

Single-Dose, Randomized, Double-Blind, Placebo-Controlled Study of ACE-011 (ActRIIA-IgG1) in Postmenopausal Women*

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ABSTRACT: The effects of ACE-011 on safety, pharmacokinetics, and bone biomarkers were evaluated in healthy, postmenopausal women. Our data indicate that ACE-011 results in a sustained increase in biomarkers of bone formation and reduction in markers of bone resorption. The activin type IIA receptor (ActRIIA) is the high-affinity receptor for activin. ACE-011 is a dimeric fusion protein consisting of the extracellular domain of the human ActRIIA linked to the Fc portion of human IgG1. ACE-011 binds to activin, preventing activin from binding endogenous receptors. A randomized, double-blind, placebo-controlled study was conducted to evaluate the safety and tolerability of ACE-011. Forty-eight healthy, postmenopausal women were randomized to receive either a single dose of ACE-011 or placebo and were followed for 4 mo. Dose levels ranged from 0.01 to 3.0 mg/kg intravenously and from 0.03 to 0.1 mg/kg subcutaneously. Safety and pharmacokinetic (PK) analyses and the biological activity of ACE-011, as assessed by markers of bone turnover, and follicle stimulating hormone (FSH) levels were measured. No serious adverse events (AEs) were reported. AEs were generally mild and transient. The PK of ACE-011 was linear over the dose range studied, with a mean half-life of 24–32 days. The absorption after subcutaneous dosing was essentially complete. ACE-011 caused a rapid and sustained dose-dependent increase in serum levels of bone-specific alkaline phosphatase (BSALP) and a dose-dependent decrease in C-terminal type 1 collagen telopeptide (CTX) and TRACP-5b levels. There was also a dose-dependent decrease in serum FSH levels consistent with inhibition of activin. ACE-011 is a novel agent with biological evidence of both an increase in bone formation and a decrease in bone resorption. ACE-011 may be an effective therapy in a variety of diseases involving bone loss.

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INTRODUCTION

ACTIVIN A IS A member of the TGF- β family that was initially identified as a gonadally derived factor involved in modulating follicle-stimulating hormone (FSH) secretion from the pituitary.^(1,2) Activin A is also known as erythroid differentiation factor (EDF) and has effects on red blood cells in the later stages of maturation.⁽³⁾ Whereas activin A is one of the most abundant TGF- β member proteins found in bone, there have been conflicting reports of its physiological role in bone metabolism. Early studies have shown that activin has a role in the regulation of osteoblast proliferation and bone formation.^(4–7) Activin A has also been reported to promote osteoblastogenesis in bone marrow cultures⁽⁸⁾ while promoting an increase in BMD in rats.⁽⁹⁾ In contrast, other studies have shown that activin A inhibits mineralized nodule formation (an indi-

cator of osteoblast differentiation) in cultured fetal rat calvarial cells.⁽¹⁰⁾ It has also been shown that activin A enhances osteoclast activity and development.^(11–13) Importantly, the use of a soluble activin receptor IIA (ActRIIA) that blocks activin inhibits osteoclast formation in culture.⁽¹³⁾ Recently, the transgenic expression of inhibin, a natural antagonist of activin, was shown to increase BMD in mice.⁽¹⁴⁾ Taken together, these data suggest that inhibition of activin may be associated with an increase in osteoblastogenesis and a decrease in osteoclastic activity, thus stimulating new bone formation.

ActRIIA is a high-affinity type II receptor within the TGF- β superfamily that avidly binds to activin.⁽¹⁵⁾ ACE-011 (ActRIIA-IgG1) is a fully human glycosylated dimeric fusion protein consisting of two extracellular domains (ECDs) of ActRIIA linked to the human IgG1 Fc domain including the hinge, CH₂, and CH₃ domains. By binding activin, ACE-011 prevents activin from binding endogenous receptors and thus acts as a decoy.

Several pharmacologic studies have been conducted in mice to assess the potential clinical efficacy of ACE-011 by

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using a murine analog of ACE-011, termed RAP-011, consisting of the ECD of ActRIIA with murine IgG. RAP-011 increased trabecular BMD and bone volume in normal mice and restored BMD in a murine ovariectomized model of postmenopausal osteoporosis.⁽¹⁶⁾ In both sham-operated and ovariectomized mice, RAP-011 treatment increased bone strength as determined by increases in femoral bending strength and energy absorbed to failure.⁽¹⁶⁾ RAP-011 also significantly increased BMD in ovariectomized mice when used in combination with bisphosphonates such as zoledronic acid. For example, the effect of RAP-011 treatment in combination with bisphosphonate treatment on BMD was additive compared with the use of either RAP-011 or bisphosphonate treatment alone.⁽¹⁷⁾ In the 5T2MM murine model of multiple myeloma, RAP-011 completely prevented 5T2MM-induced decreases in trabecular number and volume in the tibia, femur, and vertebrae compared with vehicle-treated mice.⁽¹⁸⁾ Together, these results suggest that activin may have an important role in bone formation and resorption. Therefore, it was hypothesized that ACE-011 may have the potential to stimulate bone formation and restore bone quality through activin inhibition. This report describes the results from a single-dose, placebo-controlled study of ACE-011 to determine its safety and effects on biomarkers of bone formation and resorption in healthy postmenopausal women.

MATERIALS AND METHODS

Study design

This study was a randomized, double-blind, placebo-controlled, single-dose, dose-escalation study conducted at a single study center. The primary objective of the study was to assess the safety and tolerability of ACE-011 in healthy postmenopausal women. Secondary objectives included assessment of pharmacokinetics and bioavailability of intravenous and subcutaneous administration of ACE-011 and to determine the effect of ACE-011 on biomarkers of bone formation and resorption. A total of 48 healthy postmenopausal volunteers were assigned by a computer-generated randomization scheme to receive either ACE-011 or placebo. Each cohort consisted of five active and one placebo subjects. The study included a total of eight cohorts. Six cohorts were single escalating dose levels of ACE-011 administered intravenously (IV), and two cohorts were single escalating dose levels of ACE-011 administered subcutaneously (SC). ACE-011 was manufactured and provided by Acceleron Pharma. The study center pharmacist diluted ACE-011 in 0.9% normal saline for injection according to subject weight. The placebo was 0.9% normal saline for injection and was visually indistinguishable from ACE-011.

