). *If hemoglobin (Hb) levels are >11 g/dl (6.83 mmol/l) and have not returned to <11 g/dl by day 36 (window is day 29 + 7 days) of a dosing cycle, sotatercept/placebo doses 2 to 8 may be delayed until Hb is <11 g/dl. Patients will be dosed when Hb is <11 g/dl. †If Hb levels are >11 g/dl and have not returned to <11 g/dl by day 19 (window is day 15 + 4 days) of a dosing cycle, sotatercept/placebo doses 2 to 15 may be delayed until Hb is <11 g/dl. Patient will be dosed when Hb is <11 g/dl. BMD, bone mineral density; ESA, erythropoiesis-stimulating agent; ESKD, end-stage kidney disease; PK, pharmacokinetic; qCT, quantitative computed tomography; s.c., subcutaneous.

discontinued if mean systolic BP was >200 mm Hg or mean diastolic BP was >110 mm Hg at any time (REN-002 only), or if mean pre-dose home systolic BP was >160 mm Hg and there was a >20 mm Hg systolic BP increase or a >10 mm Hg diastolic BP increase from baseline. In response to increased BP, dialysis and/or antihypertensive medications could be modified at the investigator's discretion, with mean home BP reassessed before the next dosing visit. In REN-002, post-dose home BP values were evaluated for 2 days following each dose.

Quantitative Computed Tomography

A quantitative computed tomography (qCT) scan of the hip, lumbar spine, and abdominal aorta was obtained at baseline and after the 225-day treatment phase. Trabecular volumetric BMD (mg/cm^3) was determined for 2 vertebrae within L1-4 (typically L1-2); left proximal femurs were analyzed for volumetric BMD of the cortical, trabecular, and integral bone compartments of the total hip, femoral neck, and trochanteric regions using Mindways QCT PRO software (version 4.0; Mindways Software, Austin, TX).

All study sites were provided quality assurance (QA) and CT calibration (Mindways Model 3, Mindways Software) phantoms to standardize qCT results from the multiple CT scanners. Each CT instrument calibration was collected before scanning using the QA and calibration phantoms. Study sites continued acquiring QA scans once weekly and before each scan to enable longitudinal performance monitoring of all CT instruments. These data were incorporated into the qCT analysis using QCT PRO software to account for intra-scanner calibration changes as needed.

Abdominal aorta vascular calcification was assessed using software that semi-automatically segmented the area and volume of calcifications within the region adjacent to the top of L1 through the bottom of L4 (Alice Software, PAREXEL Informatics, Waltham, MA). Number and location of slices measured were maintained across visits per patient. Agatston scores, aortic calcification volume, and square root–transformed calcium volume scores were determined, as described elsewhere.[22,23] All image quality control and blinded analyses were performed centrally by an imaging core laboratory (PAREXEL International Corp., Waltham, MA). Results are based on all available data from patients with paired qCT assessments at baseline and day 225.

Assessments and Statistical Analysis

REN-001

Primary endpoints for Part 2 were sotatercept serum pharmacokinetic parameters. Efficacy measures related to Hb were secondary endpoints and were analyzed using descriptive statistics. Efficacy analyses were conducted using the full analysis set (i.e., all randomized patients who received ≥1 dose of study treatment and had ≥1 postrandomization observation and a baseline value [only for those endpoints requiring baseline for analysis]). Placebo arms were combined for all efficacy and safety analyses. Efficacy endpoints included proportions of patients achieving an absolute Hb concentration >10 g/dl (6.21 mmol/l) at any time before rescue during the treatment period, and those requiring rescue therapy during the treatment period. Time to target Hb concentration and time to receiving rescue treatment were summarized. Exploratory endpoints based on qCT included proportions of patients with a >2% increase from baseline in BMD assessments of femoral neck, lumbar spine, and hip, and change from baseline in Agatston scores for abdominal aorta vascular calcification. No formal hypothesis testing or treatment comparison was performed for the exploratory endpoints.

REN-002

Primary endpoints were sotatercept serum pharmacokinetic parameters. Secondary endpoints included proportions of patients maintaining a mean Hb concentration of ≥10 to ≤12 g/dl (6.21–7.45 mmol/l) between days 98 and 113 and requiring rescue therapy (Hb <9 g/dl [5.59 mmol/l]) during the treatment period. Efficacy measures were analyzed using descriptive statistics among the full analysis set; safety was examined in the safety population (i.e., all randomized patients who received ≥1 dose of study treatment).

RESULTS

Patient Disposition

REN-001

In Part 1, a total of 7 patients from 4 US sites were enrolled (placebo: n = 1; sotatercept 0.1 mg/kg s.c.: n = 6). In Part 2, a total of 43 patients from 13 US sites were enrolled and received ≥1 dose of study medication by s.c. injection (placebo: n = 11; sotatercept 0.3 mg/kg: n = 9 [group 1]; 0.5 mg/kg: n = 8 [group 2]; 0.7 mg/kg: n = 9 [group 3]; 0.7/0.4 mg/kg: n = 6 [group 3a]). In the placebo group, 1 of 11 (9.1%) patients completed the study treatment (i.e., received assigned study treatment throughout the 200-day treatment phase); among the treatment groups, the sotatercept 0.3 mg/kg group had the lowest proportion of completers (2 of 9 patients [22.2%]), and the sotatercept 0.7 mg/kg group had the highest proportion of completers (4 of 9 patients [44.4%]). Rescue ESA administration due to Hb concentration <9 g/dl (5.59 mmol/l) was the primary reason for discontinuation across all treatment groups (Figure 2).

