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Multiple-Dose, Safety, Pharmacokinetic, and Pharmacodynamic Study of Sotatercept (ActRIIA-IgG1), a Novel Erythropoietic Agent, in Healthy Postmenopausal Women

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Abstract

Ligands of the transforming growth factor-beta superfamily and activin-receptor signaling play an important role in erythropoiesis. Sotatercept, an activin receptor type IIA (ActRIIA) ligand trap, is a novel, recombinant, fusion protein comprising the extracellular domain of human ActRIIA linked to the Fc portion of human immunoglobulin G1. Sotatercept, originally developed to increase bone mineral density, was noted to have robust effects on erythropoiesis. Here, we evaluated the safety, pharmacokinetic properties, and pharmacodynamic effects of sotatercept in 31 healthy postmenopausal women. Sotatercept was administered at dose level 0.1, 0.3, or 1 mg/kg every 28 days subcutaneously for up to four doses. Sotatercept was generally safe and well tolerated, and elicited clinically significant, dose-dependent increases in hemoglobin, hematocrit, and red blood cell counts that persisted for up to 4 months. The effect of sotatercept on hemoglobin was dose-limiting. Sotatercept also increased bone mineral density and biomarkers of bone formation. The sotatercept serum exposure–dose relationship was linear, with a mean terminal half-life of approximately 23 days. ActRIIA ligands are important regulators of erythrocyte production in healthy individuals. Clinical studies are ongoing to explore the potential of sotatercept to treat anemia and diseases of ineffective erythropoiesis as well as an agent to increase bone mineral density.

Keywords

activin, anemia, erythropoiesis, hemoglobin, sotatercept

Anemia is a component of many diseases as a result of defects in erythropoiesis and/or hemolysis. Anemia can result from hematologic malignancies (e.g., myelodysplastic syndromes [MDS], multiple myeloma), hereditary disorders of hemoglobin structure and synthesis (e.g., thalassemia), as a complication of nonhematologic disorders (e.g., chronic kidney disease), or as a consequence of myelosuppressive chemotherapy.

Current treatment options for anemia include blood transfusions or administration of erythropoiesis-stimulating agents (ESAs). Transfusion depends on blood availability and carries risks of infectious disease, allergic or hemolytic reactions, and iron overload.[1,2] ESAs reduce the need for transfusions and have been approved to treat certain types of anemias.

Erythropoietin (EPO) functions as a regulator of early erythropoiesis, stimulating differentiation of burst-forming unit erythroid cells and subsequently colony-forming unit erythroid cells.[3] Additionally, an in vivo study in mice has shown that EPO couples hematopoiesis with osteopoiesis, and thus either directly or indirectly promotes bone formation.[4] Factors important for regulation of later stages of erythropoiesis (erythroblastic differentiation) are still unknown. In this respect, the efficiency of EPO-replacement therapy decreases when

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Results of this study were presented in part in abstract form at the 50th Annual Meeting of the American Society of Hematology (San Francisco, CA, USA; December 6–8, 2008); at the 2009 Joint Meeting of the International Society for Clinical Densitometry-International Osteoporosis Foundation (Orlando, FL, USA; March 11–14, 2009); at the 31st American Society for Bone and Mineral Research (Denver, CO, USA; September 11–15, 2009); and at the IX International Meeting on Cancer-Induced Bone Disease (Arlington, VA, USA; October 28–31, 2009).

anemia is present due to ineffective erythropoiesis in which immature erythroid precursors undergo apoptosis. Thus, some patients with anemia are completely unresponsive to EPO, whereas others develop resistance to EPO-replacement therapy and require increased EPO doses to achieve a threshold hemoglobin level.[5,6] More importantly, the benefit–risk associated with the use of ESAs has been re-evaluated[7,8] due to increased risk of stroke,[9] and potential negative effects of ESAs on tumor progression.[10] To date, ESAs have shown only a limited response in patients with MDS or thalassemia.[11,12] Considering the limitations associated with existing treatments for anemia,[13] there is a need to develop alternatives.

Potential regulators of adult erythropoiesis contain members of the transforming growth factor-beta (TGF-beta) superfamily, including five type II receptors which have activins as ligands. The activin receptor type IIA (ActRIIA) binds activins, myostatin (growth differentiation factor [GDF]8), and GDF11 with high-affinity, and together with a type I receptor (ALK4) initiates the phosphorylation of intracellular SMAD 2/3 proteins.[14] Phosphorylated SMAD 2/3 then associates with SMAD 4, forming a complex that translocates to the nucleus to regulate gene expression.[15] Sotatercept is a novel, fusion protein comprising the extracellular domain of the human ActRIIA linked to the Fc portion (including the hinge, CH2, and CH3 regions) of human immunoglobulin (Ig) G1 (ActRIIA–IgG1)[16] and acts as a trap that binds endogenous ActRIIA ligands, inhibiting their signaling. In preclinical studies, sotatercept (or its murine counterpart) enhanced erythropoiesis in mice[17] and cynomolgus monkeys (unpublished observations) under normal physiologic conditions, and alleviated chemotherapy-induced anemia in mice.[17] A murine version of sotatercept had anabolic effects on bone formation and increased bone strength in both normal and ovariectomized mice with established bone loss,[18] and in cynomolgus monkeys,[16] via a dual anabolic-antiresorptive mechanism.[19,20] A single-dose clinical study of sotatercept demonstrated increases in erythropoiesis parameters and in biomarkers of bone formation, with decreases in biomarkers of bone resorption.[21] This multiple-dose, randomized, double-blind, placebo-controlled phase 1b study was designed to determine the safety and tolerability of multiple, escalating doses of sotatercept in healthy volunteers, and to analyze the pharmacokinetic and pharmacodynamic effects of sotatercept for dose selection for further clinical studies.