The ACE-011 dose levels tested were 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg IV, and 0.03 and 0.1 mg/kg SC and were based on nonclinical animal toxicology studies. Enrollment to subsequent IV or SC cohorts did not begin until available adverse event (AE), clinical laboratory, vital signs data, and ECG results from at least 1 wk after dosing had been reviewed, and tolerability was shown on a minimum

of four subjects in the previous cohort. Dose escalation was determined separately within each route of administration.

Subjects

Healthy, postmenopausal women between 45 and 80 yr of age were eligible if they had 12 mo of spontaneous amenorrhea or 6 mo of spontaneous amenorrhea with serum FSH levels >40 IU/liter or were at least 6 wk postsurgical bilateral oophorectomy with or without hysterectomy. Subjects with a history of any clinically significant cardiac, endocrinologic, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, renal, bone, or any other major disease were not enrolled in the study. Subjects were excluded if they had taken any drugs that may affect bone turnover, including estrogen, androgen, PTH, bisphosphonates, calcitonin, anabolic steroids, and selective estrogen receptor modulators within 6 mo of study entry or any other investigational drugs within 3 mo before dosing. Subjects were also excluded if they received fluoride therapy for >3 mo during the previous 2 yr before dosing or had received systemic glucocorticoid therapy for >1 mo within the year before dosing.

Study procedures

All subjects provided written informed consent before screening and enrollment in this study. Subjects were screened before enrollment to ensure inclusion and exclusion criteria were met. Eligible subjects were dosed on the morning of study day 1 and stayed at the clinical site for observation until the morning of study day 2 (24 h after dosing). Subjects were followed for safety for 4 mo after study drug administration.

Safety assessments including vital signs, physical examination, hematology, chemistry, electrocardiogram, urinalysis, and endocrine function testing (thyroid-stimulating hormone [TSH], free thyroxine [T₄], growth hormone [GH], adrenocorticotrophic hormone [ACTH]) were performed at baseline and at regular intervals throughout the 4-mo follow-up period. Adrenal function was assessed by intramuscular injection of 250 µg Cortrosyn (cosyntropin; Amphastar Pharmaceuticals) to measure change in cortisol levels to evaluate ACTH stimulation. Serum concentrations of ACE-011 were assessed at baseline, 0.5, 1, 2, 3, 4, 8, and 12 h, and 2, 3, 5, 8, 15, 29, 57, 85, and 120 days after dose administration. Antidrug antibody testing was performed using a bridging assay at baseline and monthly during the follow-up phase of the study. Serum concentrations of FSH were assessed as a readout for biological activity of ACE-011. Bone-specific alkaline phosphatase (BSALP), procollagen type I N-terminal propeptide (PINP), procollagen type I C terminal propeptide (PICP), and total osteocalcin were assessed regularly to look at changes in markers of bone formation, and serum C-terminal type I collagen telopeptide (CTX) and TRACP-5b were assessed at baseline and study days 3, 8, 15, 29, 57, 85, and 120 to look at changes in markers of bone resorption. Bone biomarkers were measured by immunoassay (Synarc, Lyon, France). BMD was assessed at certain time points in the highest intravenous dose levels tested. Total body and

lumbar spine areal BMD (g/cm^2) was assessed by DXA (QDR-4500W; Hologic, Bedford, MA, USA) in the highest IV dose group at baseline and 2 and 4 mo after dose administration. Adverse events and concomitant medications were assessed at all study visits after dosing. This study was conducted in accordance with the ICH guidelines and was approved by an Institutional Review Board.

Data analysis

The data from the placebo-treated subjects from all eight cohorts were treated as one group. Data from subjects who received ACE-011 were grouped by dose level and route of administration. Demographics and safety evaluations were analyzed using descriptive statistics. ACTH stimulation testing was evaluated using predetermined assessment criteria for normal adrenal function. Pharmacokinetic parameters were evaluated using descriptive statistics. Biochemical markers of bone formation and bone resorption were assessed using exploratory descriptive statistics, as well as mean percent change from baseline evaluations. The percent change from baseline of FSH and bone biomarkers by treatment group was analyzed using a single-group *t*-test. In addition, a two-group *t*-test was used to analyze the difference of percent change from baseline in FSH and bone biomarkers between treatment groups and the placebo group. 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.

RESULTS

Demographic and baseline characteristics

Table 1 summarizes the demographics and baseline characteristics of all subjects enrolled in this study. There were no notable differences among groups. The mean age ranged from 56 to 71 yr, and most ACE-011-treated subjects (88%) and all placebo subjects (100%) were postmenopausal using the 12 mo of spontaneous amenorrhea criteria. The majority of subjects were white (43% in ACE-011 group and 38% in placebo group) or Asian (43% in ACE-011 group and 38% in placebo group). Mean baseline subject weights for all cohorts ranged from 58.5 to 76.3 kg. For all cohorts, the mean baseline serum FSH ranged from 57.1 to 90.8 MIU/ml, serum BSALP from 9.1 to 15.8 ng/ml, serum PINP from 35.0 to 68.7 $\mu\text{g}/\text{liter}$, serum PICP from 64.8 to 137.4 $\mu\text{g}/\text{liter}$, serum osteocalcin from 13.7 to 24.2 ng/ml, serum CTX from 0.3 to 0.8 ng/ml, and serum TRACP-5b from 3.0 to 3.9 U/liter.