Figure 2. Patient disposition. Disposition of patients is illustrated for REN-001 Part 2 (a) and for REN-002 (b). Reasons for early discontinuation of treatment are noted; patients may have had more than 1 reason for discontinuation of study drug. *Home systolic blood pressure (BP) of >160 mm Hg plus a change from baseline of systolic BP >20 mm Hg or a diastolic BP >10 mm Hg. †Early termination from the study during the treatment phase. ‡A 76-year-old female receiving placebo died on study day 148. Cause of death was cardiomyopathy. §A 70-year-old male with a history of hypertension, myocardial infarction, and mitral valve incompetence died within 115 days of the last dose (subcutaneous [s.c.] sotatercept 0.4–0.5 mg/kg). Cause of death (severe cardiac failure) was not considered by the investigator to be treatment-related.

REN-002

Fifty patients from 16 European Union sites were randomized and received ≥1 dose of sotatercept (i.v. doses 0.1 mg/kg, n = 7; 0.2 mg/kg, n = 9; 0.1–0.4 mg/kg, n = 12; s.c. doses 0.13 mg/kg, n = 7; 0.26 mg/kg, n = 9; 0.4–0.5 mg/kg, n = 6). Thirty (60%) patients completed study treatment throughout the 99-day treatment phase; 20 (40%) patients discontinued. Among those receiving sotatercept, the s.c. 0.4 to 0.5 mg/kg dose group had the lowest proportion of completers (3 of 6 patients [50.0%]), and the i.v. 0.1 to 0.4 mg/kg dose group had the highest proportion of

Table 1. PK parameters[a] of sotatercept in REN-001 and REN-002 (noncompartmental analysis)

PK parameter, mean (CV%)	REN-001 Part 1 Sotatercept dose: 0.1 mg/kg s.c. n = 6
C_{max}, μg/ml	1.02 (56.4)
T_{max},[a] d	6.02 (3.1, 14.0)
$AUC_{0-28days}$, d•μg/ml	20.64 (55.2)
$t_{1/2,z}$,[b] d	21.07 (18.4)

	REN-001 Part 2 Sotatercept dose (s.c.)			
	0.3 mg/kg n = 9	0.5 mg/kg n = 8	0.7 mg/kg n = 9	0.7/0.4 mg/kg n = 6
$C_{max0-28days}$, μg/ml	2.40 (40.0)	3.66 (18.3)	3.97 (41.3)	7.71 (25.6)
$T_{max0-28days}$,[a] d	11.94 (2.0, 14.0)	6.96 (0.02, 14.0)	12.00 (5.0, 16.9)	18.50 (15.0, 21.0)
$AUC_{0-28days}$, d•μg/ml	50.64 (34.9)	78.03 (17.1)	87.51 (41.9)	126.96 (16.4) (n = 5)
$t_{1/2,z}$,[b] d	24.31 (13.5) (n = 8)	32.07 (61.9)	22.16 (31.6)	19.61 (14.8)

	REN-002 Sotatercept administration route and dose					
	i.v. 0.1 mg/kg n = 6	i.v. 0.2 mg/kg n = 6	i.v. 0.1 − 0.4 mg/kg n = 9	s.c. 0.13 mg/kg n = 4	s.c. 0.26 mg/kg n = 8	s.c. 0.13 − 0.5 mg/kg n = 4
$C_{max0-28days}$, μg/ml	3.56 (19.7) (n = 4)	8.61 (41.3) (n = 3)	3.50 (61.2) (n = 5)	3.87 (93.4) (n = 3)	1.97 (57.4) (n = 4)	3.16 (24.9) (n = 2)
$T_{max0-28days}$,[a] d	14.1 (14.0, 16.0) (n = 4)	14.0 (14.0, 16.0) (n = 3)	0.169 (0.004, 16.0) (n = 5)	21.0 (16.2, 28.0) (n = 3)	18.5 (16.0, 21.0) (n = 4)	21.0 (21.0, 21.1) (n = 2)
$AUC_{0-28days}$, d•μg/ml	38.36 (22.4) (n = 4)	94.11 (39.5) (n = 3)	33.17 (26.3) (n = 5)	41.50 (32.9) (n = 3)	36.07 (63.3) (n = 4)	60.50 (24.2) (n = 2)
$t_{1/2,z}$,[b] d	17.6 (26.3)	21.8 (18.1)	22.5 (23.8)	25.9 (27.6)	21.0 (25.7)	20.4 (26.0)

$AUC_{0-28days}$, area under the curve during a 28-day exposure period; $C_{max0-28days}$, maximum plasma concentration during a 28-day exposure period; CV%, coefficient of variance; PK, pharmacokinetic; s.c., subcutaneous; $t_{1/2,z}$, terminal half-life; $T_{max0-28days}$, time to C_{max} during a 28-day exposure period.
[a]T_{max}, $T_{max0-28days}$ expressed as median (range).
[b]Elimination half-life estimated by noncompartmental method using concentrations after a single dose (REN-001 part 1) and the last dose (REN-001 part 2 and REN-002).
The n reflects the number of randomized patients who underwent PK testing; actual number of patients with data available for each parameter may vary. PK parameters for REN-001 and REN-002 are based on noncompartmental analysis.

completers (10 of 12 patients [83.3%]). The most common reasons for discontinuation were lack of efficacy (n = 5) and adverse event (AE; n = 4) across all the treatment groups; 8 patients discontinued for other reasons unrelated to safety (Figure 2).

Dialysis Prescription

Patients in both studies were on a fixed dialysis protocol, and there were no changes in dialysis prescriptions during the study period. Baseline demographics, dialysis treatment history, and concomitant phosphate-binder use are reported in Supplementary Tables S1 and S2.

