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A Randomized, Double-Blind Phase 2 Clinical Trial of Blosozumab, a Sclerostin Antibody, in Postmenopausal Women with Low Bone Mineral Density

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ABSTRACT

Sclerostin, a *SOST* protein secreted by osteocytes, negatively regulates formation of mineralized bone matrix and bone mass. We report the results of a randomized, double-blind, placebo-controlled multicenter phase 2 clinical trial of blosozumab, a humanized monoclonal antibody targeted against sclerostin, in postmenopausal women with low bone mineral density (BMD). Postmenopausal women with a lumbar spine *T*-score −2.0 to −3.5, inclusive, were randomized to subcutaneous blosozumab 180 mg every 4 weeks (Q4W), 180 mg every 2 weeks (Q2W), 270 mg Q2W, or matching placebo for 1 year, with calcium and vitamin D. Serial measurements of spine and hip BMD and biochemical markers of bone turnover were performed. Overall, 120 women were enrolled in the study (mean age 65.8 years, mean lumbar spine *T*-score −2.8). Blosozumab treatment resulted in statistically significant dose-related increases in spine, femoral neck, and total hip BMD as compared with placebo. In the highest dose group, BMD increases from baseline reached 17.7% at the spine, and 6.2% at the total hip. Biochemical markers of bone formation increased rapidly during blosozumab treatment, and trended toward pretreatment levels by study end. However, bone specific alkaline phosphatase remained higher than placebo at study end in the highest-dose group. CTx, a biochemical marker of bone resorption, decreased early in blosozumab treatment to a concentration less than that of the placebo group by 2 weeks, and remained reduced throughout blosozumab treatment. Mild injection site reactions were reported more frequently with blosozumab than placebo. In conclusion, treatment of postmenopausal women with an antibody targeted against sclerostin resulted in substantial increases in spine and hip BMD. These results support further study of blosozumab as a potential anabolic therapy for osteoporosis. © 2014 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals, Inc. on behalf of American Society for Bone and Mineral Research (ASBMR)

KEY WORDS: BLOSOZUMAB; SCLEROSTIN ANTIBODY; ANABOLICS; BONE MINERAL DENSITY; OSTEOPOROSIS TREATMENT

Introduction

An estimated 200 million people worldwide are affected by osteoporosis.[1] This condition is characterized by bone fragility and susceptibility to fracture resulting from low bone mineral density (BMD), altered bone microarchitecture, and decreased bone strength.[2–5] Sclerostin, a *SOST* gene protein secreted by osteocytes, is a negative regulator of mineralized bone matrix formation and bone mass.[5–10] An antibody targeted toward sclerostin increased bone mass and strength in animals[11,12] and resulted in dose-related increases in BMD in healthy postmenopausal women.[13–18] This report summarizes the results of a randomized, double-blind, placebo-controlled phase 2 clinical trial of blosozumab, a humanized monoclonal antibody targeted to sclerostin, in the treatment of postmenopausal women with low BMD.

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Subjects and Methods

Study design

This study evaluated the efficacy and safety of blosozumab in ambulatory postmenopausal women between 45 and 85 years of age, with a lumbar spine BMD *T*-score of −2.0 to −3.5, inclusive. The primary objective was to evaluate the dose-response of blosozumab on lumbar spine BMD measured by dual-energy X-ray absorptiometry (DXA). This study included a 1-year treatment period and a 3-month follow-up period. The study was conducted at 13 sites in five countries in accordance with the ethical principles of the Declaration of Helsinki,[19] The International Conference on Harmonization Guideline for Good Clinical Practices,[20] and governing laws and regulations. Ethical Review Board approval was obtained at each clinical site. All study participants provided written informed consent prior to study enrollment. This clinical trial, NCT01144377, was sponsored by Eli Lilly and Company.[21]

The study also evaluated the effect of blosozumab on change from baseline in BMD of the hip and wrist (distal radius); total body mineral content; and biochemical markers of bone metabolism, including serum procollagen type 1 N propeptide (P1NP), osteocalcin, bone-specific alkaline phosphatase, and serum carboxy-terminal cross-linking telopeptide of type 1 collagen (CTx). The study was not designed or powered to evaluate fracture efficacy.

Patients were excluded if they had a history of the following: osteoporotic fracture; recent or long-term oral bisphosphonate treatment (defined as treatment within the last year if the previous duration was less than 1 year, or treatment within the last 3 years if previous total treatment duration exceeded 1 year); intravenous bisphosphonate treatment; treatment with therapeutic doses of systemic corticosteroids, fluoride, strontium, or parathyroid hormone (PTH); a metabolic bone disease other than primary osteoporosis; a history of Bell's palsy or other cranial nerve damage; a diagnosis of cancer within the previous 5 years, except for excised superficial basal cell or squamous cell cancers; or a known allergy to a monoclonal antibody.

At study enrollment, each patient was provided oral calcium (approximately 1000 mg/day) and vitamin D (approximately 1000 IU/day) for 4 to 8 weeks before receiving the study drug and continuing through study end. Patients meeting all enrollment criteria were randomized to double-blind treatment groups by a computer-generated random sequence interactive voice response system. Patients, investigators, study site personnel, and the sponsor study team in contact with the study sites remained blinded during the treatment phase and follow-up period, with the exception of pharmacy personnel preparing and dispensing study medication.

A medical history and physical examination were performed at baseline. Measures of vital signs and clinical assessments, including electrocardiograms and recording of adverse events, were continued throughout the study. Laboratory tests of serum calcium, 25-hydroxyvitamin D, 1, 25-dihydroxyvitamin D, intact PTH, and biochemical markers of bone turnover were performed at baseline and regular intervals throughout the study. Auditory-evoked potentials were obtained for a subset of patients at baseline and at treatment end. For all primary efficacy and safety measures, a central laboratory and reading facility maintained consistency of methods and data collection across sites.