Serum FSH

After treatment, mean FSH values decreased in the ACE-011 groups compared with the placebo group. These changes were most notable between study days 8 and 15 (Table 2; Fig. 1). The greatest changes from baseline in FSH were observed in the 1.0- and 3.0-mg/kg IV groups. On day 8, FSH decreased 27.1% ($p \leq 0.01$) and 45.2% ($p \leq 0.0001$) versus baseline in the 1.0- and 3.0-mg/kg cohorts, respectively (Table 2). In comparison, FSH was unchanged

in the placebo group on day 8 (1.1% increase; $p > 0.05$). In addition, subjects receiving ACE-011 showed significant reductions from baseline in comparison with those receiving placebo at most time points ($p \leq 0.01$ at days 3, 8, 15, 29, and 57 in the 1.0-mg/kg IV group; $p \leq 0.01$ at days 3, 8, 15, 29, 57, and 85 in the 3.0-mg/kg IV group). In these two dose groups, the greatest change in placebo-corrected mean percent change from baseline in FSH was 39.1% and 50.1% for the 1.0- and 3.0-mg/kg IV groups at day 15, respectively. After maximum decreases at days 8–15, mean FSH values in the treated groups increased gradually over the course of the study. By day 120, FSH levels had returned to baseline for the 1.0-mg/kg group and approached baseline in the 3.0-mg/kg group (Fig. 1).

Biochemical markers of bone formation and bone resorption

After a single dose of ACE-011, there was a dose-dependent increase in biochemical markers of bone formation and a decrease in biochemical markers of bone resorption. ACE-011 caused a rapid, sustained, dose-dependent increase in serum levels of BSALP. In the 0.3-mg/kg IV group, BSALP increased up to day 29 and plateaued thereafter. In the 1.0-mg/kg IV group, BSALP values increased rapidly (14.8% increase by day 3; $p \leq 0.01$), peaked at day 57 (21.1% increase; $p \leq 0.05$), and returned to baseline by day 120. In the 3.0-mg/kg IV group, the mean percent change from baseline increase peaked at day 15, with an increase over baseline of 35.9% ($p \leq 0.01$; Table 3). Mean BSALP levels continued to be elevated (16.6% increase, $p \leq 0.05$; Table 3) above baseline at day 120 in this group. The mean percent change from baseline also peaked at day 15 in the 0.1-mg/kg SC group (11.4% increase; $p \leq 0.05$; Table 3) and returned to baseline by day 120. There were no significant changes in BSALP in the placebo group at any time point. When treatment groups were compared with the placebo group, the 1.0-mg/kg IV group showed a statistically significant increase of BSALP from baseline at days 3, 8, 15, and 29 ($p \leq 0.05$), and the 3.0-mg/kg group showed a statistically significant increase at days 8, 15, 29, and 57 ($p \leq 0.05$).

ACE-011 also caused an increase in PINP. Peak mean percent changes from baseline in PINP levels were observed in the highest IV and SC dose groups by day 15. The mean percent change from baseline PINP increased 10.8% in the 1.0-mg/kg IV cohort, 15.0% in the 3.0-mg/kg IV cohort, and 10.6% in the 0.1-mg/kg SC cohort compared with a 5.2% decrease in the placebo group by day 15. In comparison with the placebo group, the 3.0-mg/kg IV group also showed a statistically significant increase of PINP in the percent change from baseline of PINP at days 8 and 15 ($p \leq 0.05$). No dose-dependent statistically significant changes were seen in PICP or osteocalcin in any cohort throughout the course of the study (Table 3).

A decrease was noted in both CTX and TRACP-5b levels. On day 15, mean percent changes from baseline in CTX levels in the three highest IV dose groups were as follows: 12.4% decrease in the 0.3-mg/kg group, 18.8% decrease in the 1.0-mg/kg group, and 24.1% decrease in the

TABLE 1. DEMOGRAPHICS AND BASELINE CHARACTERISTICS OF THE STUDY POPULATION

	ACE-011 groups								Placebo
	0.01 mg/kg IV	0.03 mg/kg IV	0.1 mg/kg IV	0.3 mg/kg IV	1.0 mg/kg IV	3.0 mg/kg IV	0.03 mg/kg SC	0.1 mg/kg SC	
Subjects enrolled	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Age (yr)	63.8 (6.8)	63.8 (11.3)	68.2 (8.3)	65.0 (6.1)	61.2 (5.2)	55.8 (3.0)	70.6 (4.3)	62.0 (10.0)	58.8 (6.3)
Race (n, %)									
American Indian/Alaska Native	0	0	0	0	0	0	0	0	1 (13%)
Asian	2 (40%)	1 (20%)	3 (60%)	4 (80%)	2 (40%)	0	3 (60%)	2 (40%)	3 (38%)
Black	0	0	1 (20%)	0	0	0	0	0	0
Native Hawaiian /other Pacific Islander	0	0	0	0	0	2 (40%)	0	0	0
White	2 (40%)	3 (60%)	1 (20%)	1 (20%)	3 (60%)	3 (60%)	1 (20%)	3 (60%)	3 (38%)
Other	1 (20%)	1 (20%)	0	0	0	0	1 (20%)	0	1 (13%)
Postmenopausal status. (n, %)									
≥12 mo of spontaneous amenorrhea	5 (100%)	4 (80%)	4 (80%)	4 (80%)	5 (100%)	4 (80%)	4 (80%)	5 (100%)	8 (100%)
≥6 mo of spontaneous amenorrhea with serum FSH levels >40 IU/liter	0	1 (20%)	0	0	0	1 (20%)	0	0	0
≥6 wk postsurgical bilateral oophorectomy with or without hysterectomy	0	0	1 (20%)	1 (20%)	0	0	1 (20%)	0	0
Weight (kg)	58.5 (9.5)	68.0 (12.6)	60.5 (13.3)	64.6 (17.1)	64.1 (10.7)	76.3 (10.6)	65.3 (9.4)	63.0 (6.7)	61.8 (10.2)
Serum FSH (MIU/ml)	86.9 (23.3)	83.3 (17.9)	74.3 (25.5)	57.1 (12.8)	78.4 (17.8)	90.8 (10.5)	58.7 (9.0)	79.0 (22.4)	88.6 (25.8)
Serum BSALP (ng/ml)	15.0 (4.0)	15.0 (5.8)	15.8 (3.2)	11.8 (6.0)	12.9 (2.5)	10.6 (1.7)	9.1 (4.1)	12.6 (2.3)	15.7 (6.7)
Serum PINP (μg/liter)	50.0 (9.3)	68.7 (33.4)	47.6 (15.3)	35.0 (14.8)	46.4 (8.3)	55.1 (26.2)	42.3 (10.5)	54.6 (19.0)	57.4 (26.2)
Serum PICP (μg/liter)	95.9 (29.9)	113.3 (47.2)	72.5 (22.6)	64.8 (19.4)	89.6 (23.1)	93.55 (21.1)	83.9 (24.7)	137.4 (78.3)	95.2 (36.8)
Serum osteocalcin (ng/ml)	17.1 (3.7)	24.2 (16.0)	15.8 (4.4)	15.7 (5.3)	20.2 (11.1)	21.6 (14.4)	13.7 (4.2)	20.1 (5.0)	18.5 (7.8)
Serum CTX (ng/ml)	0.5 (0.2)	0.6 (0.4)	0.3 (0.1)	0.5 (0.1)	0.5 (0.2)	0.6 (0.4)	0.4 (0.1)	0.8 (0.3)	0.6 (0.2)
Serum TRACP-5b (U/liter)	3.6 (0.4)	3.9 (1.5)	3.5 (0.4)	3.4 (0.8)	3.2 (0.7)	3.4 (0.6)	3.4 (0.9)	3.0 (0.8)	3.3 (0.7)