Primary Endpoint: Pharmacokinetics

REN-001

In the Part 1 noncompartmental pharmacokinetic analysis, sotatercept 0.1 mg/kg s.c. single dose was slowly absorbed into systemic circulation (median time to maximum plasma concentration, approximately 6 days), exhibiting a mean terminal half-life ($t_{1/2,z}$) of 21.1 days (n = 6; Table 1). Serum sotatercept concentration showed no changes during dialysis, indicating that the drug is nondialyzable. In the Part 2 noncompartmental analysis, sotatercept mean 28-day exposure (area under the concentration-time curve from 0 to 28 days [$AUC_{0-28days}$]) and maximum serum concentration (C_{max}) increased in an approximate dose-proportional manner from a dose of 0.3 mg/kg to 0.7 mg/kg. The 14-day dosing regimen, with loading dose in the first month (dose group 3a), led to increased AUC and C_{max} levels and higher trough concentration (not shown). Sotatercept displayed elimination rates across all dose groups ranging from 20–32 days.

REN-002

Increases in $AUC_{0-28days}$ and C_{max} were approximately dose-proportional, from 0.1 to 0.2 mg/kg for the i.v. sotatercept dosing (Table 1). A dose-dependent increase in $AUC_{0-28days}$ and C_{max} was not obvious for s.c. sotatercept dosing from 0.4 to 0.5 mg/kg, likely due to the small sample size and higher interpatient variability. Regardless of administration route, sotatercept displayed elimination rates ranging from 18 to 26 days.

Secondary Endpoints: Target Hb

REN-001

In dose groups 1–3 for up to 200 days of treatment, a general trend toward increase in mean Hb concentration and mean change from baseline in Hb concentration over time was observed across treatment groups. No consistent trend was observed in mean Hb concentration or mean change from baseline in Hb concentration over time in dose group 3a. Figure 3a summarizes the proportions of patients achieving predefined target Hb for REN-001 (Hb >10 g/dl [6.21 mmol/l] any time during the treatment period). There were no consistent trends in time to achieve target Hb across the placebo and sotatercept dose groups (range median time to target, Hb censored for rescue: 1.0–18.5 days).

Fewer patients were rescued in the sotatercept 0.5 and 0.7 mg/kg treatment groups compared with the sotatercept 0.3 mg/kg and placebo groups (Figure 3c). Because of the small group size, the chance that differences between groups existed by chance could not be excluded. All 6 sotatercept 0.7/0.4 mg/kg patients required rescue during the treatment period. Mean time to rescue was longest with sotatercept 0.7 mg/kg (100.4 days) and 0.7/0.4 mg/kg (117.5 days) and shortest with placebo (44.8 days).

REN-002

Sixteen (32%) patients maintained predefined target Hb (≥10.0 [6.21 mmol/l] and ≤12.0 g/dl [7.45 mmol/l] for visits 14–17) without rescue medication. Proportionately more patients in s.c. groups 2 (33%) and 3 (50%) met this criterion, versus i.v. dose groups 2 (11%) and 3 (25%), although the small sample size in each dose group makes comparisons difficult (Figure 3b).

Overall, during the treatment period, similar proportions of patients used rescue therapy in the i.v. (8 of 28 patients [28.6%]) and s.c. (6 of 22 patients [27.3%]) dose groups. A trend toward lower rescue therapy use was observed with higher i.v. doses (Figure 3d).

Exploratory Endpoints: Effects on Bone Endpoints (REN-001)

In 27 patients with paired qCT measurements at baseline and day 225, there were no consistent trends in the mean percent changes from baseline in lumbar spine or total hip BMD. One patient (sotatercept 0.7 mg/kg) had a ≥2% increase from baseline in total hip cortical BMD. No placebo or sotatercept 0.7/0.4 mg/kg patients had a ≥2% increase from baseline in femoral neck cortical BMD; the proportions of patients achieving a ≥2% increase from baseline in femoral neck cortical BMD increased with sotatercept dose (Figure 4). Mean percent changes from baseline in femoral neck cortical BMD were −1.2% (placebo), −1.4% (0.3 mg/kg), 1.6% (0.5 mg/kg), 1.0% (0.7 mg/kg), and −2.4% (0.7/0.4 mg/kg). Median (minimum, maximum) BMD values and other bone remodeling outcomes are presented in Supplementary Table S3.

Change in Vascular Calcification of the Abdominal Aorta (REN-001)

The highest mean percentage increase from baseline in Agatston score (48.6%) was observed with placebo (mean Agatston score: 5216 at day 225) and the smallest (7.6%) was observed with sotatercept 0.7 mg/kg (mean Agatston score: 4411 at day 225). Similar results were observed for volumetric score and square root of volumetric score. Among patients with paired assessments at baseline and day 225, proportions with a ≤15% increase from baseline in Agatston score were higher in all sotatercept treatment groups versus the placebo group. All sotatercept 0.7 mg/kg patients had a ≤15% increase from baseline in Agatston score at day 225 (Figure 5). Among patients with paired assessments of volumetric score and square root of volumetric score, the smallest mean percentage increases from baseline (9.5% and 4.6%, respectively) were observed with sotatercept 0.7 mg/kg treatment (Figure 5). Among patients with assessments of square root of volumetric score at baseline and day 225, the proportion with a ≤2.5-mm increase from baseline in square root of volume score was higher with sotatercept 0.5 mg/kg (5 of 5) and 0.7 mg/kg (6 of 8) versus placebo (4 of 6). Median (minimum, maximum) Agatston scores are presented in Supplementary Table S3.

Safety

REN-001

No dose-related increase was observed in incidence of treatment-emergent AEs (TEAEs) with sotatercept treatment. The most common TEAEs in these patients were hypertension, nausea, and fatigue (Table 2). Two deaths occurred in the placebo group: One female died on study day 148 due to cardiomyopathy and 1 male died on day 186 due to worsening coronary artery disease. There were no deaths in the sotatercept treatment groups. No association between TEAE occurrence and sotatercept mean $AUC_{0-28days}$ or C_{max} was observed.