Design and Methods

Subject Eligibility

A total of 40 healthy, postmenopausal women aged 45–85 years, and without any clinically significant medical conditions were planned to be enrolled. Subjects were required to have been taking stable doses of $\geq$500 mg of calcium supplement and 400 IU of vitamin D for $\geq$4 weeks before the first dose of the allocated study drug. Prohibited medications included bone-active medications such as selective estrogen receptor modulators, fluoride therapy, teriparatide, bisphosphonates, systemic glucocorticoid therapy, or other investigational drugs. All subjects were fully informed of the investigational nature of the study, and written informed consent was obtained according to federal and local institutional guidelines. The study was approved by an independent institutional review board [Aspire IRB, 9320 Fuerte Drive, Suite 105, La Mesa, CA 91941], conducted in accordance with the Declaration of Helsinki and applicable guidelines on good clinical practice, and registered at ClinicalTrials.gov (number NCT00709540). The study was conducted at West Coast Clinical Trials (Cypress, CA).

Study Design

This was a phase 1b, single-center, randomized, double-blind, placebo-controlled, multi-dose, dose-escalating study in healthy postmenopausal women. Four cohorts of 10 subjects each were to be randomly assigned to receive either sotatercept or placebo at an 8:2 ratio within each cohort. Sotatercept was to be administered at dose levels 0.1, 0.3, 1, and 2 mg/kg in Cohorts 1–4, respectively, and sotatercept or placebo were to be administered subcutaneously every 28 days for a total of four doses. Sotatercept was supplied in phosphate-buffered saline at a concentration of approximately 50 mg/mL.

The primary objective of the study was to determine the safety and tolerability of multiple escalating doses of sotatercept in healthy postmenopausal women. Secondary objectives were to determine: the pharmacokinetic properties of sotatercept associated with each dose; the effect of sotatercept on red blood cell (RBC) parameters; and the effects of sotatercept on bone mineral density (BMD) and on biochemical markers of bone formation and resorption. The primary pharmacodynamic endpoints used were BMD evaluated by a dual energy X-ray absorptiometry (DXA) scan and biochemical markers of bone remodeling. EPO samples were prospectively collected for the subjects enrolled in Cohort 3 (1 mg/kg sotatercept); for the other cohorts, EPO results were obtained retrospectively after the study was completed using frozen stored samples.

Safety Assessment

Safety was assessed in terms of adverse events (AEs), physical examination, vital signs, electrocardiograms, and clinical laboratory testing (hematology, chemistry, urinalysis, and anti-drug antibodies). Exposure to study drug and reasons for discontinuation of study treatment or placebo were monitored. Endocrine function testing

(thyroid-stimulating hormone, free thyroxine, growth hormone, and adrenocorticotropic hormone) was performed at baseline and at regular intervals throughout the 3-month follow-up period. Hematologic changes were monitored by measuring RBC count, hemoglobin, hematocrit, and reticulocytes prior to dosing, and after 7 and 14 days of each of the four planned treatment cycles. Adrenal function (adrenocorticotropic hormone stimulation test) was assessed by intramuscular injection of 250 μg cosyntropin and the change in cortisol levels was measured. Levels of anti-drug and neutralizing antibodies were measured using enzyme-linked immunosorbent assays (ELISA) at baseline and every month during the follow-up phase of the study. All subjects, clinical site staff, and study team members were masked to the subject's treatment assignment of sotatercept or placebo.

Information on AEs was collected throughout the study. AEs were defined as any potentially adverse medical event, regardless of whether they were considered to be related to the study drug. Events included any unfavorable or unintended sign, or an abnormal laboratory finding, symptom, or disease temporally associated with the use of the study drug. This included any newly occurring event or previous condition that had increased in severity or frequency since starting treatment with the study drug. Treatment-emergent AEs were defined as AEs that were newly acquired or worsened during or after administration of the study drug. Per protocol, a decrease in follicle-stimulating hormone (FSH) level below the normal limit for postmenopausal women was not considered an AE because this was an expected pharmacodynamic effect of sotatercept. AEs were graded as mild, moderate, severe, or life-threatening in severity, and were assessed as having no, unlikely, possible, probable, or definite relationship to the study drug. Serious AEs (SAEs) were defined per protocol as those that were fatal, life-threatening, requiring or prolonging hospitalization, or resulting in persistent or significant disability or incapacity, congenital anomalies or birth defects, or other important medical events. Specific safety events including thrombotic events that could be associated with an increase in hemoglobin levels were assessed by history, physical examination, and AE reporting.