Blosozumab was administered by subcutaneous (s.c.) injections delivering 180 mg every 4 weeks (Q4W), 180 mg every 2 weeks (Q2W), or 270 mg every 2 weeks (Q2W) (Fig. 1). Matching placebo injections were administered every 2 weeks such that all study patients, regardless of treatment arm, received three subcutaneous injections at their study visit every 2 weeks. Each injection totaled 1.5 mL in volume. These injections were administered in the lower abdomen and outer thigh by clinical study personnel. A fifth s.c. treatment arm, blosozumab 270 mg every 12 weeks, was later added through protocol addendum, and is not included in this report.

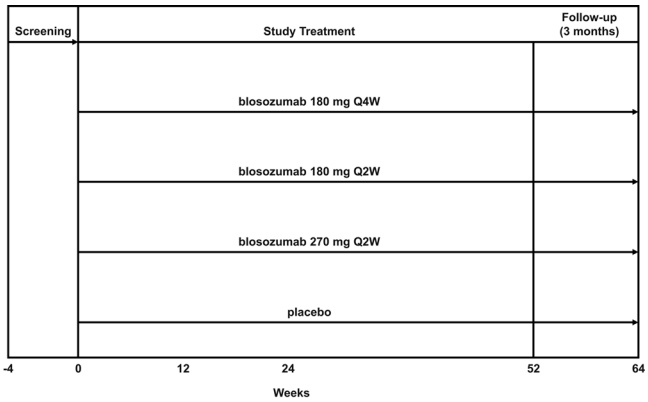

Fig. 1. Study design of the randomized, double-blind, placebo-controlled, multicenter phase 2 clinical trial of blosozumab in postmenopausal women with low bone mineral density.

The sample size was determined based on simulations to achieve a greater than 90% power in detecting a change of 0.05 g/cm^2 in lumbar spine BMD between blosozumab and placebo at week 52. Data from the phase 3 trial of an approved anabolic, teriparatide,[22,23] and data observed in a phase 1 multiple-dose study of blosozumab,[13] were used for simulations. Increases in lumbar spine BMD of 0.03 g/cm^2 at week 12, 0.05 g/cm^2 at week 24, and 0.06 g/cm^2 at week 52 with blosozumab treatment were assumed, corresponding to increases of 3.78%, 6.22%, and 8.26%, respectively. With placebo, increases in lumbar spine BMD of 0 g/cm^2 at week 12, 0.01 g/cm^2 at week 24, and 0.01 g/cm^2 at week 52 were assumed. In addition, a common SD of 0.04 g/cm^2 for lumbar spine BMD and a compound symmetry variance-covariance structure with a correlation of 0.5 were used in the mixed-effects repeated measures model. With at least 20 evaluable patients per treatment group, the study has 93% power in detecting a difference in lumbar spine BMD between blosozumab and placebo at week 52 (two-sided 0.05 significance level). Assuming a dropout rate of 30%, approximately 30 patients per treatment group were randomized.

The active treatment in this study, blosozumab, a humanized monoclonal antibody targeted to sclerostin, is regulated and restricted for distribution by the U.S. Food and Drug Administration (FDA) under an Investigational New Drug Application (IND), and is proprietary property of Eli Lilly and Company. Both the screening assay and neutralizing assay used in immunogenicity analyses were developed by Eli Lilly and Company. These assays are not commercially available and are proprietary property of Eli Lilly and Company. Therefore, access to both blosozumab and the immunogenicity assays used in the conduct of this trial is restricted.

Statistical analysis

Efficacy and safety analyses were conducted according to a prespecified statistical analysis plan, using the full analysis set of

study data, which included all data from all randomized patients receiving at least one dose of the assigned treatment. Missing data were not imputed.

All tests of treatment effect were conducted at a two-sided alpha level of 0.05, unless otherwise stated, using SAS™ version 9 (SAS Institute, Cary, NC, USA) or later.

The primary efficacy analysis of change from baseline in lumbar spine BMD at week 52 compared with placebo was performed using a mixed-effects repeated measures model analysis of covariance. Mixed-effects repeated measures takes into account within-subject and between-subject variability, and is appropriate for longitudinal data.[24] Factors in the model included treatment, time, and the interaction of treatment-by-time as fixed effects, with baseline lumbar spine BMD as a covariate. Pairwise comparisons of the difference in lumbar spine BMD changes (two-sided 0.05 significance level, Dunnett's multiplicity-adjusted for multiple treatment arms) between the blosozumab regimens and placebo were constructed and analyzed for the week 52 primary endpoint. Additional time points were evaluated as secondary analyses without multiplicity adjustment among the different times. However, within each time point, the comparisons among multiple treatment arms were multiplicity-adjusted. Analyses of percent changes of lumbar spine and hip BMD were also performed.

The change from baseline for laboratory parameters and vital signs was evaluated using a mixed-effects repeated measures model.[24] Comparisons of treatment groups were made based on least squares means at each visit.[25]

Interim analyses were conducted by an assessment committee independent of the study team, in accordance with the study protocol and the prespecified statistical analysis plan. Adverse events were evaluated by blinded investigators to determine if they were treatment-emergent adverse events and categorized by severity level using the lowest-level term from *The Medical Dictionary for Regulatory Activities*.[20] The proportion of patients experiencing treatment-emergent adverse events was compared among all treatments groups and pairwise using Fisher's exact test.

Results

Study patients

Overall, 120 postmenopausal women enrolled and 106 patients completed the primary treatment phase; 1 additional patient discontinued during follow-up (Fig. 2). There were no statistically significant differences between the treatment groups in the number of patients who discontinued the study. The baseline characteristics of the study population were similar across treatment groups (Table 1).