Serious AEs (SAEs) were reported in 13 patients (Table 2). The SAEs reported by >1 sotatercept patient were gastroenteritis and hypertensive crisis. Two SAEs were considered treatment-related (atrial fibrillation: n = 1 [sotatercept 0.3 mg/kg]; anemia [reported by the investigator as an SAE instead of lack of efficacy, per protocol]: n = 1 [sotatercept 0.3 mg/kg]).

BP assessments at clinic visits or periodic interdialytic ambulatory home monitoring showed no dose-related trends versus placebo. Hypertension events

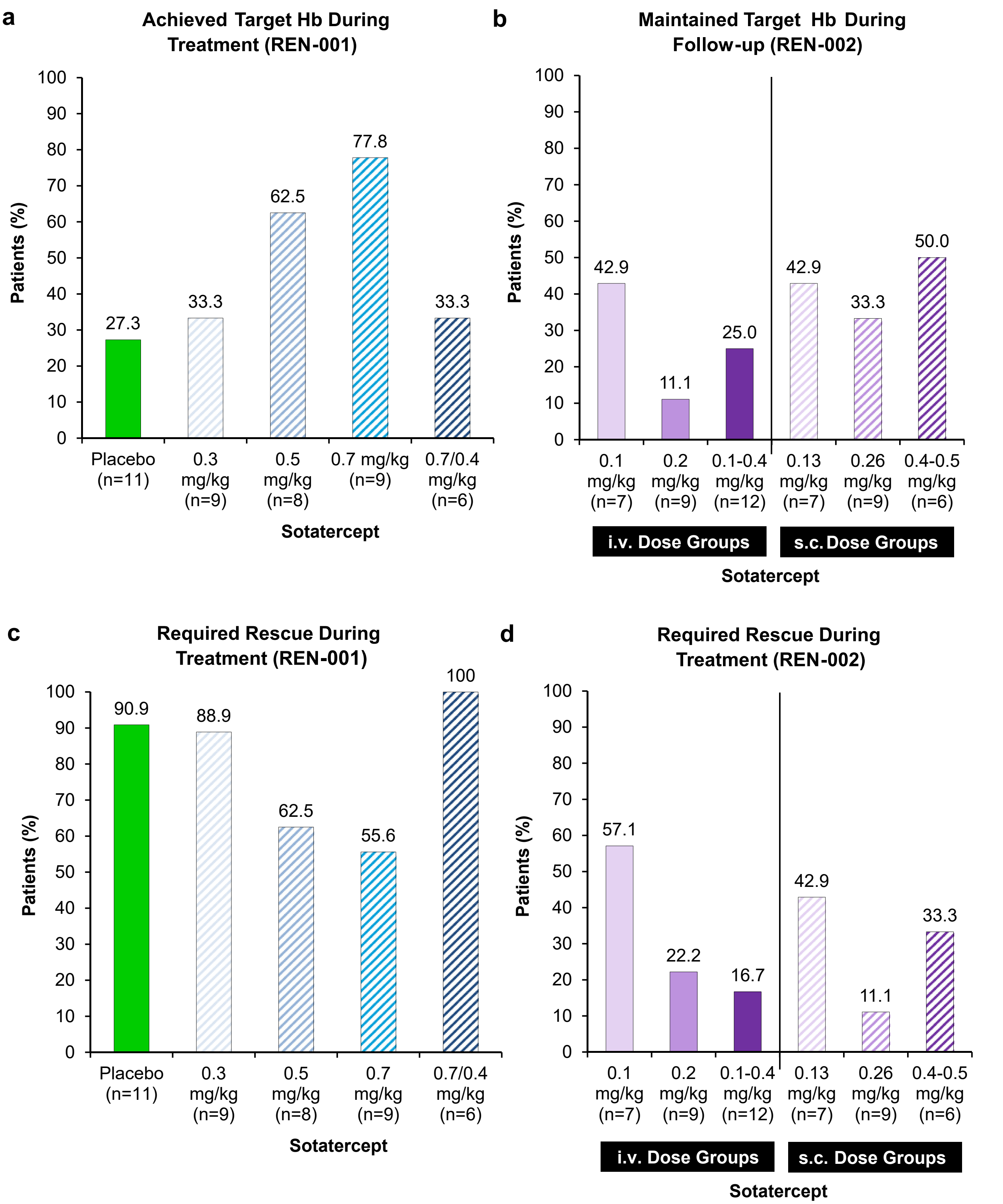

Figure 3. Hemoglobin endpoints are shown for REN-001 (a) and REN-002 (b), and rescue treatment is shown for REN-001 (c) and REN-002 (d). For REN-001, the target Hb was >10 g/dl (6.21 mmol/l) at any time before rescue during the treatment period; for REN-002, the target Hb was ≥10 (6.21 mmol/l) to ≤12 g/dl (7.45 mmol/l) at any time before rescue between days 98 and 115. If Hb was <9 g/dl (5.59 mmol/l), or at the discretion of the investigator if deemed necessary for the safety and well-being of the patient, a blood transfusion or erythropoietin-stimulating agent therapy was given. Because of the small group size, the chance that differences between groups existed because of chance could not be excluded.