Cohorts were evaluated sequentially, and dose escalation was only permitted after each dose level was approved by a safety review team, based on data from a minimum of eight subjects within each cohort. Information reviewed included safety data through to day 36 (i.e., 7 days after the second dose) for the most recent dose cohort, as well as cumulative safety data from all previous cohorts. If ≥ 3 subjects in a cohort experienced any severe AE or a SAE that was at least possibly related to sotatercept, then subsequent study drug administration within the cohort or dose-level escalation would be postponed or terminated in order to further evaluate the cumulative safety of sotatercept.

Pharmacokinetic and Pharmacodynamic Assessments

Pharmacokinetic characterization of sotatercept was based on its serum concentrations determined by ELISA at multiple times during the study. Blood samples for pharmacokinetic testing were to be collected from subjects at the following time points: day 1 (pre-dose, 6 and 12 hours after dosing); days 2, 3, 4, 8, 15, and 29 (pre-dose); day 57 (pre-dose); day 85 (pre-dose, 6 and 12 hours after dosing); and days 86, 87, 88, 92, 99, 113, 141, and 169/early termination. Due to early study drug discontinuation, samples collected from the 0.3 and 1 mg/kg groups followed a modified schedule. Human serum samples were stored at approximately −70°C at West Coast Clinical Trials. Sotatercept serum concentrations were measured via a validated competitive ELISA using anti-human activin RIIA antibodies (R&D Systems, Minneapolis, MN). The standard curve range for the assay is from the lower limit of quantification (8 ng/mL) to 400 ng/mL. At quality control concentration levels, the inter-batch assay precision was 8.5–14.7% and the inter-batch assay accuracy was −3.6% to 6.7% difference from nominal concentrations.

Pharmacodynamic assessments including biomarkers of bone formation, such as serum FSH levels, B-cell-specific activator protein (BSAP), and procollagen type 1 N-terminal propeptide (all of which were determined throughout the study), as well as biomarkers of bone resorption, such as cross-linked C-telopeptide (CTX) and TRACP-5b. All bone biomarkers were determined by immunoassays (Synarc, Lyon, France) performed multiple times throughout the study. BMD was measured by DXA scans of the full hip and lumbar spine at baseline, after the last administration of study drug, and at the end of the follow-up period.

Statistical Analysis

Baseline values were defined as the last observation before administration of sotatercept or placebo. Data from subjects treated with sotatercept were grouped by dose level/cohort, whereas data from placebo-treated subjects were pooled across all cohorts. Demographic characteristics, tolerability, pharmacokinetic parameters, clinical laboratory data, and pharmacodynamic parameters (FSH levels, bone biomarkers, and BMD) were analyzed using descriptive statistics. Categorical data were summarized as frequencies and percentages. Continuous data were summarized as means, standard deviation, and range. The percentage change from baseline of FSH levels and bone biomarkers within each treatment group were analyzed using single-group *t*-test. Two-group *t*-tests were used to compare percentage changes from baseline in FSH levels and bone biomarkers between each dose of

sotatercept and placebo. The mean percent change from baseline was computed for each cohort as the mean change at a particular time point from the baseline value, and expressed as a percentage relative to the baseline value. All pharmacokinetic analyses were performed using standard techniques as implemented in WinNonlin® Professional version 5.0.1 (Pharsight Corporation, Mountain View, CA). All statistical analyses were performed using SAS® version 9.1 (SAS Corporation, Cary, NC).

Results

A total of 31 postmenopausal women, ranging in age from 49 to 81 years, were enrolled at a single clinical center in the United States (Supplemental Table). The majority of subjects were white (64.5%) and their body mass index ranged from 18.9 to 30.8 kg/m^2. The mean baseline hemoglobin levels ranged from 12.7 to 13.3 g/dL, and serum FSH levels ranged from 77.9 to 88.7 mIU/mL across all dose cohorts. Three cohorts were enrolled in a sequential manner and were to receive four doses of sotatercept. Eight subjects in each cohort received sotatercept at doses of 0.1 mg/kg (four doses), 0.3 mg/kg (three doses), or 1 mg/kg (two doses); seven subjects received placebo. One placebo-treated subject discontinued for personal reasons after the first dose and was replaced. Demographic and baseline characteristics were generally balanced across the treatment groups (Supplemental Table).

All 31 subjects were included in the safety analysis. Two subjects treated with 0.3 mg/kg sotatercept were excluded from the FSH analysis because they did not meet the protocol-specified definition of postmenopausal. Dose escalation was discontinued when one subject treated with 1 mg/kg sotatercept experienced a dose-limiting toxicity (described below) which resulted in the discontinuation of further study drug administration for all subjects at all dose levels.

Pharmacokinetics

Observed mean concentration profiles of sotatercept in serum after multiple SC administrations over the dose range of 0.1–1.0 mg/kg were adequately described by a one-compartment model with first-order absorption and elimination (Figure 1), suggesting the pharmacokinetics of sotatercept are linear and time-independent over the test-dose range. The pharmacokinetic parameters, apparent total clearance (CL/F), elimination half-life ($t_{1/2}$), and apparent volume of distribution (V/F), were generated by fitting all available multiple-dosing concentration data to the one-compartment model for each subject. As summarized in Table 1, the mean value ranged from 3.05 to 3.90 mL/d/kg for CL/F, from 21 to 23 days for $t_{1/2}$, and from 97 to 103 mL/kg for V/F (Table 1), with no apparent dose-dependency.