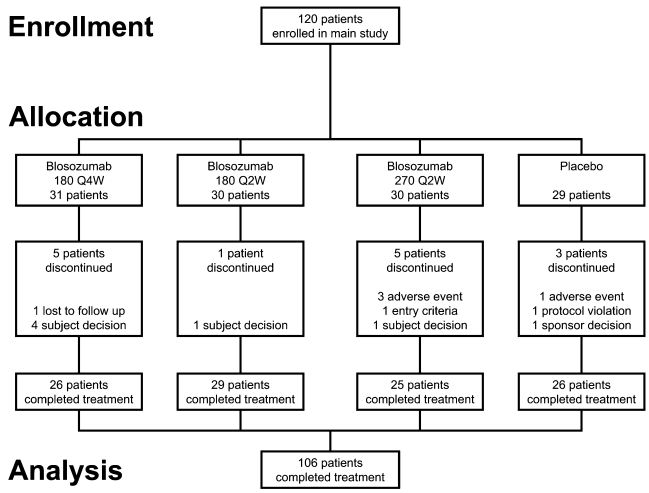

Fig. 2. Enrollment of the study patients through 52 weeks of treatment.

Efficacy

Blosozumab treatment resulted in statistically significant dose-related increases in lumbar spine BMD. The changes were apparent after 12 weeks of treatment, and the mean increase after 52 weeks of treatment at the primary study endpoint was 8.4% above baseline in women assigned to blosozumab 180 mg Q4W, 14.9% above baseline with blosozumab 180 mg Q2W, and 17.7% above baseline with blosozumab 270 mg Q2W (Fig. 3). When compared with placebo, these mean increases in lumbar spine BMD from baseline to week 52 were statistically significant for all blosozumab treatment groups ($p < 0.001$). In women receiving placebo, lumbar spine BMD declined from baseline to week 52 by a mean of 1.6%.

There were also statistically significant dose-related increases in total hip and femoral neck BMD. At 52 weeks of treatment,

Table 1. Baseline Characteristics for All Study Patients

	Placebo	Blosozumab 180 mg Q4W	Blosozumab 180 mg Q2W	Blosozumab 270 mg Q2W
n	29	31	30	30
Age, years (mean ± SD)	66.0 ± 9.2	66.8 ± 9.0	64.2 ± 8.2	66.1 ± 7.7
Race, *n* (%)				
White	16 (55.2)	17 (54.8)	17 (56.7)	17 (56.7)
Black	1 (3.4)	0	0	0
Asian, Japanese	12 (41.4)	14 (45.2)	13 (43.3)	13 (43.3)
BMI, kg/m^2 (mean ± SD)	23.8 ± 5.6	23.1 ± 3.7	23.7 ± 3.8	24.6 ± 4.7
LS *T*-score (mean ± SD)	−2.8 ± 0.5	−2.8 ± 0.5	−2.8 ± 0.4	−2.7 ± 0.5
FN *T*-score (mean ± SD)	−2.1 ± 1.0	−2.2 ± 0.7	−2.1 ± 0.9	−1.9 ± 0.6
25-hydroxyvitamin D, nmol/L (mean ± SD)	67.8 ± 15.9	68.9 ± 24.2	68.6 ± 18.8	68.4 ± 23.9
1,25-dihydroxyvitamin D, pmol/L (mean ± SD)	161.9 ± 68.2	156.3 ± 55.9	189.2 ± 63.4	160.9 ± 56.3

Q4W = every 4 weeks; Q2W = every 2 weeks; BMI = body mass index; LS = lumbar spine; FN = femoral neck.

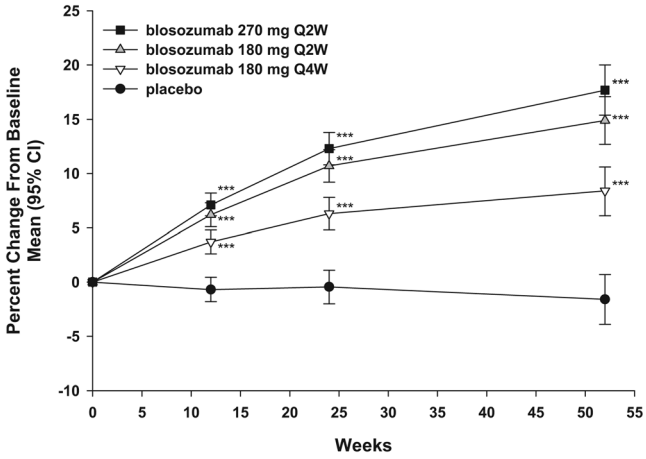

Fig. 3. Percent change (mean, 95% CI) in bone mineral density of the lumbar spine from baseline to week 52 for all study patients according to study group. The least squares mean percent change (mean, 95% CI) in bone mineral density of the lumbar spine from baseline to week 52 is shown. Asterisks (*) indicate statistically significant differences ($^{*}p < 0.050$, $^{**}p < 0.010$, $^{***}p < 0.001$) for each study group as compared with placebo.

