ORIGINAL ARTICLE

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Modeling-Based Bone Formation After 2 Months of Romosozumab Treatment: Results From the FRAME Clinical Trial

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ABSTRACT

The bone-forming agent romosozumab is a monoclonal antibody that inhibits sclerostin, leading to increased bone formation and decreased resorption. The highest levels of bone formation markers in human patients are observed in the first 2 months of treatment. Histomorphometric analysis of bone biopsies from the phase 3 FRAME trial (NCT01575834) showed an early significant increase in bone formation with concomitant decreased resorption. Preclinical studies demonstrated that most new bone formation after romosozumab treatment was modeling-based bone formation (MBBF). Here we analyzed bone biopsies from FRAME to assess the effect of 2 months of romosozumab versus placebo on the surface extent of MBBF and remodeling-based bone formation (RBBF). In FRAME, postmenopausal women aged ≥55 years with osteoporosis were randomized 1:1 to 210 mg romosozumab or placebo sc every month for 12 months, followed by 60 mg denosumab sc every 6 months for 12 months. Participants in the bone biopsy substudy received quadruple tetracycline labeling and underwent transiliac biopsies at month 2. A total of 29 biopsies were suitable for histomorphometry. Using fluorescence microscopy, bone formation at cancellous, endocortical, and periosteal envelopes was classified based on the appearance of underlying cement lines as modeling (smooth) or remodeling (scalloped). Data were compared using the Wilcoxon rank-sum test, without multiplicity adjustment. After 2 months, the median percentage of MBBF referent to the total bone surface was significantly increased with romosozumab versus placebo on cancellous (18.0% versus 3.8%; $p = 0.005$) and endocortical (36.7% versus 3.0%; $p = 0.001$), but not on periosteal (5.0% versus 2.0%; $p = 0.37$) surfaces, with no significant difference in the surface extent of RBBF on all three bone surfaces. These data show that stimulation of bone formation in the first 2 months of romosozumab treatment in postmenopausal women with osteoporosis is predominately due to increased MBBF on endocortical and cancellous surfaces. © 2021 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: BONE HISTOMORPHOMETRY; BONE MODELING AND REMODELING; OSTEOPOROSIS; THERAPEUTICS; WNT/β-CATENIN/LRPS CELL/TISSUE SIGNALING

Introduction

Romosozumab is a humanized monoclonal antibody that binds to sclerostin, an extracellular Wnt inhibitor that prevents the binding of Wnt ligands to low-density lipoprotein receptor-related protein (LRP) 5 and 6 and the activation of the canonical Wnt signaling pathway.[1] The inhibition of sclerostin thereby results in activation of canonical Wnt signaling, which has a dual effect on bone, increasing bone formation and decreasing bone resorption. In phase 2 and phase 3 clinical trials, bone formation markers are highest in the first 2 months of treatment and return to baseline levels over 12 months of treatment.[2,3] In contrast, bone resorption markers are decreased and sustained across 12 months of treatment. Histomorphometric analyses of bone biopsies obtained from a substudy of the FRAME trial (NCT01575834) at month 2 and month 12 of treatment are

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consistent with the temporal changes in bone biomarkers, with bone formation parameters significantly increased at month 2 and resorption parameters decreased at months 2 and 12 on cancellous and endocortical surfaces.[4]

Studies in rats and monkeys have demonstrated the same temporal response in bone formation to romosozumab, with maximal bone formation occurring early in the treatment followed by progressive attenuation.[5,6] An analysis of active bone-forming surfaces in the cancellous and endocortical compartments as either modeling or remodeling (based on the characteristics of the underlying cement lines) early in the course of romosozumab treatment demonstrated that the increased bone formation was primarily modeling-based.[7,8] Studies in rats have demonstrated that a modeling-based bone formation (MBBF) response to sclerostin antibody is initiated by activation of bone-lining cells on quiescent bone surfaces followed by proliferation of osteoprogenitors and recruitment to the bone surface to sustain the life span of the modeling-based formation surface.[9-11]

To determine if the early increase in bone formation in response to romosozumab in postmenopausal women is largely modeling-based as demonstrated in nonclinical studies, bone biopsies obtained at month 2 from the FRAME biopsy substudy were analyzed to evaluate the extent of MBBF and remodeling-based bone formation (RBBF) on cancellous, endocortical, and periosteal surfaces.