Values are mean (SD) except for race and postmenopausal status where values are n and % of total n in cohort.

TABLE 2. FSH RESULTS AT DAY 8

	ACE-011								
	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	0.03 mg/kg	0.1 mg/kg	Placebo
	IV (n = 5)	SC (n = 5)	SC (n = 5)						
Mean FSH (MIU/ml)	90.2 (34.2)	81.0 (12.9)	67.6 (25.3)	48.8 (12.2)	57.0 (14.3)	49.9 (8.0)	57.9 (6.9)	67.0 (17.7)	90.0 (29.1)
Mean change from baseline (MIU/ml)	3.4 (12.3)	-2.3 (6.1)	-6.6 (4.6)	-8.3 (2.7)	-21.3 (7.8)	-40.9 (5.3)	-0.8 (5.5)	-12.1 (11.3)	1.4 (7.5)
Mean percent change from baseline (%)	2.1 (11.2)	-1.4 (9.6)	-10.6 (9.6)	-15.0 (5.3)*	-27.1 (8.0)*	-45.2 (4.2)†	-0.5 (10.3)	-14.0 (10.8)‡	1.1 (9.0)

Values are mean (SD).

* $p \leq 0.01$.

† $p \leq 0.001$.

‡ $p \leq 0.05$.

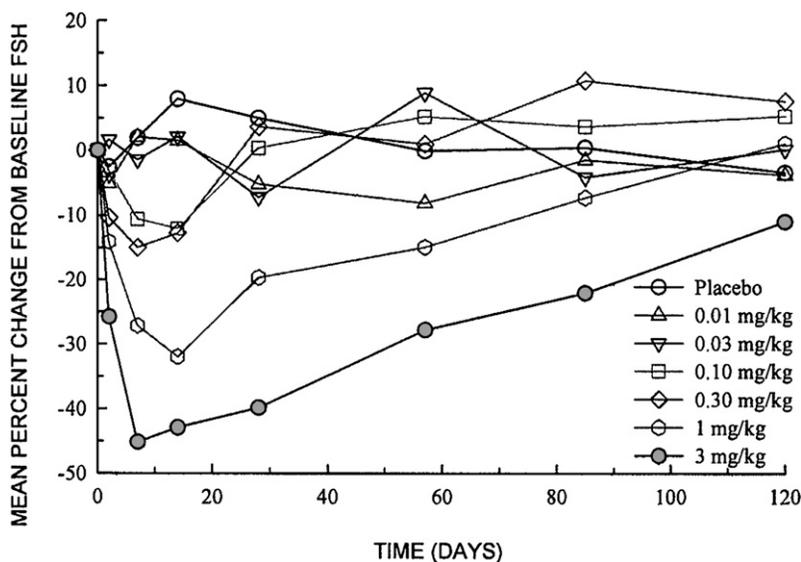


FIG. 1. Mean percent change from baseline in serum concentrations of FSH after intravenous administration of single doses of ACE-011 to healthy postmenopausal female volunteers. Mean data from IV cohorts are expressed over time. Dosing occurred on day 1. There were five active subjects in each cohort, except in the 0.01-mg/kg cohort after day 29, in which there were four active subjects, and six subjects in the placebo IV group.

3.0-mg/kg group. In the 0.1-mg/kg SC cohort, the mean percent change from baseline in CTX levels on day 15 was an 11.2% decrease ($p \leq 0.05$; Table 4). The maximum mean percent change from baseline CTX in the 3.0-mg/kg cohort was a 30.6% decrease by day 8 (Table 4). In comparison, the mean change from baseline in the placebo group was a 1.1% increase on day 8 and a 2.6% increase on day 15. Although subjects were asked to be fasting, serum samples were obtained as outpatients. CTX levels are highly variable based on fed versus fasting state. Some of the variability of the assay may be caused by testing in a fed state.⁽¹⁹⁾

On day 29, mean percent change from baseline in TRACP-5b levels in the three highest IV dose groups were as follows: 0.9% decrease (0.3 mg/kg), 3.5% decrease (1.0 mg/kg), and 6.2% decrease (3.0 mg/kg). In comparison, the mean percent change from baseline in the placebo group was a 0.6% decrease on day 29 (Table 4).