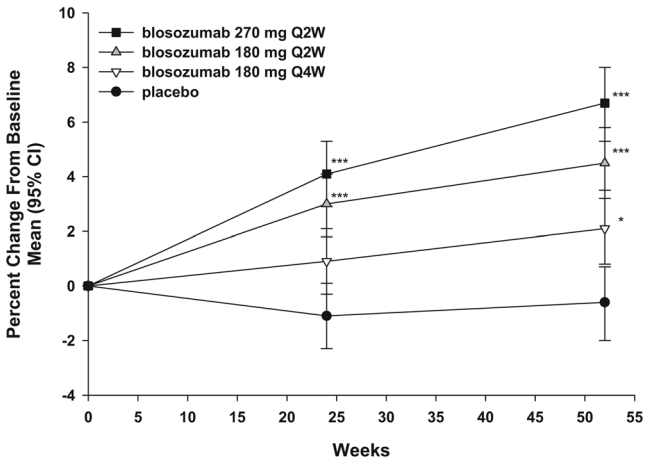

Fig. 4. Percent change (mean, 95% CI) in bone mineral density of the total hip from baseline to week 52 for all study patients according to study group. The least squares mean percent change (mean, 95% CI) in bone mineral density of the total hip from baseline to week 52 is shown. Asterisks (*) indicate statistically significant differences ($^{*}p < 0.050$, $^{**}p < 0.010$, $^{***}p < 0.001$) for each study group as compared with placebo.

total hip BMD increased from baseline by a mean of 2.1% in women assigned to blosozumab 180 mg Q4W, 4.5% for women assigned to blosozumab 180 mg Q2W, and 6.7% for women assigned to blosozumab 270 mg Q2W (Fig. 4). Femoral neck BMD increased from baseline by a mean of 2.7% in women assigned to blosozumab 180 mg Q4W, 3.9% in women assigned to blosozumab 180 mg Q2W, and 6.3% for women receiving blosozumab 270 mg Q2W. When compared with placebo, the mean increases in total hip BMD from baseline to week 52 were statistically significant for all blosozumab treatment groups. However, when compared with placebo, the mean increases in femoral neck BMD from baseline to week 52 were statistically significant only for patients receiving blosozumab 180 mg Q2W and 270 mg Q2W. In women receiving placebo, total hip and femoral neck BMD decreased from baseline to week 52 by a mean of 0.7% and 0.6%, respectively.

There were no statistically significant changes in wrist BMD observed in the study treatment groups. At the one-third radius, mean 1.5% and 1.9% decreases in BMD were observed for the two blosozumab 180 mg treatment groups at week 52. However, in the blosozumab 270 mg Q2W treatment group, a 0.9% mean increase from baseline was observed at week 52, which was not statistically significant when compared with placebo ($p = 0.11$). A mean 1.4% decrease in one-third radius BMD from baseline was observed at the end of the treatment period for the placebo group.

At baseline, 95.6% of the women randomized to blosozumab treatment had a lumbar spine *T*-score less than or equal to –2.0. At the end of treatment, a positive shift in the lumbar spine *T*-score to greater than –2.0 was observed in 72.4% of the women receiving blosozumab 180 mg Q2W, and 88.5% of the women receiving blosozumab 270 mg Q2W.

Total body bone mineral content

Total body bone mineral content (BMC), a measure of treatment effect on the skeleton, increased from baseline to week 52 by a mean of 1.7%, 4.2%, and 7.3% in women assigned to blosozumab 180 mg Q4W, 180 mg Q2W, and 270 mg Q2W, respectively. For women randomized to placebo, total body BMC declined from baseline by a mean of 1.9% over 52 weeks of treatment. The corresponding mean percent changes from baseline in BMC of the head (skull) subregion were an increase of 1.6%, 1.4%, and 4.0% in women assigned to blosozumab 180 mg Q4W, 180 mg Q2W, and 270 mg Q2W, respectively. The changes in BMC of the head for women randomized to placebo were a mean decrease of 2.2% from baseline during 52 weeks of treatment.

Biochemical markers of bone turnover

Treatment with blosozumab resulted in increased serum concentrations of biochemical markers of bone formation, including serum P1NP, osteocalcin, and bone-specific alkaline phosphatase, measured prior to dose of study drug. Serum concentrations of P1NP increased toward a peak level within 4 weeks of blosozumab treatment, remained significantly above baseline through 24 weeks for all but one blosozumab treatment group, as compared with placebo, and then trended toward pretreatment concentrations by study end (Fig. 5). Osteocalcin and serum bone-specific alkaline phosphatase concentrations increased early and significantly from baseline during blosozumab treatment, as compared with placebo, and were approaching baseline by study end (Fig. 5). However, the blosozumab 270 mg Q2W group maintained an increase in bone-specific alkaline phosphatase concentration significantly greater than placebo through week 52 (Fig. 5). Serum concentrations of CTx, a biochemical marker of bone resorption, decreased from baseline during blosozumab treatment, with a trough concentration less than placebo occurring by 2 weeks, a concentration similar to placebo at 12 weeks, and a concentration less than placebo at study end (Fig. 5).