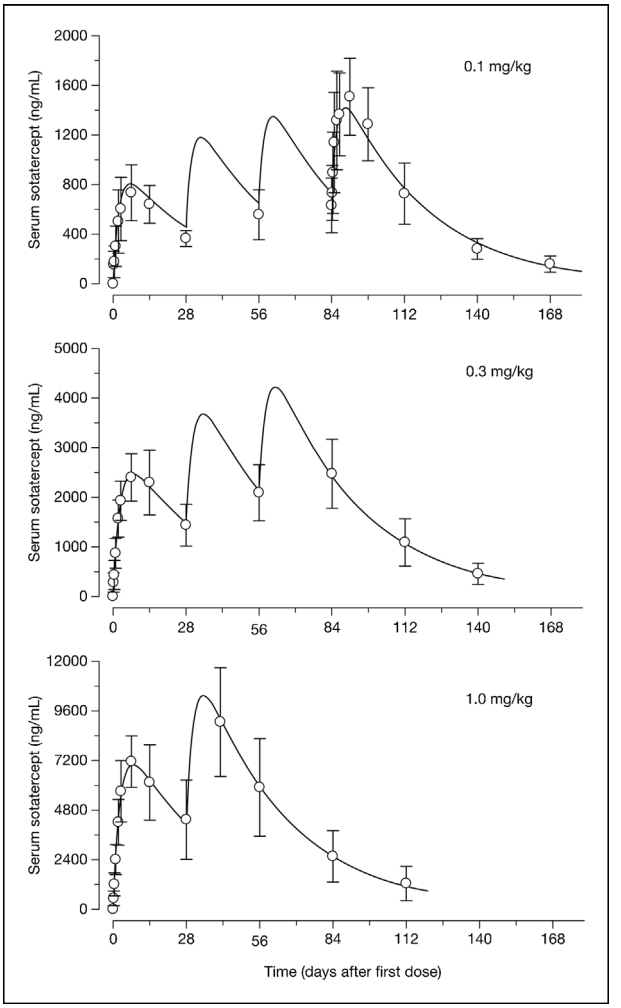

Figure 1. Sotatercept serum concentration versus time profiles after multiple doses. The symbols and vertical bars represent the observed mean ± SD concentrations (n = 8 for each dose level) and the lines represent the predicted concentrations by the one-compartment model.

Noncompartmental analyses were also performed to estimate the systemic sotatercept exposure after the first dose and the terminal $t_{1/2}$ after the last dose. At all three doses, the maximum plasma concentration (C_{max}) after the first dose of sotatercept occurred after a median time of approximately 7 days after the dose. The mean C_{max} values after the first dose, as well as the mean area under the sotatercept plasma concentration–time curve from days 0 to 28 ($AUC_{0–28d}$), increased linearly and in a manner proportional to the dose (Table 1). The mean accumulation ratio was calculated for the 0.1 mg/kg dose cohort that received all four planned doses. The mean ratios of dose four on day 85 to dose one on day 1 for C_{max} and $AUC_{0–28d}$ were 2.27 and 2.25, respectively, indicating moderate accumulation of multiple monthly doses of sotatercept. The mean terminal $t_{1/2}$ was approximately 23 days for all dose groups, consistent with the values estimated by the one-compartment model.

Table 1. Pharmacokinetic Parameters of Sotatercept[a]

Parameter	Sotatercept 0.1 mg/kg (n = 8)	Sotatercept 0.3 mg/kg (n = 8)	Sotatercept 1 mg/kg (n = 8)
C_{max}, ng/mL	746 ± 224	2459 ± 601	7394 ± 1183
t_{max}, days	7.00 (2.95–14.00)	6.99 (6.95–13.98)	7.01 (6.96–26.93)
AUC_{0-28d}, d × ng/mL	15,492 ± 3,906	54,547 ± 13,183	147,952 ± 33,851
Absorption rate, d^{-1}	0.45 ± 0.28	0.38 ± 0.16	0.32 ± 0.12
CL/F, mL/d/kg	3.22 ± 0.89	3.05 ± 0.96	3.90 ± 1.73
V/F, mL/kg	97 ± 19	99 ± 20	103 ± 20
$t_{1/2}$, days	22.3 ± 7.4	23.3 ± 4.5	20.9 ± 7.7

AUC_{0-28d}, C_{max}, and t_{max} were estimated for the first dose by noncompartment analysis, and other parameters were estimated by fitting the multiple-dosing concentration data to a one-compartment model.

[a]Data are presented as mean ± standard deviation except t_{max} which is presented as median (range).

Pharmacodynamics

Effects on erythropoiesis. A rapid, clinically relevant, and durable effect on erythropoiesis was evident after administration of multiple subcutaneous doses of sotatercept, as demonstrated by significant dose-dependent increases from baseline in mean hemoglobin, hematocrit, and RBC counts. The mean change from baseline in hemoglobin levels after multiple doses of sotatercept is presented as a function of dose level in Table 2 and Figure 2A. The mean baseline hemoglobin levels ranged from 12.7 to 13.3 g/dL across all dose cohorts and increases were evident as early as 7 days after the initial dose. The maximum change in hemoglobin levels is shown in Figure 2B. Increases in hemoglobin ≥1 g/dL were maintained for 45–110 days after the last dose of sotatercept in all active treatment arms (Figure 2A).