Materials and Methods

Details of the design of the FRAME trial and bone biopsy substudy have been previously described and are outlined in Fig. 1.[2,4] Briefly, from 7180 participants (women aged ≥55 years with postmenopausal osteoporosis), 7.5-mm-diameter transiliac biopsies from 14 placebo and 15 romosozumab-treated patients were analyzed after 2 months of treatment. Quadruple fluorochrome labeling was performed as described by Chavassieux and colleagues.[4] Only the second set of tetracycline labels administered just before biopsy at month 2 was used in the analysis of MBBF and RBBF. Biopsies were processed as previously described.[4] From the biopsy blocks, unstained 10-μm sections were collected from three section levels separated by 150 μm (Charles River Laboratories Montreal ULC, Senneville, Canada) and used in the analyses. Active bone-forming surfaces were measured and identified by single- and double-tetracycline labels (total labeled surface) by fluorescence microscopy. Using an ocular linear test system randomly rotated between fields of view for unbiased sampling of bone surfaces, the underlying cement line was classified as smooth (signifying bone modeling) or scalloped (signifying bone remodeling) at each line intersection of the ocular sampling grid with either single- or double-tetracycline labels. Analysis was performed at 200× magnification. Intercept classification was recorded on cancellous, endocortical, and periosteal surfaces of the iliac biopsies (Fig. 2). Endocortical surfaces were defined as surfaces on the inner cortical surface and surfaces within one trabecular width from the inner cortical surface.

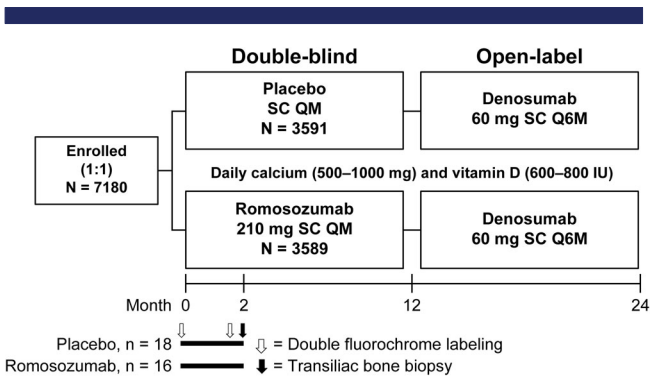

Fig 1. FRAME bone biopsy substudy design. Patients in the bone biopsy substudy underwent transiliac bone biopsies at month 2 ($n = 34$) and received quadruple labeling (double labeling at baseline and before biopsy). Twenty-nine biopsies of 34 obtained biopsies were suitable for histomorphometric analysis (placebo, $n = 14$; romosozumab, $n = 15$). Five biopsies of the 34 obtained biopsies were excluded due to reasons including crush artifact or insufficient cancellous bone surface. Q6M = every 6 months; QM = every month.

Statistical analysis

Data were expressed as median (quartiles 1 and 3). The effect of 2 months of romosozumab versus placebo on MBBF and RBBF at each envelope referent to bone surface and labeled surface was compared using the Wilcoxon rank-sum test, without multiplicity adjustment.

Results

Baseline demographics for patients in FRAME and in the FRAME bone biopsy substudy are shown in Table 1. Baseline