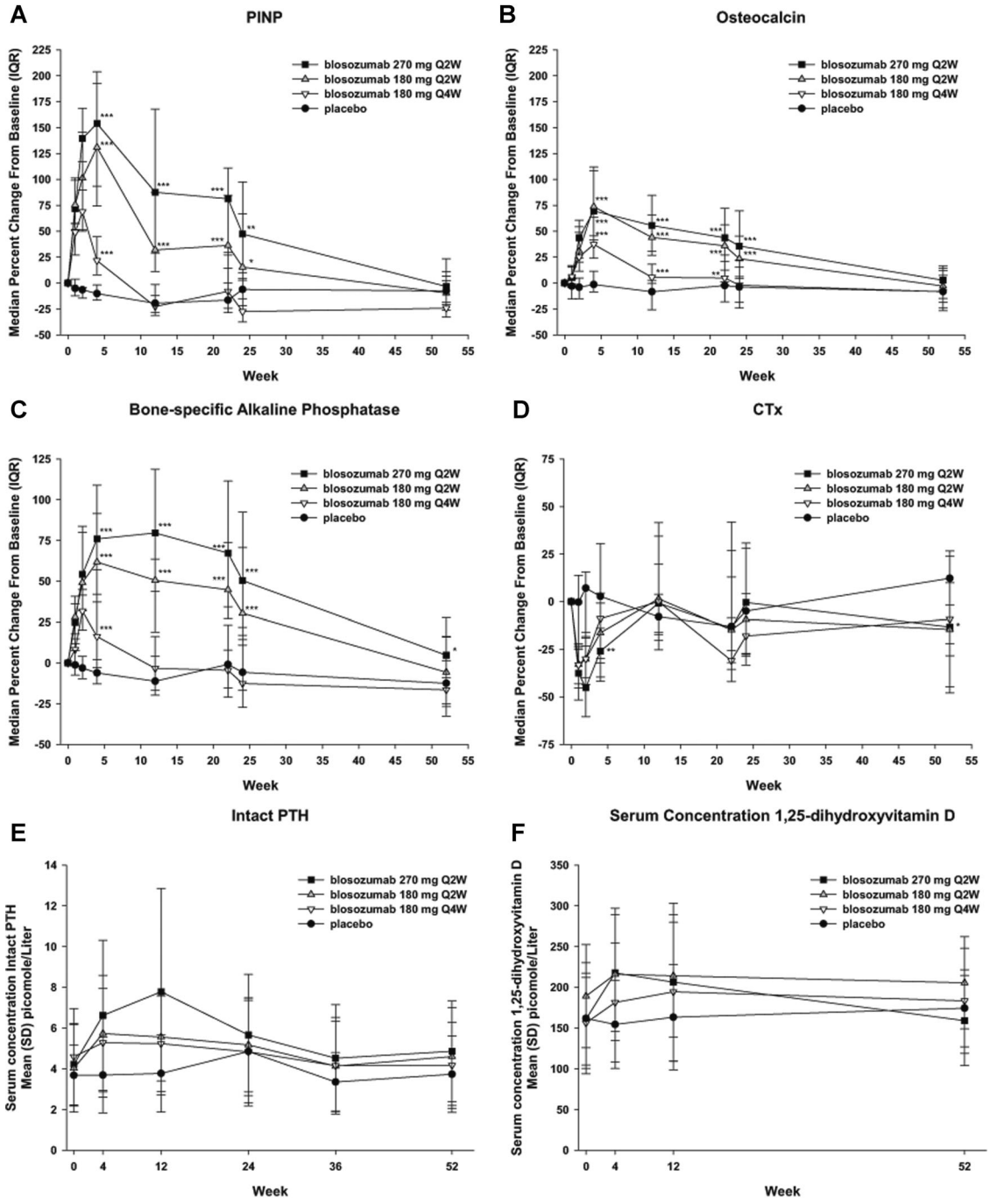

Fig. 5. Median percent change (IQR) in biochemical markers of bone turnover from baseline to week 52 and serum concentration of intact PTH and 1,25-dihydroxyvitamin D from baseline to week 52. Median percent change (IQR) in predose serum concentrations of biochemical markers of bone turnover from baseline to week 52 for all study patients: serum P1NP (*A*); osteocalcin (*B*); bone-specific alkaline phosphatase (*C*); and serum CTx (*D*). Asterisks (*) indicate statistically significant differences ($^{*}p < 0.050$, $^{**}p < 0.010$, $^{***}p < 0.001$) for each study group as compared with placebo. In *A*, in addition to designations of statistical significance provided on the figure, all values for median percent change from baseline in P1NP at weeks 1 and 2 are statistically significant at $p < 0.001$ as compared with placebo. In *B*, in addition to designations of statistical significance provided on the figure, all values for median percent change from baseline in osteocalcin at week 1 are statistically significant at $p < 0.050$ as compared with placebo, and $p < 0.001$ at week 2 as compared with placebo. In *C*, in addition to designations of statistical significance provided on the figure, the values at week 1 for median percent change from baseline in bone-specific alkaline phosphatase are statistically significant at $p < 0.050$ for blosozumab 180 mg Q4W as compared with placebo, and $p < 0.001$ for blosozumab 180 mg Q2W and blosozumab 270 mg Q2W. At week 2, all values for median percent change from baseline in bone-specific alkaline phosphatase are statistically significant at $p < 0.001$ for blosozumab as compared with placebo. In *D*, in addition to designations of statistical significance provided on the figure, all values for median percent change from baseline in CTx are statistically significant at $p < 0.001$ as compared with placebo at weeks 1 and 2. In (E), approximately one-half of the patients in each treatment group had an iPTH assessment at week 24. IQR = interquartile range; P1NP = procollagen type 1 N propeptide; CTx = carboxy-terminal cross-linking telopeptide of type 1 collagen.

Safety

Other than mild injection site reactions reported more frequently with blosozumab than placebo, the frequency of adverse events during treatment and the 3-month follow-up period was similar across all treatment groups. Mild injection-site reactions, including pruritus, swelling, erythema, bruising, and pain, were reported by 22.6% to 40.0% of women receiving blosozumab and 10.3% of women receiving placebo, and were not associated with the development of anti-drug antibodies. A complete table of treatment-emergent adverse events is included as Supporting Table 1.

There were no patient deaths during the study. Nine patients reported serious adverse events during the treatment period, with only 1 evaluated by a blinded investigator as possibly related to the study drug. This patient, randomized to placebo, experienced a cerebral infarction after 3 weeks of treatment. Breast cancer was reported in 4 women receiving blosozumab: 2 women (270 mg Q2W group) within 3 months of initiating blosozumab treatment, 1 woman (180 mg Q2W group) 3 months after the last dose of blosozumab, and 1 woman (180 mg Q4W group) approximately 1 year after the last dose of blosozumab. All 4 women were Japanese and enrolled in two study sites in Japan. A retrospective exploration of these 4 patients' medical histories provided additional information. One patient, with bone metastases detected at the time of the breast cancer diagnosis, had a mammogram report indicating microcalcifications prior to study enrollment. Two patients had not had a screening mammogram for over 4 years prior to the study, and 1 patient had never had a mammogram. The tumors were heterogeneous with respect to histopathology, receptor status, and stage. None of the investigators considered this serious adverse event to be related to blosozumab treatment.