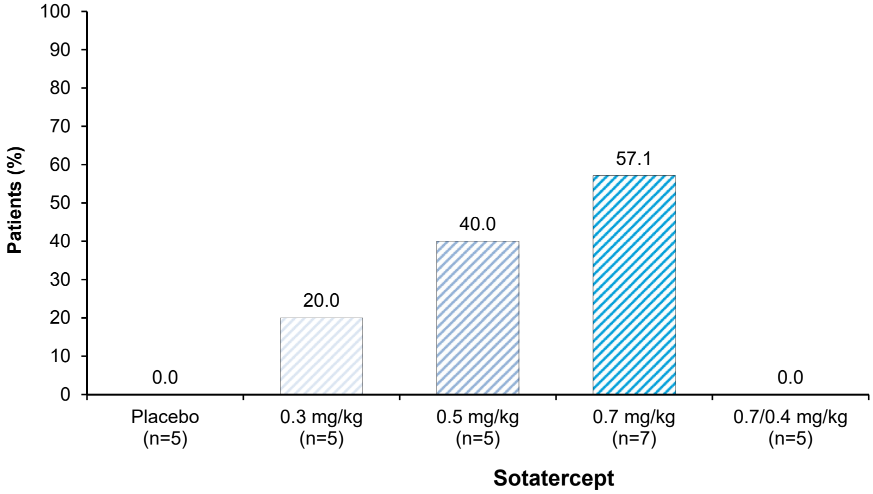

Figure 4. Patients with a ≥2% increase in femoral neck cortical bone mineral density in REN-001. Percentages are based on the number of patients with quantitative computed tomography of femoral neck cortical bone mineral density at baseline and day 225.

were the most frequently reported TEAEs in sotatercept patients. All hypertension TEAEs were mild/moderate in severity; none were considered treatment-related.

One patient (sotatercept 0.7 mg/kg) had at least 1 Hb measurement >12 g/dl (7.45 mmol/l), and 1 patient (sotatercept 0.5 mg/kg) had a rise in Hb concentration >2 g/dl (1.24 mmol/l) during any 4-week treatment period. Patients with these Hb increases did not experience AEs of hypertension or increased BP.

No persistent or dose-related changes were noted in laboratory parameters (including chemistries, lipids, HbA1c, sex steroids, and non–red blood cell–related hematology parameters) or electrocardiographic parameters.

REN-002

Forty-one patients experienced ≥1 TEAE; <10% had treatment-related AEs or a TEAE leading to treatment discontinuation. Approximately one-fourth experienced SAEs (Table 2). The most commonly reported TEAEs were hypertension, muscle spasms, headache, arteriovenous fistula site complication, and influenza (Table 2). No association between TEAE occurrence and sotatercept $AUC_{0-28days}$ or C_{max} was observed.

Although 40% of patients had Hb >12 g/dl (7.45 mmol/l) after the first dose, and 44% had a change from baseline in Hb >1.0 g/dl (0.62 mmol/l), neither occurrence appeared to be dose-related; 8% had Hb >13 g/dl (8.07 mmol/l) without discontinuation of study medication, and 2% had Hb >13 g/dl (8.07 mmol/l), leading to treatment discontinuation. These increases in Hb >12 g/dl (7.45 mmol/l) or >13 g/dl (8.07 mmol/l) were not accompanied by hypertension or increased BP AEs. Mean changes from baseline in home systolic and diastolic BP values across all patients showed considerable variability within and across dose groups, with no clear trend.

One death occurred. A 70-year-old male with a history of hypertension, myocardial infarction, and mitral valve incompetence died within 115 days of the last dose (s.c. sotatercept 0.4–0.5 mg/kg). Cause of death (severe cardiac failure) was not considered by the investigator to be treatment-related.

Anti-Sotatercept Antibodies

REN-001

Anti-sotatercept antibodies were detected in 3 patients during Part 2 (Supplementary Table S4). One patient (sotatercept 0.7 mg/kg) tested positive on day 1 of dose cycle 1 and remained positive through day 309 and at 3 unscheduled follow-up visits. One patient (sotatercept 0.7/0.4 mg/kg) tested positive on day 1 of dose cycle 2 and then tested negative through day 112; another receiving the same dose tested positive on day 1 of dose cycle 2 and through day 15. No TEAEs of hypersensitivity were reported in any patient positive for anti-sotatercept antibodies. Anti-sotatercept antibodies were not factors in the decisions related to repeated dosing. The anti-sotatercept antibodies did not have an impact on safety or efficacy, and the Hb response did not differ in these patients.

REN-002

Anti-sotatercept antibodies were detected in 7 patients (Supplementary Table S4); among these, 5 were in the i.v. dosing groups and 2 were in the s.c. sotatercept 0.13 mg/kg dosing group. In all antidrug antibody-positive patients, the first confirmed positive sample was observed ≈28 days after the first dose. Antidrug antibody titer tended to decrease over time, and most antidrug antibody-positive patients were antibody-negative