The increase in hemoglobin in response to sotatercept among these subjects was correlated with increased dose level. Twenty-two of 24 subjects (92%) treated with sotatercept achieved a maximum hemoglobin increase of ≥1.5 g/dL from baseline, including 75% of subjects treated with 0.1 mg/kg sotatercept and 100% of those treated with 0.3 and 1 mg/kg sotatercept (Table 3). In addition, the number of doses required to reach this threshold also reflects a dose effect. All eight subjects treated with 1 mg/kg sotatercept achieved an increase in hemoglobin of ≥1.5 g/dL after a single dose of sotatercept. Two of the eight subjects treated with 0.3 mg/kg sotatercept required one dose, with the remainder requiring at least two doses (Table 3). All of the subjects treated with 0.1 mg/kg sotatercept required at least two doses to achieve a ≥1.5 g/dL increase in hemoglobin. The median time required to reach this threshold was inversely proportional to the dose level, also indicating dose dependency (Table 3).

Table 2. Mean Change in Hemoglobin Levels From Baseline

Evaluation Time	Treatment Group: Placebo (n = 7[a])	Sotatercept 0.1 mg/kg (n = 8)	Sotatercept 0.3 mg/kg (n = 8)	Sotatercept 1 mg/kg (n = 8)
Baseline level, g/dL (range)	13.20 (11.9–15.1)	13.11 (12.1–14.4)	13.30 (12.4–13.9)	12.71 (8.4–14.1)
Mean change from baseline, g/dL (range)				
Day 8	0.17 (−0.3 to 0.7)	0.68 (0.2–1.2)	0.85 (0.2–1.6)	1.21 (0.4–2.0)
Day 15	−0.27 (−1.1 to 0.8)	0.43 (−0.3 to 0.9)	0.44 (−0.6 to 2.4)	1.75 (0.9–3.1)
Day 29	0.27 (−0.1 to 0.5)	0.61 (−0.1 to 1.0)	1.21 (0.1–2.8)	2.68 (1.7–4.4)[b]
Day 36	0.56 (−0.3 to 1.3)	0.64 (0.4–1.1)	1.89 (1.0–3.3)	2.96 (1.5–6.4)
Day 43	−0.02 (−0.8 to 0.5)	0.89 (−0.1 to 1.7)	1.21 (0.4–2.2)	2.85 (1.9–4.1)
Day 57	0.27 (−0.6 to 1.0)	1.28 (0.9–1.8)	1.64 (1.1–3.1)[b]	2.09 (1.2–3.2)
Day 64	0.53 (0.3–0.8)	1.11 (0.7–1.8)	2.49 (2.0–3.8)	2.21 (1.6–3.6)
Day 71	−0.10 (−1.0 to 1.1)	1.34 (0.6–1.9)	2.09 (0.7–3.8)	1.66 (0.7–3.2)
Day 85	0.38 (−0.1 to 1.3)	1.18 (0.4–1.9)[b]	2.55 (1.9–4.1)	1.86 (0.0–3.6)
Day 92	0.00 (0.0–0.0)[c]	1.04 (0.2–1.6)	1.60 (1.6–1.6)[c]	3.80 (3.8–3.8)[c]
Day 99	−0.20 (−0.3 to −0.1)	1.21 (0.3–2.0)	3.20 (3.2–3.2)[c]	1.28 (−0.3 to 3.2)
Day 113	−0.02 (−1.1 to 0.5)	1.30 (0.4–2.5)	1.29 (0.7–1.8)	1.04 (−0.6 to 3.2)
Day 141	0.30 (−0.8 to 1.4)	0.95 (0.5–2.0)	0.34 (−1.8 to 0.8)	2.30 (1.4–3.2)
Day 169	0.20 (0.2–0.2)[c]	0.06 (−0.7 to 0.9)	—	2.00 (2.0–2.0)[c]

[a]One placebo subject discontinued study prematurely after the first dose.

[b]Four, three, and two doses of 0.1, 0.3, and 1 mg/kg sotatercept, respectively, were administered; the last dose was administered on protocol days 85, 57, and 29, respectively.