There was a slight initial decrease in serum calcium (0.01 to 0.05 mmol/L, equivalent to 0.04 to 0.20 mg/dL) in the blosozumab treatment groups that was notable at week 4, with the maximum decrease occurring by week 12. Thereafter, serum calcium fluctuated around baseline for the duration of the study and follow-up period in all treatment groups. As expected with the decrease in serum calcium concentrations, there was a corresponding increase in intact PTH concentrations (0.61 to 3.57 pmol/L, equivalent to 5.8 to 34.0 pg/mL) (Fig. 5E) The increase was noted at week 4 and continued through week 24, returning to normal levels by week 36 and remaining normal through the follow-up period. These observed changes in calcium concentrations were likely a result of rapid bone mineral increase associated with blosozumab treatment, and the changes in PTH were a physiological response to changes in serum calcium concentrations. There were no adverse events associated with the changes in calcium or PTH.

An increase in 1,25-dihydroxyvitamin D concentration was observed in patients during the treatment period. The increase in 1,25-dihydroxyvitamin D concentration appeared to be dose-related in the blosozumab groups, with the peak mean increase of 56.8 pmol/L occurring in the blosozumab 270 mg Q2W group at week 4 (Fig. 5F). At week 12, mean increases in serum concentration of 1,25-dihydroxyvitamin D were 32.0 to 32.7 pmol/L from baseline in the blosozumab 180 mg groups, and 45.4 pmol/L in the blosozumab 270 mg Q2W group. Mean serum concentrations of 1,25-dihydroxyvitamin D declined toward baseline at the end of treatment, with the blosozumab 270 mg Q2W group essentially reaching the level of pretreatment concentration at week 52.

Serum concentration of 25-hydroxyvitamin D was measured at baseline (Table 1) and at the end of the treatment phase. An increase in serum concentration of 25-hydroxyvitamin D was observed in all groups during the treatment period. At the end of treatment, a mean increase from baseline of 2.1 to 12.2 nmol/L was observed in the blosozumab treatment groups, whereas a mean increase from baseline of 10.0 nmol/L was seen in the placebo group. There were no adverse events associated with these changes in vitamin D metabolites.

There were no clinically relevant changes in systolic or diastolic blood pressure, heart rate, or any electrocardiogram parameter at any blosozumab dose during treatment or during the follow-up period.

Thirty-two patients (35%) developed anti-drug antibodies after exposure to blosozumab. The highest incidence was noted in the blosozumab 180 mg Q4W and 180 mg Q2W groups, with increasing occurrence observed over the course of treatment. The development of anti-blosozumab antibodies appeared to be inversely dependent on dose and dose frequency. Only 1 patient (180 mg Q2W group) developed anti-blosozumab antibodies that had an effect on blosozumab exposure and efficacy. Briefly, the treatment-emergent anti-drug antibody was first detected at week 24 with blosozumab serum concentration more than 10-fold lower than the expected level. Based on a validated screening assay, the anti-drug antibody titer reached its maximal level ($>$1:160000) at the end of the treatment, when blosozumab could no longer be detected in serum. The anti-drug antibodies in this patient were found to be neutralizing to blosozumab using a validated neutralizing assay. The BMD responses at the end of treatment were relatively small in this patient, with increases from baseline of approximately 3.2% and 0.2% in lumbar spine BMD and total hip BMD, respectively. There were no adverse events associated with the development of anti-drug antibodies in any of the patients, including the 1 patient with reduced blosozumab exposure.

Pretreatment and posttreatment brainstem auditory-evoked potential testing was performed in a subset of 44 patients. One woman in the blosozumab 180 mg Q2W group began the study with a normal auditory-evoked potential and ended with an observed abnormality. This abnormality was described as probable conductive loss, thought to be secondary to a technical effect, such as ear wax blocking the auditory canal. The results of auditory-evoked potential testing were otherwise unremarkable, as judged by a blinded expert clinician.

Discussion

The dose-related increases in lumbar spine BMD observed at 52 weeks of treatment with blosozumab met the primary objective of the study. Injection of blosozumab, a humanized IgG4 monoclonal antibody designed to neutralize sclerostin, at doses of 180 mg Q4W, 180 mg Q2W, and 270 mg Q2W, increased BMD up to 17.7% at the lumbar spine, and up to 6.7% at the total hip, compared with pretreatment levels. In this patient population at risk for osteoporotic fracture, based on baseline BMD, 72% to 89% of women assigned to one of the every 2 weeks-dosing blosozumab treatment groups experienced an increase in spine BMD to within the range observed in young adult women (*T*-score greater than –2.0). Significant increases in BMD at both the lumbar spine and total hip were clearly shown in the higher dose groups.

Observed increases in total body BMC suggest a net increase in bone at skeletal sites without a disproportionate effect of

treatment on the head (skull). Cranial nerve function testing using brainstem auditory-evoked potentials did not detect clinically significant abnormalities. This testing was performed because compression of the VII and VIII cranial nerves by bone overgrowth is observed in individuals with sclerosteosis and van Buchem disease who have complete loss or deficiency of sclerostin.[26,27] Hence, this finding formed the basis for excluding patients with Bell's palsy or other cranial nerve damage from study enrollment, because preexisting cranial nerve damage might confound the interpretation of safety data in this study.