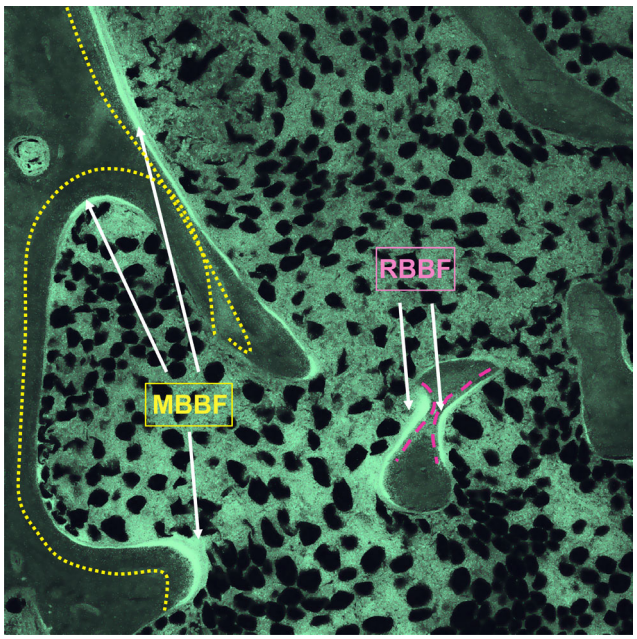

Fig 2. Confocal transiliac bone biopsy image from romosozumab-treated patient. Confocal microscope image of transiliac bone biopsy (original magnification 200×) from a romosozumab-treated patient showing modeling-based bone formation (MBBF) at the endocortical surface on the left (dotted lines = cement lines), and remodeling-based bone formation (RBBF) on trabecular bone surfaces where bone resorption previously occurred on the right (dashed lines = cement lines). Higher-resolution images could not be obtained due to the COVID-19 pandemic.

Table 1. Baseline Characteristics and Demographics

	FRAME		FRAME bone biopsy substudy month 2 cohort	
	Placebo $N_1 = 3591$	Romosozumab $N_1 = 3589$	Placebo $N_2 = 18$	Romosozumab $N_2 = 16$
Age, years	70.0 (65.0, 76.0)	70.0 (65.0, 76.0)	72.5 (63.0, 75.0)	70.0 (65.0, 73.0)
Body mass index, kg/m^2	24.3 (21.6, 27.2)	24.2 (21.6, 27.3)	24.2 (21.8, 27.2)	24.1 (22.8, 26.1)
Bone mineral density *T*-score				
Lumbar spine	−2.78 (−3.44, −2.05)	−2.82 (−3.44, −2.08)	−3.12 (−3.55, −2.20)	−2.77 (−3.41, −1.97)
Total hip	−2.52 (−2.78, −2.16)	−2.53 (−2.80, −2.18)	−2.39 (−2.72, −2.21)	−2.47 (−2.61, −2.01)
Femoral neck	−2.72 (−2.92, −2.57)	−2.73 (−2.93, −2.58)	−2.67 (−2.88, −2.55)	−2.72 (−2.87, −2.61)
Bone turnover markers				
P1NP, µg/L	52.4 (38.9, 62.7)[a]	50.3 (36.2, 65.9)[a]	37.1 (18.5, 54.2)	40.8 (29.9, 57.2)
CTX, ng/L	516.5 (325.0, 672.5)[a]	551.0 (338.0, 706.0)[a]	458.0 (353.0, 660.0)	385.0 (301.0, 441.0)

N_1 = number of patients randomized; N_2 = number of randomized patients in the bone biopsy substudy that received at least one dose of investigational product and had at least one evaluable biopsy at month 2. Twenty-nine biopsies of 34 obtained biopsies were suitable for histomorphometric analysis (placebo, $n = 14$; romosozumab, $n = 15$). Five biopsies of the 34 obtained biopsies were excluded due to reasons including crush artifact or insufficient cancellous bone surface.

Baseline characteristics were similar between the romosozumab and placebo groups in both patient populations.

[a]Bone turnover markers were only assessed in patients enrolled in the bone turnover substudy (placebo, $n = 66$; romosozumab, $n = 64$). All data are shown as median (quartile 1, quartile 3).