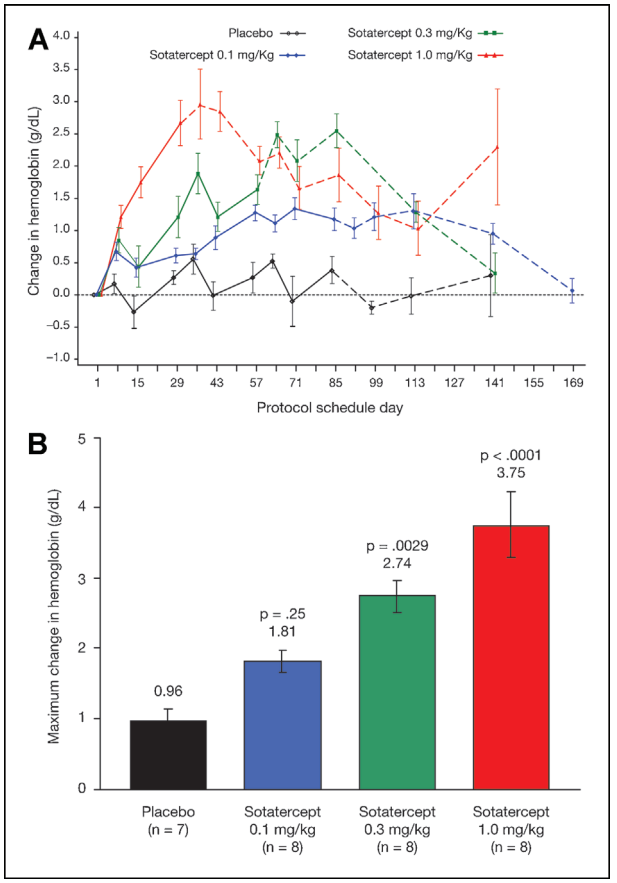

Figure 2. Effects of sotatercept on hemoglobin levels and red blood cell counts. (A) Mean change in hemoglobin levels from baseline over time (days post-first dose) by treatment group. For each dose, the solid line represents the dosing period, and the dashed line represents the follow-up period (i.e., visits after the last dose). The last dose of 0.1 mg/kg (four doses), 0.3 mg/kg (three doses), and 1 mg/kg sotatercept (two doses) was administered on study days 85, 57, and 29, respectively. Error bars show standard error of the mean (SEM). (B) Mean maximum change in hemoglobin from baseline after the initial dose by treatment group. Error bars indicate SEM. p-values show differences compared with placebo.

The elevated hemoglobin levels returned toward baseline levels by the end of the study (Figure 2A). Three subjects treated with 1 mg/kg sotatercept experienced elevated hemoglobin levels substantially above the normal range and underwent phlebotomies. In one of these subjects, phlebotomy was necessary to treat an SAE (described below). The other two subjects were asymptomatic, but underwent phlebotomy as a preventive measure. Changes in hematocrit and RBC count after the administration of sotatercept were consistent with the changes in hemoglobin levels. There were no clinically significant changes in white blood cell, platelet, or reticulocyte counts in subjects treated with sotatercept or placebo (data not shown).

An increase in mean EPO levels was observed 1 month after the first and second doses of 1 mg/kg sotatercept (mean change: 17.3 and 38.5 mIU/mL, respectively). Smaller mean increases in EPO levels were observed in 0.1 and 0.3 mg/kg dose cohorts at these times, although these increases were within the normal range.

A more detailed hematologic evaluation was undertaken in selected subjects. Peripheral smears showed no evidence of hemolysis or abnormal RBC morphology after sotatercept treatment. Hemoglobin electrophoresis was performed in a subject whose hemoglobin peaked at 20 g/dL. The results showed normal adult hemoglobin (98.1% hemoglobin A and 1.9% hemoglobin A_2) with no evidence of fetal hemoglobin (0% hemoglobin F). Janus kinase 2 (JAK2) activity was also assessed in the three subjects with elevated hemoglobin levels who underwent phlebotomy after sotatercept treatment. The negative results in each subject suggest that the elevated hemoglobin levels were not due to acquired myeloproliferative disorders.[22]

Effects on bone. Sotatercept caused sustained dose-dependent increases in a biomarker of bone formation without increasing bone resorption biomarkers. Serum levels of the bone formation biomarker BSAP exhibited rapid, sustained increases after sotatercept administration, with maximum mean increases from baseline of 12.5%, 24.5%, and 33.0% in subjects treated with 0.1, 0.3, and 1 mg/kg sotatercept, respectively. The mean BSAP levels remained elevated throughout the treatment period, in most subjects peaking at approximately 7–14 days after each dose of sotatercept. BSAP levels returned to near or

Table 3. Proportion of Subjects Achieving a ≥1.5 g/dL Increase in Hemoglobin, With Median Time and Exposure

	Treatment Group			
		Sotatercept		
	Placebo (n = 7)	0.1 mg/kg (n = 8)	0.3 mg/kg (n = 8)	1 mg/kg (n = 8)
Subjects achieving a ≥1.5 g/dL increase in hemoglobin, n (%)	0	6 (75)	8 (100)	8 (100)
Time to threshold, days, median (range)	—	70.0 (43–99)	39.5 (8–64)	15.0 (8–28)
No. of doses of sotatercept				
1 dose		0	2	8
2 doses		2	5	—
3 doses		3	1	—
4 doses		1	—	—

below baseline levels during the follow-up period. The mean levels of the bone resorption biomarker CTX decreased in a sustained manner after each dose of 0.3 and 1 mg/kg sotatercept, with mean percentage changes of −8.4% and −25.5%, respectively.

Sotatercept also dose-dependently increased BMD of the total hip from baseline to the end of study (i.e., 6, 5, and 4 months for sotatercept doses 0.1, 0.3, and 1 mg/kg, respectively). Most notable was a significant and rapid mean increase of 2.4% ($P = .006$) in subjects treated with 1 mg/kg sotatercept compared with a 0.7% decrease in the placebo group ($P < .01$). Mean BMD of lumbar spine increased slightly by 0.4–1.0% from baseline to the end of the study in all active treatment groups, compared with a 0.5% mean decrease in the placebo group.