The serum concentration of P1NP, a biochemical marker of bone formation, increased rapidly during the first 4 weeks of blosozumab treatment, while concurrently the serum concentration of CTx, a biochemical marker of bone resorption, decreased rapidly within the first 2 weeks of treatment to a concentration below that observed with placebo. Although P1NP concentration later trended toward pretreatment levels, CTx concentration remained reduced to study end. The changes in biochemical markers of bone turnover observed with blosozumab treatment, namely increases in biochemical markers of bone formation and the decrease in biochemical marker of bone resorption, are consistent with a skeletal anabolic response to blosozumab therapy. The reasons for transient changes in biochemical markers of bone formation during treatment are unclear. Sampling of marker concentrations occurred predose, during trough concentrations of blosozumab, with no measure of marker concentrations between dosing. The trend in bone formation markers toward baseline later during treatment might be a result of new bone formation in the skeleton reducing stresses and strains within the skeleton, thereby reducing a positive signal for bone formation. In addition, negative counter-regulation of bone formation by molecules such as Dickkopf-related protein 1 (DKK-1) might reduce bone formation.[3] The significant decrease in biochemical markers of bone resorption observed with blosozumab treatment may be related to an inhibitory effect on the RANK-L-RANK osteoclastogenic signaling pathway. In osteoblasts and osteocytes, Wnt-β-catenin signaling is required for expression of the RANK-L decoy receptor osteoprotegerin (OPG).[28] Additionally, sclerostin may upregulate the expression of RANK-L.[29] It is plausible that blosozumab, as an antibody targeted to sclerostin, may decrease RANK-L and increase OPG, with a reduction in the RANK-L to OPG ratio, decreasing bone resorption.[3,29–31]

Observed changes in laboratory assessments are consistent with physiologic efflux of calcium into mineralizing new bone, and were not associated with patient symptoms or adverse events. Further evaluation of the cardiovascular safety of drugs targeting sclerostin, specifically vascular calcification, has been suggested in the literature.[32] The authors cite increasing recognition of Wnt signaling in vascular pathophysiology, and raise the question of whether sclerostin directly affects vascular calcification. However, toxicology studies conducted for the blosozumab development program showed no effect on the vasculature and no effect on cardiovascular risk. A review of patients with sclerosteosis and van Buchem disease does not reveal increased cardiovascular risk factors.[26,27] There have been no reports of vascular calcification in *SOST* knockout mice.[33] The role of Wnt signaling in vascular pathophysiology is an emerging area of exploration, and data from larger phase 3 study populations and increased patient-years of exposure to sclerostin antibodies may provide insight.

Changes in serum concentrations of intact PTH (iPTH) and vitamin D metabolites among patients treated with blosozumab are consistent with the observed physiologic movement of calcium from blood to bone during bone mineralization. This pattern of iPTH response to blosozumab is similar to the dose-related trend reported in the blosozumab phase 1 study by McColm and colleagues.[13] Linking the observed increase in iPTH with the anabolic effect of blosozumab requires consideration of physiologic variables in calcium movement and intricacies of Wnt signaling, and will require further study.

The imbalance in breast cancer cases reported in patients receiving blosozumab has been extensively explored. Screening mammography was not included in the protocol, and is not routinely obtained in some study site locations. Based on timing of the breast cancer diagnosis and size of the tumors, the investigators determined the breast cancers were likely preexisting. Preclinical rat and monkey toxicity studies of blosozumab have not shown an effect on mammary gland histology or increased cell proliferation. Sclerostin mRNA does not appear to be widely expressed in human breast cancer tissue,[34] but additional investigation is ongoing. In addition, in one report of 63 patients with sclerosteosis followed for 38 years, there was no evidence of an increased risk of cancer in general or breast cancer in particular.[35]

Anti-drug antibodies have been noted to occur with low-dose therapy of therapeutic antibodies,[36] a finding in the present study. However, the occurrence of anti-drug antibodies was not associated with adverse events and, in all but 1 anti-drug antibody–positive patient, it did not appear to affect blosozumab exposure or anabolic bone activity of blosozumab.

The findings that blosozumab increases bone formation, decreases bone resorption, and increases spine and total hip BMD are consistent with a recently published report of an antibody targeted to sclerostin.[18] Although blosozumab and romosozumab are both sclerostin antibodies, they are structurally diverse, and indirect comparisons must be made with caution. While there have been no direct comparisons, the blosozumab trial explored high doses, achieved large increases in BMD at the lumbar spine and hip, and included measures of total body and skull BMC. The potential significance of the findings we report herein is the substantial anabolic effects on the skeleton achieved with blosozumab treatment, supporting further investigation of blosozumab as a potential therapy for osteoporosis. Further study of blosozumab will continue to assess the efficacy and safety of blosozumab in the treatment of osteoporosis.

In conclusion, injections of blosozumab for 1 year resulted in substantial anabolic effects on the skeleton and were well tolerated. These findings support further investigation of blosozumab as a potential therapy for osteoporosis.