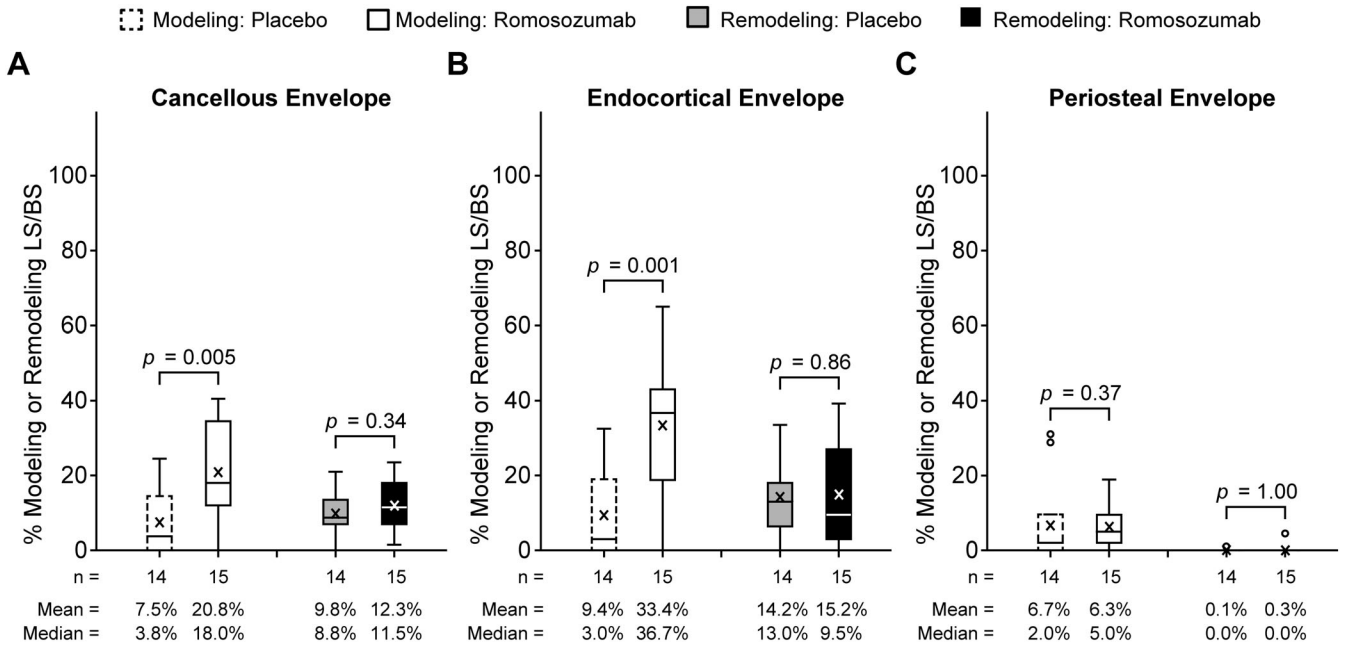

Fig 3. Bone modeling and remodeling after 2 months of romosozumab versus placebo: referent to total bone surface. Box plots show 25th and 75th percentiles (lower and upper edges of the box), median (horizontal bar inside the box), mean values (x), outliers (°), and minimum to maximum ranges excluding any outliers (error bars). Outliers are defined as values either greater than 1.5 IQR above 75th percentile or less than 1.5 IQR below 25th percentile. n = number of patients with evaluable histomorphometry data at the time point of interest. Nominal p values are the treatment difference (romosozumab versus placebo) and are based on the Wilcoxon rank-sum test without multiplicity adjustment. BS = bone surface; IQR = interquartile range; LS = labeled surface.

characteristics were similar between the romosozumab and placebo groups in both patient populations. At month 2, there were 34 subjects with evaluable biopsies obtained (placebo = 18, romosozumab = 16); of these, 29 were evaluable for histomorphometry (placebo = 14, romosozumab = 15).

After 2 months, the median percentage of MBBF referent to the total bone surface was significantly increased with romosozumab versus placebo on cancellous (18.0% versus 3.8%; $p = 0.005$) and endocortical (36.7% versus 3.0%; $p = 0.001$), but not on periosteal (5.0% versus 2.0%; $p = 0.37$) surfaces, with no significant difference in the surface extent of RBBF on all three bone surfaces (Fig. 3). When expressed referent to the total labeled surface, the significant increase in MBBF resulted in a reversal of the proportions of the median