An exploratory assessment of total body and lumbar spine areal BMD (g/cm^2) was assessed by DXA in the highest IV dose group at baseline and 2 and 4 mo after dose

administration. There were no statistically significant changes observed in this small subset of subjects.

Safety and tolerability

There were no serious adverse events (AEs), and no AEs leading to early termination of this study. Treatment-emergent AEs occurring in more than one subject in any dose level included headache, infusion site reaction, injection site hemorrhage, and toothache. Five of the six reported infusion site reaction or injection site hemorrhage AEs were related to infiltration of the IV site and was likely caused by both IV pump malfunctions and lack of IV nurse specialists on that particular day of infusions. There were no injection site reactions reported after SC administration. There were no clinical signs or symptoms of tissue toxicity at the infiltration sites. Toothache was reported by four subjects in this study, and all of the events were judged to be unlikely or unrelated to ACE-011. Two of the subjects reported a history of toothache before enrollment, and in three of the subjects, toothache resolved within a few days

TABLE 3. PERCENT CHANGE FROM BASELINE IN BONE FORMATION MARKERS

Time point (after dose)	ACE-011 groups								Placebo
	0.01 mg/kg IV	0.03 mg/kg IV	0.1 mg/kg IV	0.3 mg/kg IV	1.0 mg/kg IV	3.0 mg/kg IV	0.03 mg/kg SC	0.1 mg/kg SC	
Serum BSALP	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Day 3	0.7 (7.8)	4.4 (7.1)	7.7 (7.5)	10.1 (7.0)*	14.8 (5.2) [†]	8.1 (3.5) [†]	-1.0 (3.9)	7.2 (9.2)	0.0 (12.0)
Day 8	2.4 (9.8)	0.3 (8.5)	10.2 (3.4) [†]	12.7 (7.2)*	16.2 (6.2) [†]	16.7 (7.5) [†]	1.3 (8.8)	7.6 (6.8)	-1.1 (12.6)
Day 15	3.4 (14.1)	0.6 (15.2)	5.8 (13.0)	14.6 (10.2)*	19.0 (7.2) [†]	35.9 (14.7) [†]	-1.6 (5.8)	11.4 (6.7)*	-0.3 (11.2)
Day 29	-8.0 (11.4)	-5.4 (9.0)	-6.8 (11.2)	14.7 (12.6)	20.7 (12.3)*	22.6 (8.2) [†]	-2.5 (3.9)	11.3 (10.3)	0.4 (8.2)
Day 57	-2.2 (14.3)	-2.6 (9.8)	0.5 (8.9)	11.5 (15.2)	21.1 (15.2)*	30.5 (16.5) [†]	-7.0 (5.0)*	9.4 (9.8)	6.1 (15.0)
Day 85	-9.8 (9.6)	-9.3 (20.8)	-0.6 (12.8)	12.1 (16.8)	17.0 (8.6) [†]	20.0 (15.6)*	0.3 (4.1)	4.9 (7.3)	7.4 (16.0)
Day 120	-10.6 (25.6)	-6.6 (15.3)	-6.3 (9.9)	12.5 (23.4)	7.0 (7.7)	16.6 (9.9)*	-4.8 (5.4)	-6.1 (8.1)	7.6 (14.8)
Serum PINP	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Day 3	6.3 (12.0)	1.4 (7.2)	-4.8 (2.9)*	3.5 (17.2)	2.3 (12.4)	-0.3 (14.7)	-0.2 (11.4)	1.2 (9.2)	-3.1 (10.9)
Day 8	2.9 (17.9)	-11.9 (6.2) [†]	-7.9 (6.6)	5.3 (13.2)	5.6 (18.3)	10.1 (5.3) [†]	-1.0 (9.2)	-4.8 (13.9)	-2.6 (11.7)
Day 15	3.1 (16.3)	-6.8 (3.6) [†]	-14.2 (7.5) [†]	-1.2 (20.5)	10.8 (15.9)	15.0 (9.6)	-0.3 (13.1)	10.6 (20.8)	-5.2 (14.4)
Day 29	1.0 (15.7)	-8.2 (8.7)	-5.4 (11.2)	3.1 (24.3)	11.2 (24.0)	6.4 (16.2)	-0.4 (15.9)	6.4 (11.9)	7.5 (12.7)
Day 57	1.0 (21.4)	-14.0 (9.6)*	-16.9 (7.8) [†]	0.9 (35.4)	10.7 (23.7)	-15.7 (16.2)	-12.1 (12.7)	-7.7 (9.6)	0.2 (18.7)
Day 85	-2.2 (22.5)	-14.2 (20.0)	-18.6 (16.1)	16.6 (50.5)	-16.9 (14.9)	-19.2 (19.4)	-8.7 (11.5)	-14.3 (16.3)	-1.3 (18.9)
Day 120	-2.4 (22.0)	-20.7 (5.1) [†]	-13.8 (18.0)	21.3 (55.6)	11.5 (71.4)	-8.1 (21.1)	-2.7 (16.3)	9.9 (17.5)	2.0 (20.1)
Serum PICP	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Day 3	2.7 (17.3)	4.3 (5.6)	0.4 (13.1)	-4.9 (17.1)	-4.8 (16.7)	-8.4 (19.7)	7.9 (26.7)	-8.3 (17.7)	3.5 (19.1)
Day 8	-7.4 (26.2)	0.4 (16)	2.3 (12.3)	0 (13.6)	-12.6 (14.3)	3.4 (16.1)	13.5 (21.8)	-8.5 (9.1)	-0.7 (14.1)
Day 15	4.7 (22.7)	7.8 (25.1)	-4.4 (16.2)	-3.2 (28.2)	2.4 (10.4)	8.0 (27.1)	7.7 (31.3)	-5.2 (15.1)	-0.5 (14.5)
Day 29	7.2 (16.0)	4.5 (8.7)	21.5 (27.0)	3.3 (27.5)	8.4 (18.2)	3.4 (29.2)	4.2 (25.1)	3.8 (12.6)	-2.5 (19.3)
Day 57	19.9 (34.2)	0.7 (11.8)	4.6 (13.8)	9.0 (28.9)	18.6 (14.6)*	3.1 (22.3)	2.3 (15.4)	-6.0 (26.8)	5.1 (11.0)
Day 85	28.1 (44.1)	-12.1 (21.0)	-5.1 (16.3)	10.2 (23.8)	3.0 (21.7)	0.0 (25.3)	3.8 (29.3)	-1.0 (22.3)	-3.9 (17.7)
Day 120	2.7 (22.6)	-12.2 (16.1)	3.3 (32.6)	3.8 (20.0)	10.5 (22.7)	3.0 (26.9)	13.0 (31.2)	-12.7 (33.0)	-5.6 (15.0)
Serum OC	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Day 3	-3.4 (14.9)	-2.8 (8.2)	1.7 (7.2)	-0.4 (6.3)	-7.3 (10.3)	-14.7 (10.8)	2.3 (4.7)	11.9 (11.5)	-4.4 (5.4)
Day 8	6.4 (21.6)	3.8 (4.8)	12.2 (7.6)	10.1 (11)	3.1 (8.9)	1.1 (10.7)	11 (9.5)	14.5 (15.5)	3.6 (7.8)
Day 15	4.4 (24.9)	1.3 (4)	2.9 (10.9)	6.3 (10.9)	4 (10.1)	6.5 (2.7)	10.7 (14.1)	14 (17.9)	-0.1 (11.5)
Day 29	12.6 (18.8)	8.5 (18.2)	1.6 (14.1)	12.7 (6.1)	-0.2 (11.9)	11.6 (9.6)	14.9 (11.7)	21.4 (12.8)	3.9 (10.5)
Day 57	7.1 (10.5)	-8.4 (15.5)	5.8 (5.2)	10.3 (20.6)	4.2 (21.9)	14.5 (18.5)	9.5 (8.1)	9 (16.6)	7.2 (12.1)
Day 85	-3.8 (12.8)	-7.2 (10)	-8.7 (5.8)	8 (17.7)	-1.2 (13)	0.6 (21.1)	10.4 (13.3)	13.7 (14.2)	1.4 (10.5)
Day 120	-0.3 (29.1)	-6.7 (6.5)	5.2 (18.7)	19.8 (29)	-6.7 (8.4)	4.2 (32.3)	13.1 (16.7)	19.4 (26.3)	7.7 (16.1)