Disclosures

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References

1. International Osteoporosis Foundation. Epidemiology [Internet]. Nyon, Switzerland: IOF; 2014 [cited 2014 Sep 15]. Available from: http://www.iofbonehealth.org/epidemiology.
2. Kanis JA, McCloskey EV, Johansson H, et al. A reference standard for the description of osteoporosis. Bone. 2008;42:467–75.
3. Baron R, Hesse E. Update on bone anabolics in osteoporosis treatment: rationale, current status, and perspectives. J Clin Endocrinol Metab. 2012;97:311–25.
4. [No authors listed]. Consensus Development Conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med. 1993;94(6):646–50.
5. Costa AG, Bilezikian JP. Sclerostin: therapeutic horizons based upon its actions. Curr Osteoporos Rep. 2012;10:64–72.
6. Atkins GJ, Findlay DM. Osteocyte regulation of bone mineral: a little give and take. Osteoporos Int. 2012;23:2067–79.
7. Moester MJC, Papapoulos SE, Lowik CWGM, et al. Sclerostin: current knowledge and future perspectives. Calcif Tissue Int. 2010;87:99–107.
8. Veverka V, Henry AJ, Slocombe PM, et al. Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of Wnt-mediated bone formation. J Biol Chem. 2009;284:10890–900.
9. Kogawa M, Wijenayaka AR, Ormsby R, et al. Sclerostin regulates release of bone mineral by osteocytes by induction of carbonic anhydrase. J Bone Miner Res. 2013;28:2436–48.
10. Burgers TA, Williams BO. Regulation of Wnt/β-catenin signaling within and from osteocytes. Bone. 2013;54:244–9.
11. Ma YL, Page T, Zeng QQ, et al. Blosozumab, a humanized monoclonal antibody, and a chimeric rodent monoclonal antibody against sclerostin robustly increase bone formation activity in intact monkeys and ovariectomized rats. J Bone Miner Res. 2012;27(Suppl 1):[Oral presentation at: Annual Meeting American Society for Bone and Mineral Research (ASBMR); 2012 Oct 12-15; Minneapolis, MN, USA; Presentation Number: 1062]. Available from: http://www.asbmr.org/meetings/annualmeeting/AbstractDetail.aspx?aid=580ec783-5e46-4f61-bb0d-0b63f8ee361d.
12. Das S, Sakthiswary R. Bone metabolism and histomorphometry changes in murine models treated with sclerostin antibody: a systematic review. Curr Drug Targets. 2013;14:1667–74.
13. McColm J, Hu L, Womack T, et al. Single- and multiple-dose randomized studies of blosozumab, a monoclonal antibody against sclerostin, in healthy postmenopausal women. J Bone Miner Res. 2014;29:935–43.
14. Benson CT, Robins D, Recker R, et al. Effect of blosozumab on bone mineral density: results of a phase 2 study of postmenopausal women with low bone mineral density. Presented at the European Calcified Tissue Society Conference ECTS 2013, Lisbon, Portugal. Bone Abstracts. 2013;1:OC5.3. DOI: 10.1530/boneabs.01.OC5.3 Available from: http://www.bone-abstracts.org/ba/0001/ba0001oc5.3.htm.
15. Matsumoto T, Robins D, Alam J, et al. Effect of blosozumab on bone mineral density in Japanese and non-Japanese postmenopausal women with low bone mineral density. IBMS Bonekey. 2013; 10: Article number 333. [Presented at the International Bone and Mineral Society/Japan Society of Bone and Mineral Research 2013, Kobe, Japan. Abstract OC902]. Available from: http://www.nature.com/bonekey/knowledgeenvironment/2013/130522/bonekey201367/pdf/bonekey201367.pdf.
16. Lewiecki EM. Sclerostin monoclonal antibody therapy with AMG 785: a potential treatment for osteoporosis. Expert Opin Biol Ther. 2011;11:117–27.
17. Padhi D, Jang G, Stouch B, et al. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res. 2011;26:19–26.
18. McClung M, Grauer A, Boonen S, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med. 2014;370:412–20.
19. World Medical Assembly. Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects. Available from: www.wma.net/en/30publications/10policies/b3/. Accessed October 2, 2013.
20. International Conference on Harmonisation. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. Guideline for Good Clinical Practice. E6(R1). 10 June 1996. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf.
21. Public Clinical Trial Registration. A phase 2 randomized study of LY 2541 546 versus placebo in postmenopausal women with low bone mineral density: an evaluation of the dose response relationship using bone mineral density. Available from: http://clinicaltrials.gov/show/NCT01144377.
22. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med. 2001;344(19):1434–41.
23. Marcus R, Wang O, Satterwhite J, Mitlak B. The skeletal response to teriparatide is largely independent of age, initial bone mineral density, and prevalent vertebral fractures in postmenopausal women with osteoporosis. J Bone Miner Res. 2003;18(1):18–23.
24. Mallinckrodt CH, Lane PW, Schnell D, Peng Y, Mancuso JP. Recommendations for the primary analysis of continuous endpoints in longitudinal clinical trials. Drug Inf J. 2008;42:303–19.
25. Searle SR, Speed FM, Milliken GA. Population marginal means in the linear model: an alternative to least squares means. Am Stat. 1980;34(4):216–21.
26. Beighton PH, Hamersma H, Brunkow ME, *SOST*-related sclerosing bone dysplasias. In: Pagon RA, Adam MP, Bird TD, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2014. 2002 Jun 04 [updated 2013 Jan 10; cited 2014 Sep 16]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1228/.
27. van Lierop AH, Hamdy N, van Egmond MJ, Bakker E, Dikkers FG, Papapoulos SE. Van Buchem disease: clinical, biochemical, and densitometric features of patients and disease carriers. J Bone Miner Res. 2013;28:848–54.
28. Glass DA. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell. 2005;8:751–64.
29. Wijenayaka AR, Kogawa M, Lim HP, Bonewald LF, Findlay DM, Atkins GJ. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. PLoS One. 2011;6(10):e25900. DOI: 10.1371/journal.pone.0025900.
30. Rossini M, Gatti D, Adami S. Involvement of WNT/B-catenin signaling in the treatment of osteoporosis. Calcif Tissue Int. 2013;93:121–32.

31. Silva I, Branco JC. Rank/Rankl/opg: literature review. Acta Reumatol Port. 2011;36:209–18.
32. Evenepoel E, D'Haese P, Brandenburg V. Romosozumab in postmenopausal women with osteopenia. N Engl J Med. 2014; 370(17):1664–5. DOI: 10.1056/NEJMc1402398.
33. Li X, Ominsky MS, Niu Q, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res. 2008;23(6):860–9.
34. National Center for Biotechnology Information. Gene Expression Omnibus public functional genomics data repository. Available from: http://www.ncbi.nlm.nih.gov/geo/.
35. Hamersma H, Gardner J, Beighton P. The natural history of sclerosteosis. Clin Genet. 2003;63:192–7.
36. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther. 2008;84: 548–58.