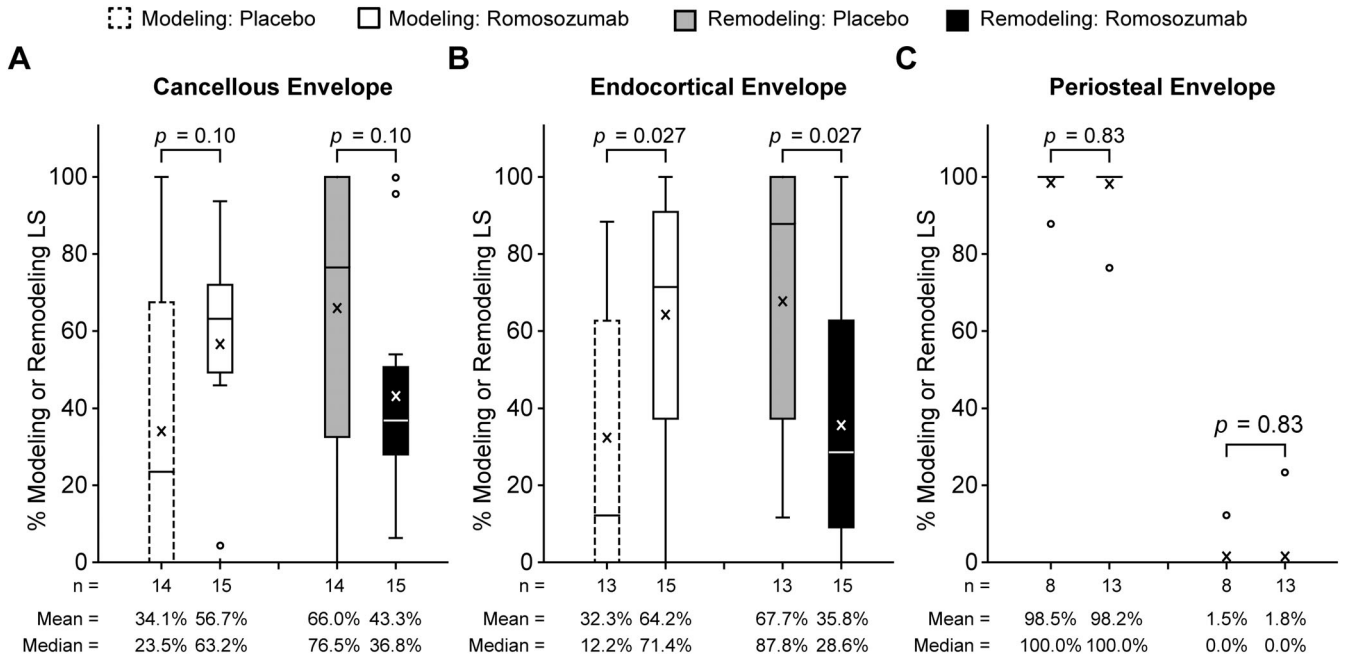

Fig 4. Bone modeling and remodeling after 2 months of romosozumab versus placebo: referent to labeled surface. Box plots show 25th and 75th percentiles (lower and upper edges of the box), median (horizontal bar inside the box), mean values (x), outliers (°), and minimum to maximum ranges excluding any outliers (error bars). Outliers are defined as values either greater than 1.5 IQR above 75th percentile or less than 1.5 IQR below 25th percentile. n = number of patients with evaluable histomorphometry data at the time point of interest. Nominal p values are the treatment difference (romosozumab versus placebo) and are based on the Wilcoxon rank-sum test without multiplicity adjustment. IQR = interquartile range; LS = labeled surface.

percentages of MBBF/RBBF with romosozumab versus placebo in both cancellous (63.2%/36.8% versus 23.5%/76.5%) and endocortical (71.4%/28.6% versus 12.2%/87.8%) envelopes (Fig. 4).

Discussion

Similar to the findings in both rat and monkey, the early increase in bone formation in response to romosozumab in postmenopausal women is due to MBBF. The magnitude of effect of romosozumab on MBBF increased approximately fourfold on cancellous surface and 12-fold on endocortical surface compared with placebo, underscoring the robust effects of romosozumab on cancellous and cortical bone. The increased endocortical MBBF would lead to increased cortical thickness. Indeed, after 12 months of romosozumab, cortical thickness was significantly increased in transiliac biopsies from the FRAME bone biopsy substudy.[4]

Romosozumab did not affect bone formation on the iliac periosteal surface in contrast to the findings in weight-bearing long bones in ovariectomized cynomolgus monkeys at clinically relevant exposures based on area under the curve (AUC).[6] Bone-forming effects of romosozumab on periosteal surfaces may require mechanical loading and may only manifest in weight-bearing sites or, less likely, may not be as prominent in humans.