Values are mean (SD). In the 0.01-mg/kg cohort, $n = 4$ at days 57–120.

* $p \leq 0.05$.

[†] $p \leq 0.01$.

of onset. The majority of treatment-emergent AEs were mild in severity and were judged to be unrelated to the study drug (i.e., no relationship or unlikely relationship). Some hematology results (increases in red blood cells [RBCs], hemoglobin, hematocrit, reticulocytes) were consistent with a dose-dependent increase in blood volume. The maximum absolute change and percent change from baseline for each cohort are presented in Table 5. None of the hematology results were reported as AEs. There were some transient changes in liver function tests (LFTs) that were reported as AEs unlikely related to administration of ACE-011. The most notable changes in LFTs were observed in one subject in the 3.0-mg/kg cohort. In this subject, there were increases in alanine aminotransferase (ALT; 143 U/liter), aspartate aminotransferase (AST; 120 U/liter), and gamma glutamyl transferase (GGT; 118 U/liter) on day 15, which resolved back within the normal range by day 29. Changes in liver function tests that were above the normal ranges were also noted in the placebo group. There were also some transient changes in glucose, uric acid, amylase, and lipase levels in some subjects in

both the ACE-011 and placebo groups. There were no clinically significant changes from baseline in vital signs, physical examination, endocrine function, or ECG data. Testing for anti-ACE-011 antibodies was performed on a monthly basis to the end of the study at day 120, and all samples were reported negative for a specific antibody response.

Pharmacokinetics

Serum concentrations for ACE-011 increased in a dose-related manner and decayed at essentially the same rate for the 0.1- through 3.0-mg/kg doses after IV administration. Mean values for C_{max} , AUC(0-t), and AUC(inf) also increased in a dose-related manner. Over the range of 0.1–3.0 mg/kg, the mean clearance (CL) ranged from 0.092 to 0.128 ml/h/kg, the mean volume of distribution (V_z) ranged from 73.7 to 110 ml/kg, and the mean $t_{1/2}$ ranged from 23.7 to 31.8 days with no apparent dependence on any of these three parameters on dose. The pharmacokinetics of ACE-011 seemed to be linear after IV administration of single doses ranging from 0.01 to 3.0 mg/kg.