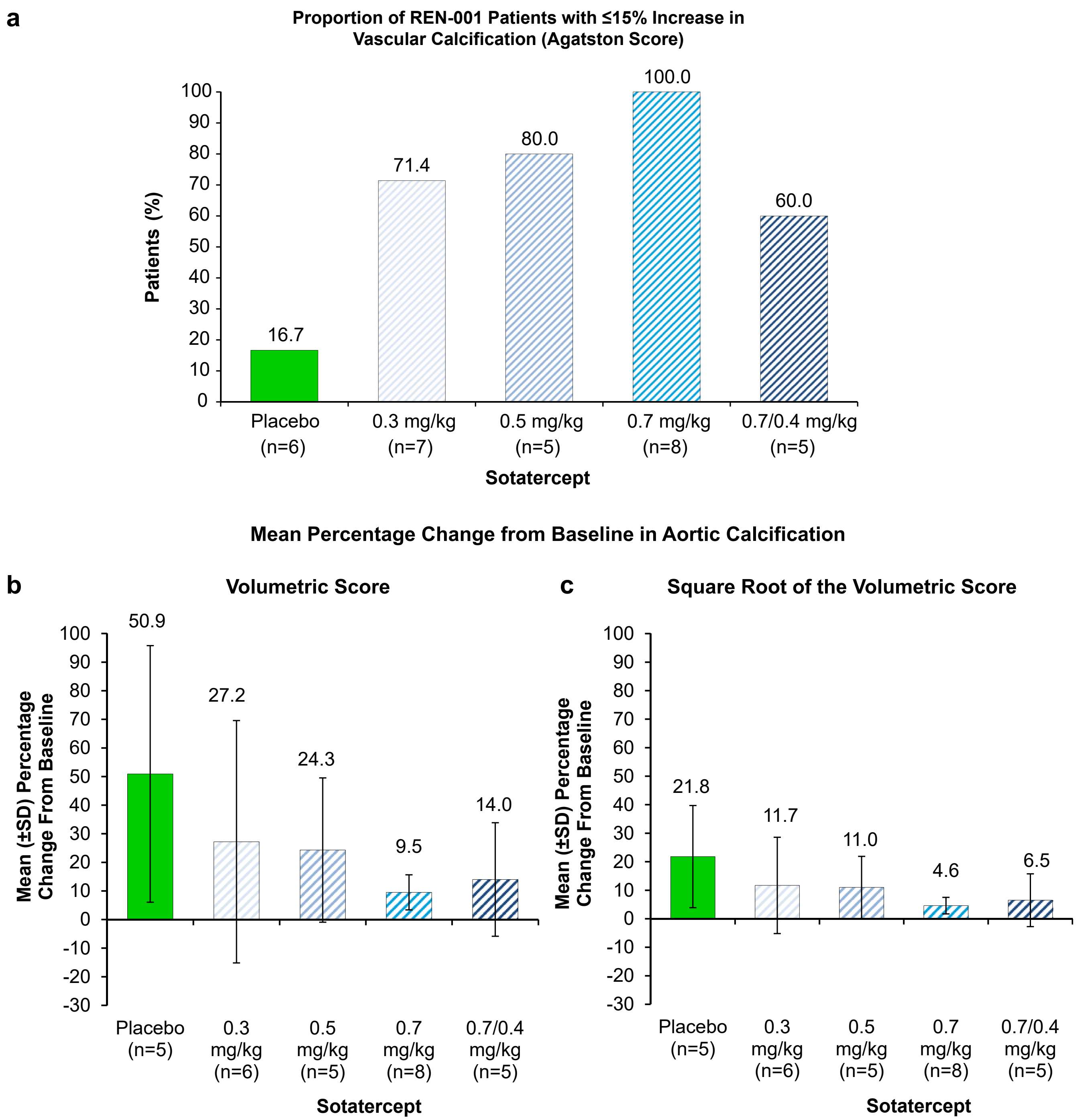

Figure 5. The proportion of patients with a ≤15% increase in vascular calcification of the abdominal aorta by the Agatston score in REN-001 are shown (a). Mean percent change from baseline in aortic calcification is shown based on the volumetric score (b) and the square root of the volumetric score (c). Data are based on patients with assessment of the Agatston score at baseline and day 225.

at study end (6 of 7 patients [85.7%]). Antidrug antibody positivity was not related to hypersensitivity AEs or other safety concerns.

DISCUSSION

Sotatercept, a ligand trap based on the ActRIIA receptor for selected proteins of the TGF-β superfamily, including activin A,[10] was originally conceived as a potential treatment for CKD-related anemia because of effects on late-stage erythropoiesis observed in preclinical models[24] and phase I healthy volunteers.[13,14] However, preclinical models demonstrated that the ActRIIA receptor is activated in skeletal, vascular, and heart tissue in CKD.[19] Therefore, in REN-001, we also explored the impact of sotatercept on bone and vascular calcification endpoints. Targeting ActRIIA activity with sotatercept treatment in ESKD patients in two phase 2 studies demonstrated an acceptable safety

Table 2. Overview of TEAEs in patients receiving sotatercept in REN-001 and REN-002

Patients, n (%)	Placebo n = 11	REN-001 Sotatercept dose (s.c.) 0.3 mg/kg n = 9	0.5 mg/kg n = 8	0.7 mg/kg n = 9	0.7/0.4 mg/kg n = 6	All sotatercept N = 32
Any TEAE	7 (63.6)	8 (88.9)	6 (75.0)	8 (88.9)	4 (66.7)	26 (81.3)
TEAE related to study drug	3 (27.3)	2 (22.2)	0 (0.0)	3 (33.3)	1 (16.7)	6 (18.8)
Serious TEAE	2 (18.2)	4 (44.4)	0 (0.0)	4 (44.4)	1 (16.7)	9 (28.1)
Any TEAE leading to discontinuation	1 (9.1)	0 (0.0)	0 (0.0)	2 (22.2)	1 (16.7)	3 (9.4)
Death	2[a] (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TEAEs in ≥3 patients in any treatment group						
Hypertension	1 (9.1)	4 (44.4)	0 (0.0)	4 (44.4)	0 (0.0)	8 (25.0)
Nausea	1 (9.1)	1 (11.1)	1 (12.5)	4 (44.4)	0 (0.0)	6 (18.8)
Fatigue	2 (18.2)	2 (22.2)	0 (0.0)	1 (11.1)	2 (33.3)	5 (15.6)