Serum FSH. A dose-dependent decrease in mean FSH levels was observed after administration of sotatercept versus the placebo group, an expected pharmacodynamic effect of sotatercept consistent with activin inhibition. Mean FSH level decreases from baseline to day 15 were 2.0%, 19.3%, and 31.2% after the initial dose of 0.1, 0.3, and 1 mg/kg sotatercept, respectively, as compared with a 2.2% decrease in the placebo group. In all three sotatercept-treated groups, the mean FSH levels increased during the follow-up period to within the range observed in the placebo group at the end of the study (data not shown).

Safety

The nature, frequency, and severity of AEs observed in sotatercept-treated subjects were generally similar to those observed in placebo-treated subjects, with the exception of the erythropoietic effects described above. The most frequently reported AEs were increases in hemoglobin and hematocrit (Table 4). Headache was reported in all treatment groups; however, the incidence of headache diminished with higher sotatercept doses.

The majority of AEs were mild and did not require medical intervention. The rate of moderate AEs was low; most AEs were associated with the erythropoietic effects of sotatercept and primarily occurred in subjects treated with 1 mg/kg sotatercept. Mild-to-moderate increases in hemoglobin and/or hematocrit were reported as AEs in seven of the eight subjects in this group and all were considered related to the study drug.

Sotatercept was associated with dose-dependent effects on blood pressure that appear to be associated with the increases in hemoglobin levels. Blood pressure increased in all three sotatercept-treated groups in a dose-dependent fashion, with an onset approximately 2–3 days after each dose. Most of these increases in blood pressure were not associated with clinical signs or symptoms, although hypertension was reported as an AE in three sotatercept-treated subjects. Two of these subjects had a prior history of hypertension. Blood pressure generally returned to baseline levels by the end of the study in all three sotatercept-treated groups.

Two SAEs were reported, one of which was considered treatment-related and fulfilled the criteria for dose-limiting toxicity. One subject treated with 1 mg/kg sotatercept experienced moderate, persistent, and progressive hypertension, with blood pressure ranging from 143/113 to 180/120 mmHg at 1 week after the second dose of sotatercept. This SAE was attributed to a rapid and significant increase in hemoglobin levels (20.0 g/dL vs. 13.5 g/dL at baseline). The hypertension resolved after therapeutic phlebotomy and a short course of antihypertensive treatment, and this was considered to be a dose-limiting toxicity. The second SAE (a planned and elective total knee replacement) occurred in a subject treated with 0.3 mg/kg sotatercept and was considered unrelated to sotatercept. Two additional subjects were asymptomatic, but underwent preventative phlebotomy because of elevated hemoglobin levels.

There were no clinically significant changes from baseline in physical examinations, chemistry parameters, endocrine function, adrenal function, urinalysis, or electrocardiography data. No splenic enlargement was identified during physical examinations. All samples tested were negative for anti-ActRIIA antibodies.

Discussion

Results of the present study indicate that sotatercept, an ActRIIA ligand trap, achieves clinically relevant increases in hemoglobin, hematocrit, and RBC counts in healthy postmenopausal women in a dose-dependent manner. These results suggest that ActRIIA ligands are important negative regulators of erythrocyte production in humans under normal physiologic conditions. The erythrocytic effects of sotatercept observed in the present study occurred without detectable changes in white blood cell, or platelet counts. Sotatercept also rapidly increased BMD and biomarkers of bone formation without upregulating bone resorption, a characteristic of bone anabolic agents such as parathyroid hormone. On the basis of these results, sotatercept is a potential treatment for patients with impaired erythropoiesis, with or without concomitant bone loss.

The multiple-dosing sotatercept concentration profiles in serum were adequately described by a one-compartment model with first-order absorption and elimination, and the estimated clearance and half-life were independent of dose levels. This observation suggests that sotatercept is cleared from the body by the linear elimination pathway, which leads to a dose-proportional increase in the exposure to systemic sotatercept from 0.1 to 1 mg/kg. In addition, sotatercept has a prolonged half-life (approximately 23 days), allowing a less frequent dosing schedule and contributing to the sustained erythroposis response.

Table 4. Most Frequent Adverse Events[a]

	Treatment Group			
		Sotatercept		
Adverse Event	Placebo (n = 7)	0.1 mg/kg (n = 8)	0.3 mg/kg (n = 8)	1 mg/kg (n = 8)
Arthralgia	2 (28.6)	0	2 (25.0)	0
Asthenia	0	1 (12.5)	2 (25.0)	0
Decreased appetite	1 (14.3)	0	2 (25.0)	0
Diarrhea	0	2 (25.0)	0	0
Dizziness	0	0	2 (25.0)	2 (25.0)
Fatigue	2 (28.6)	0	1 (12.5)	2 (25.0)
Headache	2 (28.6)	4 (50.0)	3 (37.5)	2 (25.0)
Hematocrit increased	0	0	0	6 (75.0)
Hemoglobin increased	0	0	0	7 (87.5)
Hot flash	0	2 (25.0)	0	1 (12.5)
Limb injury	0	1 (12.5)	0	2 (25.0)
Muscle spasms	0	1 (12.5)	0	2 (25.0)
Oropharyngeal pain	2 (28.6)	0	0	0
Paresthesia	1 (14.3)	0	0	3 (37.5)
Red blood cell count increased	0	0	0	3 (37.5)
Viral upper respiratory tract infection	0	4 (50.0)	0	0

[a]All data are presented as no. of subjects experiencing an adverse event (%).