TABLE 4. PERCENT CHANGE FROM BASELINE IN BONE RESORPTION MARKERS

Time point (after dose)	0.01 mg/kg IV	0.03 mg/kg IV	0.1 mg/kg IV	0.3 mg/kg IV	1.0 mg/kg IV	3.0 mg/kg IV	0.03 mg/kg SC	0.1 mg/kg SC	Placebo
Serum CTX	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Day 3	2.9 (31.8)	-3.9 (15.2)	13.3 (19.6)	1.7 (12.8)	-19.4 (9.9)*	-5.8 (15.9)	-5.4 (14.6)	5.3 (9.3)	12.0 (17.9)
Day 8	12.4 (29.8)	-4.5 (14.7)	-11.1 (17.9)	4.8 (18.5)	-1.3 (11.6)	-30.6 (27.2)	-13.8 (29.0)	-7.0 (9.4)	1.1 (12.6)
Day 15	3.4 (22.4)	-9.0 (20.5)	2.9 (15.1)	-12.4 (16.7)	-18.8 (19.4)	-24.1 (27.0)	-10.0 (15.5)	-11.2 (6.8) [†]	2.6 (23)
Day 29	10.1 (29.0)	-6.7 (23.7)	3.3 (29.9)	11.4 (31.3)	-28.7 (15.2)*	-7.4 (38.7)	1.8 (30.0)	-7.3 (4.9) [†]	6.9 (19.4)
Day 57	12.6 (37.8)	-7.1 (13.1)	-2.7 (18.1)	11.3 (34.3)	-23.7 (13.6) [†]	16.0 (35.0)	-2.5 (40.3)	-1.2 (10.3)	8.9 (18.3)
Day 85	-12.4 (34.6)	-18.8 (23.2)	4.6 (49.9)	12.0 (32.4)	-7.1 (21.1)	14.7 (40.8)	2.5 (32.8)	0.9 (28.0)	15.7 (44.2)
Day 120	16.0 (16.9)	9.3 (7.8)	8.7 (43.0)	30.6 (25.4) [†]	20.4 (82.1)	22.3 (54.1)	11.3 (31.9)	-16.1 (19.4)	33.0 (66.9)
Serum TRACP-5b	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Day 3	6.7 (3.9) [†]	1.7 (5.0)	4.5 (5.8)	6.4 (4.1) [†]	12.2 (4.1)*	6.5 (1.4)*	2.3 (7.7)	0.0 (3.9)	5.5 (3.0)*
Day 8	5.7 (8.2)	3.5 (6.5)	-2.5 (5.5)	2.4 (5.2)	5.8 (4.3) [†]	1.1 (3.5)	3.8 (4.9)	5.3 (11.1)	4.5 (5.0) [†]
Day 15	2.2 (9.2)	-0.5 (15.6)	-4.6 (9.5)	0.3 (9.0)	7.1 (5.2) [†]	5.6 (8.6)	3.2 (1.6)*	-0.6 (3.0)	6.4 (3.4)*
Day 29	-6.5 (8.5)	-0.5 (8.9)	-6.4 (3.0)*	-0.9 (7.6)	-3.5 (5.5)	-6.2 (7.2)	6.2 (10.2)	-3.0 (10.4)	-0.6 (5.0)
Day 57	-0.8 (10.6)	-1.5 (8.8)	-8.7 (5.1) [†]	4.1 (8.8)	-1.6 (7.0)	3.5 (12.3)	1.9 (8.1)	-1.5 (8.0)	1.4 (4.8)
Day 85	-15.5 (7.5) [†]	-4.5 (6.0)	-8.0 (4.8) [†]	4.4 (15.0)	0.2 (10.0)	1.4 (14.5)	3.8 (6.1)	-4.1 (19.1)	2.2 (10.3)
Day 120	-15.4 (14.2)	-6.4 (10.6)	-9.6 (2.6)*	0.9 (13.3)	-1.5 (8.2)	-0.7 (12.3)	2.1 (9.5)	21.0 (23.1)	2.8 (23.2)

Values are mean (SD). In the 0.01-mg/kg cohort, $n = 4$ at days 57-120.

* $p \leq 0.01$.

[†] $p \leq 0.05$.

TABLE 5. MAXIMUM CHANGE AND MAXIMUM PERCENT CHANGE FROM BASELINE OF HEMATOLOGIC PARAMETERS

	0.01 mg/kg IV	0.03 mg/kg IV	0.1 mg/kg IV	0.3 mg/kg IV	1 mg/kg IV	3 mg/kg IV	0.03 mg/kg SC	0.1 mg/kg SC	Placebo
Hematocrit (%)	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Baseline	41.2 (2.4)	40.3 (2.6)	40.3 (3.4)	39.9 (1.1)	38.5 (1.8)	40.9 (2.4)	37.5 (2.3)	37.0 (1.8)	40.7 (1.2)
Max change	1.4 (1.1)	1.0 (2.4)	3.2 (2.3)	5.1 (1.7)	6.6 (1.9)	7.5 (2.3)	2.2 (1.7)	2.6 (0.9)	1.8 (0.9)
Max % change	3.4 (2.8)	2.7 (5.9)	8.2 (6.6)	12.8 (4.3)	17.1 (4.4)	18.3 (5.7)	6.0 (4.8)	7.1 (2.1)	4.4 (2.3)
Hemoglobin (g/dl)	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Baseline	13.7 (0.8)	13.8 (0.9)	13.6 (1.1)	13.5 (0.4)	13.3 (0.7)	13.7 (0.6)	12.8 (0.9)	12.6 (0.5)	13.7 (0.5)
Max change	0.4 (0.5)	0.3 (0.9)	1.0 (0.7)	1.7 (0.6)	1.7 (0.6)	2.4 (0.7)	0.7 (0.6)	0.8 (0.4)	0.4 (0.3)
Max % change	3 (3.5)	2.1 (6.3)	7.4 (6.0)	12.6 (4.1)	12.5 (4.0)	17.6 (5.0)	5.6 (4.6)	6.3 (2.9)	3.2 (2.3)
Red blood cell (10^{12} /liter)	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 5	N = 8
Baseline	4.7 (0.1)	4.4 (0.4)	4.4 (0.5)	4.3 (0.1)	4.3 (0.3)	4.7 (0.4)	4.0 (0.2)	4.0 (0.2)	4.4 (0.2)
Max change	0.2 (0.1)	0.1 (0.3)	0.4 (0.2)	0.6 (0.2)	0.8 (0.4)	0.9 (0.2)	0.3 (0.2)	0.3 (0.1)	0.2 (0.1)
Max % change	3.2 (2.8)	2.9 (5.8)	8.5 (6.6)	13.4 (4.1)	18.9 (8.4)	18.6 (4.6)	6.5 (5.1)	8.2 (2.4)	4.5 (2.8)
Reticulocyte (%)					N = 5	N = 5			N = 2
Baseline					1.3 (0.4)	1.0 (0.5)			1.5 (0.1)
Max change					1.5 (0.5)	1.4 (0.3)			0.7 (0.6)
Max % change					128.2 (73.0)	176.7 (90.3)			47.4 (36.7)

Values are mean (SD). In the 0.01-mg/kg cohort, $n = 4$ at days 57-120. Percent reticulocyte was collected in the 1- and 3-mg/kg IV cohorts only.