	REN-002 Sotatercept administration route and dose i.v. 1.1 mg/kg n = 7	i.v. 0.2 mg/kg n = 9	i.v. 0.1–0.4 mg/kg n = 12	s.c. 0.13 mg/kg n = 7	s.c. 0.26 mg/kg n = 9	s.c. 0.4–0.5 mg/kg n = 6	All sotatercept N = 50
Any TEAE	7 (100.0)	9 (100.0)	10 (83.3)	3 (42.9)	6 (66.7)	6 (100.0)	41 (82.0)
TEAE related to study drug	1 (14.3)	1 (11.1)	0 (0.0)	1 (14.3)	0 (0.0)	1 (16.7)	4 (8.0)
Serious TEAE	3 (42.9)	3 (33.3)	3 (25.0)	0 (0.0)	1 (11.1)	3 (50.0)	13 (26.0)
Any TEAE leading to discontinuation	0 (0.0)	3 (33.3)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	4 (8.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1[b] (16.7)	1 (2.0)
TEAEs in ≥3 patients in any treatment group							
Hypertension	1 (14.3)	2 (22.2)	2 (16.7)	0 (0.0)	2 (22.2)	2 (33.3)	9 (18.0)
Muscle spasms	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	3 (33.3)	2 (33.3)	6 (12.0)
Arteriovenous fistula site complication	2 (28.6)	0 (0.0)	0 (0.0)	2 (28.6)	1 (11.1)	0 (0.0)	5 (10.0)
Headache	0 (0.0)	1 (11.1)	1 (8.3)	0 (0.0)	0 (0.0)	3 (50.0)	5 (10.0)
Influenza	1 (14.3)	1 (11.1)	0 (0.0)	1 (14.3)	0 (0.0)	2 (33.3)	5 (10.0)
Epistaxis	1 (14.3)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.0)
Hematuria	2 (28.6)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.0)
Nausea	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	3 (6.0)
Urinary tract infection	2 (28.6)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.0)

TEAE, treatment-emergent adverse event.
[a]In REN-001, 2 deaths occurred in the placebo group. A 53-year-old male died on day 186 of coronary artery disease, and a 76-year-old female died on day 148 of cardiomyopathy. Both patients had a history of cardiovascular disease.
[b]In REN-002, a 70-year-old male died in the s.c. sotatercept 0.4–0.5 mg/kg group on day 132, of cardiac failure. The patient had a history of cardiovascular disease and the event was not considered related to study treatment.

profile and dose-related slowing of vascular calcification progression, consistent with previously reported results in CKD-MBD models using sotatercept's murine analog RAP-011.[19,20] Improvements were seen in serum Hb concentration and BMD endpoints, although less consistently.

In both studies, sotatercept was nondialyzable and was eliminated slowly, regardless of administration route. Sotatercept demonstrated an acceptable safety profile and was well tolerated up to 225 days (up to eight 28-day or fifteen 14-day dose cycles). Across both studies, no increase in TEAE incidence was observed with sotatercept related to dose or administration route. TEAE occurrence was not associated with sotatercept mean $AUC_{0-28days}$ or C_{max}. Interdialytic ambulatory home BP monitoring, repeated at regular intervals, did not reveal dose-dependent changes over time. A higher proportion of all REN-001 sotatercept treatment groups achieved the target Hb increase versus the placebo group; Hb response was dose-related when doses were given monthly. This dose effect was lost with administration every 2 weeks. REN-002 Hb findings were similar, with generally better responses seen among the s.c. versus i.v. dose groups. No increase in Hb response or maintenance of Hb response was apparent with sotatercept when given in higher and/or more frequent doses, suggesting that sotatercept's Hb-stimulatory effect may be limited in ESKD patients.

REN-001 results from the placebo group were as expected for CKD-MBD, with increasing trabecular bone (e.g., increase in lumbar spine BMD), decreasing cortical BMD (e.g., decrease in femoral neck cortical BMD), and progressive vascular calcification.[15–17] Sotatercept demonstrated a ≥2% increase from baseline in femoral neck cortical BMD in a dose-dependent manner when administered monthly; no treatment effects on total hip or lumbar spine BMD were apparent. Mixed findings in the assessed skeletal endpoints indicate that sotatercept's effects on bone volume in the CKD-MBD setting are not fully understood. Furthermore, despite recent progress, BMD is not a well-established marker of bone disease in patients with ESKD.

Sotatercept showed a dose-dependent trend toward slowing progression of abdominal aortic vascular calcification. The highest mean percentage increase from baseline in Agatston score (48.6%) was observed with placebo; the smallest (7.6%) was observed with sotatercept 0.7 mg/kg. Similar results were observed for volumetric score and square root of volumetric score.

REN-001 parameters measured to determine sotatercept's pharmacodynamic effects (e.g., Hb, BMD, Agatston score) generally showed dose-dependency when it was administered monthly (0.3–0.7 mg/kg). Paradoxically, this effect was lost when sotatercept was administered with higher and/or more frequent doses (every 2 weeks). A ligand and receptor pair within the TGF-β superfamily can have opposing effects, depending on the tissue and biologic context.[25] Therefore, it is plausible that opposing effects could be observed when the ActRIIA receptor is highly presented with sotatercept administration, possibly by changing the relative inhibition of various ligands in the circulation or at the tissue level (e.g., changing their signaling profile through various native TGF-β superfamily receptors).

Interpretation of our findings is limited by the small number of patients in each dose group, differences in baseline characteristics, unmeasured variables (e.g., dialysate calcium), relatively short treatment duration, and background co-medication patterns. Patient selection criteria were based on presence of ESA-responsive anemia, not CKD-MBD. Selected sotatercept dose levels were based on Hb dynamics and its predicted effects on Hb. Overall, our findings may have been influenced by high variability, curtailing our ability to detect "signal" versus "noise."

In summary, although highly preliminary and derived from a small number of patients, current vascular and bone findings with sotatercept treatment, along with preclinical findings of activin's role in vascular and bone changes and RAP-011 in decreasing vascular calcification in CKD,[19–21] suggest that targeting ActRIIA activity may be valuable in slowing vascular disease progression in CKD patients. We believe that these highly preliminary data hold promise for modulating activin biology in CKD. Further preclinical and clinical studies targeting ActRIIA activity in CKD may be warranted.

DISCLOSURE

DWC is a consultant and speaker for Fresenius Medical Care North America and a consultant for GlaxoSmithKline and MediBeacon. HNS, ACG, and JNC are employees of Celgene Corporation. WTS was an employee of Celgene Corporation at the time the study was conducted. MLS is an employee of Acceleron Pharma Inc. FD has served as an advisor for Sandoz and an investigator for Hexal AG. HHM and KAH have been Celgene Corporation consultants.