Although still under investigation, the mechanism underlying the erythropoietic effects of sotatercept are thought to differ from that of conventional ESAs. Sotatercept administration resulted in a rapid onset of its erythropoietic effects in a dose-dependent manner at the three dose levels tested. These kinetics suggests that sotatercept may be at least partly targeting late-stage RBC precursors downstream of the early stage proliferative effects of ESAs.[23] Furthermore, as more mature erythroblasts are released into circulation, a feedback mechanism is potentially activated to supply early progenitors by increasing EPO concentration, which might explain the paradoxical delayed increase in EPO after 1 month in the presence of an increase in hemoglobin.

Beyond the emerging safety concerns about ESAs, there is a significant need for agents to treat anemia for which conventional ESAs are not effective. In particular, ESAs have not been widely tested in thalassemia, or have shown only limited responses in this disease.[12] Based on our current knowledge, ESAs may offer only limited benefit in diseases characterized by ineffective erythropoiesis because endogenous EPO levels are typically elevated in such diseases and EPO-stimulated proliferation of erythroid precursors contributes to a vicious cycle that can eventually lead to extramedullary erythropoiesis, secondary bone pathologies, and splenomegaly.[24] Indeed, JAK2 inhibitors have been proposed as a therapeutic means to inhibit EPO-driven erythroid proliferation in beta-thalassemia major and beta-thalassemia intermedia.[25,26] Alternatively, an agent that promotes late-stage erythroid differentiation, as sotatercept appears to do in healthy individuals, could help to correct the ineffective erythropoiesis characteristic of thalassemias.

Although ESAs are not approved for the treatment of MDS by the United States Food and Drug Administration, the major treatment guidelines support the use of ESAs in some low-risk patients. High doses of ESAs, given alone or in combination with granulocyte colony-stimulating factor analogs, are widely used to treat some MDS patients with: normal cytogenetics; $<15\%$ ringed sideroblasts; and pretreatment serum EPO levels <500 U/L.[11] ESA therapy is seldom effective in patients with MDS and pretreatment serum EPO levels >500 U/L, and it is currently unclear if ESAs represent disease-modifying treatment for lower-risk MDS or if their use for MDS is associated with safety issues.[27] Thus, there is a need for additional agents that can treat anemia in patients with MDS.

Sotatercept is thought to exert its pharmacodynamic effects by inhibiting the signaling pathways mediated by one or more ActRIIA ligands.[28] For example, there are multiple isoforms of activin[29] that can bind to ActRIIA, a type 2 receptor, and trigger recruitment and activation of the corresponding type 1 receptors (activin receptor-like kinases) leading to SMAD protein activation and transcriptional regulation of their target genes.[30] When multiple ligands of the TGF-beta superfamily can activate the same receptor, signaling specificity is thought to arise from specific ligand expression patterns, receptor affinity, regulation by co-receptor proteins, and susceptibility to endogenous ligand traps. Inhibins are two protein isoforms that are closely related to activins. They function as important antagonists of activins by forming an inactive complex between inhibin, ActRIIA, and the co-receptor

beta-glycan.[31] The pharmacodynamic effects of sotatercept on FSH levels, bone, and erythropoiesis in the present study are consistent with the involvement of activin/ActRIIA signaling based on our current understanding. The rapid and dose-dependent reduction in serum FSH levels observed after administering sotatercept to postmenopausal women is consistent with the well-established role of activin in FSH secretion,[32] and resembles the FSH-inhibiting effect of inhibins. There is also compelling evidence that activins and inhibins exert opposing effects to regulate bone status in vivo,[33] and the anabolic effects of sotatercept on bone status observed in the present study resemble the predominantly osteoblastic (as opposed to antiresorptive) effects of continuous inhibin exposure on bone.

The physiologic roles of activins and ActRIIA signaling in adult erythropoiesis are not fully understood. Many studies have documented the erythropoietic effects of activin and inhibin in transformed cell lines or other in vitro models;[34–37] however, there is a paucity of data regarding the hematopoietic role of activin in vivo.[38,39] Intriguingly, elevated levels of phosphorylated SMAD2/3, a key transcriptional mediator of ActRIIA ligands, have been reported in hematopoietic progenitors from MDS patients.[40] This raises the possibility that excessive ActRIIA signaling contributes to ineffective erythropoiesis and anemia in this disease. Our current results suggest that ActRIIA ligands are important negative regulators of erythrocyte levels in healthy individuals.

In conclusion, we have shown that sotatercept achieves clinically relevant increases in hemoglobin, hematocrit, and RBC counts in healthy postmenopausal women. It will be important to determine if sotatercept can achieve these increases and provide a clinical benefit in patients with anemia and diseases of ineffective erythropoiesis. Clinical studies are now ongoing to further evaluate the effects of sotatercept on hematologic parameters in patients with chronic kidney disease and in other diseases of ineffective erythropoiesis including MDS and beta-thalassemia.

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