The MBBF in placebo patients largely reflects the smooth cement lines that extend from the scalloped cement line of a remodeling site. Hattner and colleagues[12] initially reported the presence of MBBF in adult humans, with approximately 3% of the total extent of cement lines being smooth. They speculated this may reflect bone formation arising in quiescent surfaces, overfilling of the remodeling sites, or extension of the remodeling sites. Estimates of MBBF on cancellous bone in placebo patients in the current study are similar to those reported by Hattner and colleagues.[12]

At month 2, there was no measurable effect on RBBF, although resorption markers are moderately decreased as early as 2 weeks, which is consistent with the observed reductions in eroded and osteoclastic surfaces at month 2.[2,4] With the lag time between initiation of bone resorption and initiation of bone formation being more than 2 months, remodeling bone formation parameters may not show significant changes at the 2-month time point.[13] These effects on resorption parameters are sustained through month 12 and are associated with decreased activation frequency.

MBBF accounted for approximately 60% to 70% of the bone-forming surfaces after 2 months of romosozumab treatment. In contrast, after 3 months of teriparatide treatment, MBBF represented approximately 25% to 35% of active bone-forming surfaces on cancellous and endocortical surfaces, with extended remodeling sites (overfilling) contributing approximately 60% to 70% to the modeling surfaces.[14] With teriparatide, increased formation is coupled with increased bone resorption and reflects an augmentation of the bone turnover as shown by the activation frequency, leading to an increase in remodeling space and cortical porosity and transient loss in bone mass.[15-19] The consequence of the dual effect of romosozumab over 12 months of treatment, increasing MBBF and reducing bone resorption, without increasing cortical porosity,[4] is that this mode of action leads to rapid and large increase in bone mass without increasing remodeling space, followed by a progressive reduction in remodeling space due to decreased activation frequency. Taken

together, these actions likely contribute to the observed rapid reductions in fracture risk reported in the FRAME and ARCH clinical trials of romosozumab[2,20] and underscore the clinical relevance of increasing bone formation while reducing bone resorption, a unique dual effect resulting from romosozumab administration.

Limitations to this study include the relatively low number of patients and the inability to assess weight-bearing sites of the skeleton, as samples were taken from the iliac crest.

In conclusion, increase in bone formation in the first 2 months of romosozumab treatment in postmenopausal women with osteoporosis is predominantly due to increased MBBF at endocortical and cancellous envelopes in human bone.

Disclosures

EFE has received grant support from Amgen and served as an advisor for Amgen, Novartis, Eli Lilly, UCB, EffRx, Ascendis, and Takeda. RC has received speaker fees and consulted for Amgen, UCB, Eli Lilly, Arrow, Kyowa Kirin, MSD, BMS, Janssen-Cilag, Sanofi, Chugai, Pfizer, Mylan, Medac, Novartis, and AbbVie. RWB is a former employee of Amgen. JPB has received research support from Mereo BioPharma, Radius Health, and Servier; has served as a consultant for Amgen and Servier; and has served on speakers' bureaus for Amgen. SH is a former employee of Amgen and an Amgen stockholder. YS and DB are employees and stockholders of Amgen. CL is an employee and stockholder of UCB Pharma. PC has received travel grants from Amgen and UCB.

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Authors' roles: EFE was involved in the study conceptualization, methodology, data curation, formal data analysis and supervision, and writing of the original draft and reviewing and editing of subsequent drafts of the manuscript. RWB was involved in writing of the original draft and reviewing and editing of subsequent drafts of the manuscript. YS was involved in data curation, formal analysis, and reviewing and editing of manuscript drafts. RC, JB, SH, DB, CL, and PC were involved in reviewing and editing of manuscript drafts. All authors approved the final version of the manuscript.

Data Availability Statement

Qualified researchers may request data from Amgen clinical studies. Complete details are available at the following: https://wwwext.amgen.com/science/clinical-trials/clinical-data-transparency-practices/clinical-trial-data-sharing-request/.

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