ACKNOWLEDGMENTS

These studies were sponsored by Celgene Corporation. The authors thank the patients and their families as well as the investigators and subinvestigators for their contributions to the studies. The authors thank Sarah Warner, PhD, of Parexel International, for her support of the methodology for capture and analysis of the qCT data presented in this report, and Theodore Reiss, MD, of Celgene Corporation, for his review of the report. The authors received editorial support in the preparation of this report from Kristin Carlin, RPh, MBA, of Peloton Advantage, LLC, an OPEN Health company, Parsippany, NJ, USA, sponsored by Celgene Corporation, Summit, NJ, USA. The authors, however, directed and are fully responsible for all content and editorial decisions for this report.

DATA SHARING

Celgene is committed to responsible and transparent sharing of clinical trial data with patients, healthcare practitioners, and independent researchers for the purpose of improving scientific and medical knowledge as well as fostering innovative treatment approaches. For more information, please visit: https://www.celgene.com/research-development/clinical-trials/clinical-trials-data-sharing/.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Baseline demographic characteristics and dialysis treatment history in REN-001 and REN-002.

Table S2. Concomitant phosphate binding use at baseline.

Table S3. Vascular calcification and bone modeling outcomes.

Table S4. Summary of patients who developed anti-sotatercept antibodies.

CONSORT Statement.

REFERENCES

1. Babitt JL, Lin HY. Mechanisms of anemia in CKD. *J Am Soc Nephrol*. 2012;23:1631–1634.
2. Jacobson LO, Goldwasser E, Fried W, Plzak L. Role of the kidney in erythropoiesis. *Nature*. 1957;179:633–634.

3. McGonigle RJ, Wallin JD, Shadduck RK, Fisher JW. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney Int*. 1984;25:437–444.
4. Fishbane S. Anemia and cardiovascular risk in the patient with kidney disease. *Heart Fail Clin*. 2008;4:401–410.
5. Eschbach JW, Egrie JC, Downing MR, et al. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. *N Engl J Med*. 1987;316:73–78.
6. Singh AK, Szczech L, Tang KL, et al. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med*. 2006;355:2085–2098.
7. Besarab A, Bolton WK, Browne JK, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *N Engl J Med*. 1998;339:584–590.
8. Drueke TB, Locatelli F, Clyne N, et al. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. *N Engl J Med*. 2006;355:2071–2084.
9. Pfeffer MA, Burdmann EA, Chen CY, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med*. 2009;361:2019–2032.
10. Pearsall RS, Canalis E, Cornwall-Brady M, et al. A soluble activin type IIA receptor induces bone formation and improves skeletal integrity. *Proc Natl Acad Sci U S A*. 2008;105:7082–7087.
11. Iancu-Rubin C, Mosoyan G, Wang J, et al. Stromal cell-mediated inhibition of erythropoiesis can be attenuated by sotatercept (ACE-011), an activin receptor type II ligand trap. *Exp Hematol*. 2013;41:155–166.
12. Carrancio S, Markovics J, Wong P, et al. An activin receptor IIA ligand trap promotes erythropoiesis resulting in a rapid induction of red blood cells and haemoglobin. *Br J Haematol*. 2014;165:870–882.
13. Ruckle J, Jacobs M, Kramer W, et al. Single-dose, randomized, double-blind, placebo-controlled study of ACE-011 (ActRIIA-IgG1) in postmenopausal women. *J Bone Miner Res*. 2009;24:744–752.
14. Sherman ML, Borgstein NG, Mook L, et al. Multiple-dose, safety, pharmacokinetic, and pharmacodynamic study of sotatercept (ActRIIA-IgG1), a novel erythropoietic agent, in healthy postmenopausal women. *J Clin Pharmacol*. 2013;53:1121–1130.
15. Fang Y, Ginsberg C, Sugatani T, et al. Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. *Kidney Int*. 2014;85:142–150.
16. Tsuruya K, Eriguchi M, Yamada S, et al. Cardiorenal syndrome in end-stage kidney disease. *Blood Purif*. 2015;40:337–343.
17. Hruska KA, Sugatani T, Agapova O, Fang Y. The chronic kidney disease–mineral bone disorder (CKD-MBD): advances in pathophysiology. *Bone*. 2017;100:80–86.
18. Malluche HH, Monier-Faugere MC. Renal osteodystrophy: What's in a name? Presentation of a clinically useful new model to interpret bone histologic findings. *Clin Nephrol*. 2006;65:235–242.
19. Williams MJ, Sugatani T, Agapova OA, et al. The activin receptor is stimulated in the skeleton, vasculature, heart, and kidney during chronic kidney disease. *Kidney Int*. 2018;93:147–158.
20. Agapova OA, Fang Y, Sugatani T, et al. Ligand trap for the activin type IIA receptor protects against vascular disease and renal fibrosis in mice with chronic kidney disease. *Kidney Int*. 2016;89:1231–1243.
21. Sugatani T, Agapova OA, Fang Y, et al. Ligand trap of the activin receptor type IIA inhibits osteoclast stimulation of bone remodeling in diabetic mice with chronic kidney disease. *Kidney Int*. 2017;91:86–95.
22. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832.
23. Hokanson JE, MacKenzie T, Kinney G, et al. Evaluating changes in coronary artery calcium: an analytic method that accounts for interscan variability. *AJR Am J Roentgenol*. 2004;182:1327–1332.
24. Murata M, Eto Y, Shibai H, et al. Erythroid differentiation factor is encoded by the same mRNA as that of the inhibin beta A chain. *Proc Natl Acad Sci U S A*. 1988;85:2434–2438.
25. Horbelt D, Denkis A, Knaus P. A portrait of transforming growth factor beta superfamily signalling: Background matters. *Int J Biochem Cell Biol*. 2012;44:469–474.