As shown in Fig. 2, a log-log plot of the mean AUC(inf) versus dose is linear with a slope essentially equal to 1, showing a dose-proportional increase in AUC with dose (i.e., linear pharmacokinetics). In addition to the six dose levels ranging from 0.01 to 3.0 mg/kg administered intravenously, a limited range (0.03 and 0.1 mg/kg) of ACE-011 administered subcutaneously was tested. Consistent with the complete absorption after subcutaneous administration, AUC values for the 0.03- and 0.1-mg/kg SC doses are superimposable with those from the same doses given intravenously. The SC results were similar for the corresponding

IV dose levels showing a linear relationship, and a mean $t_{1/2}$ of ~30 days, with no apparent dependence on dose.

DISCUSSION

This study showed that a single dose of ACE-011 induces a rapid and sustained increase in BSALP, a marker of bone formation, whereas not inducing and possibly decreasing markers of bone resorption in healthy postmenopausal women. The rapid, sustained increase in serum levels of BSALP and decrease in serum levels of CTX are consistent

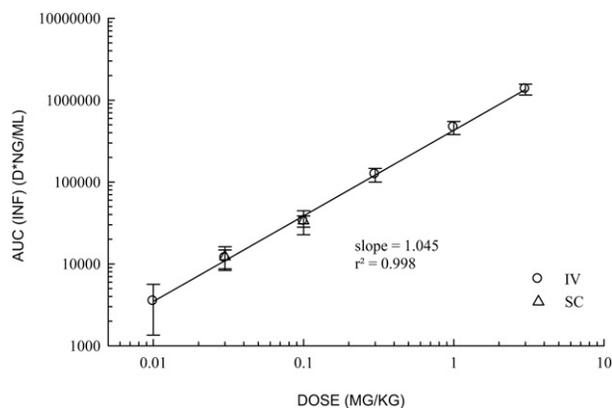


FIG. 2. Relationship between mean \pm SD AUC(inf) and dose after intravenous and subcutaneous administration of single doses of ACE-011 to healthy postmenopausal female volunteers. Mean data from IV and SC cohorts are expressed over time. Dosing occurred on day 1. There were five active subjects in each cohort, except in the 0.01-mg/kg IV cohort after day 29, in which there were four active subjects.

with observations of a primarily anabolic mechanism underlying increased bone mass and strength observed in preclinical studies. If sustained, this decoupling of the bone resorption and bone formation process has the potential for a large net increase in bone mass and strength. The effects of ACE-011 are reversible, because biomarker levels return to baseline at 4 mo of follow-up, when ACE-011 has cleared from circulation. The PK and pharmacodynamic (PD) data showed similar results for both the IV and SC routes of administration, which supports the convenience of SC dosing in future studies.

There were no significant safety concerns noted in this study. Activin is associated with erythroid differentiation and has been shown to have effects on RBCs in the later stages of maturation. Between days 15 and 85, ACE-011 caused a transient increase in the number of RBCs, reticulocytes, hematocrit, and hemoglobin, which may be associated with activin inhibition. There were no AEs related to hematologic changes, and these changes could be beneficial to some patients such as cancer patients with impaired hematopoiesis. FSH values decreased significantly after administration of ACE-011; however, because this study was performed in postmenopausal women, the changes observed in FSH values were not clinically significant and did not result in any AEs. The decreases observed in FSH values are consistent with activin inhibition and show a PD effect of ACE-011. Although low FSH levels may affect the reproductive status of premenopausal women, the consequences of long-term inhibition of FSH in postmenopausal women are unknown.⁽²⁰⁾ Recent data have suggested that high FSH levels may be associated with postmenopausal bone loss.⁽²¹⁾

A potential risk of ACE-011 is the development of anti-drug antibodies, which in this case could cross-react with the endogenous ActRIIA receptor, causing neutralization of its activity. There was no evidence of specific anti-ACE-011 antibody or neutralizing antibody formation in this study.

Generally two classes of agents have been approved for decreasing fracture risk: antiresorptive agents and anabolic agents.⁽²²⁾ Antiresorptive agents including bisphosphonates, estrogen, calcitonin, and selective estrogen receptor modulators (SERMs) are useful clinically to decrease bone resorption, but all of these agents have limitations. Whereas bisphosphonates cause a marked and sustained decrease in markers of bone resorption, they also cause a corresponding decrease in markers of bone formation.^(23,24) Oral bisphosphonates have been associated with gastrointestinal side effects, particularly esophagitis.^(25,26) Bisphosphonate use, particularly after IV administration in cancer patients, is associated with osteonecrosis of the jaw.^(27–29) Estrogen therapy has been associated with increased risk of endometrial cancer and breast cancer and is also often not well tolerated by patients.^(30,31) Calcitonin and SERMs have only a moderate effect on bone resorption.^(32,33) Teriparatide [PTH(1–34)] is the only approved anabolic agent for osteoporosis and also has several limitations.⁽³⁴⁾ Teriparatide increases bone formation markers but also increases bone resorption markers.^(35,36) A main limitation for patients is the requirement to be treated by daily subcutaneous injection. Teriparatide is limited to 18–24 mo of treatment because of an increased incidence of osteosarcoma in rodents exposed to high doses. Moreover, teriparatide is contraindicated in cancer patients.⁽³⁷⁾

The preclinical and clinical results for ACE-011 show a distinct profile from existing therapies to treat bone loss. The changes described above on biomarkers of bone formation and bone resorption show that ACE-011 may decouple bone formation and bone resorption processes after a single dose. These effects on bone biomarkers are distinct from those observed with both currently available antiresorptive and anabolic agents. ACE-011 may have the potential to treat a variety of diseases involving a loss in bone mass and strength. The data generated thus far with ACE-011 support further research and development of this promising compound.

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