Published in final edited form as:

Connect Tissue Res. 2010 April ; 51(2): 123–131. doi:10.3109/03008200903108472.

RANKL Inhibition Improves Bone Properties in a Mouse Model of Osteogenesis Imperfecta

Renee Bargman,

NYPH-Weill Cornell Medical Center, New York, New York, USA

Alice Huang,

Hospital for Special Surgery, New York, New York, USA

Adele L. Boskey,

Hospital for Special Surgery, New York, New York, USA

Cathleen Raggio, and

Hospital for Special Surgery, New York, New York, USA

Nancy Pleshko

Hospital for Special Surgery, New York, New York, USA

Abstract

Recently, a new class of agents targeting the receptor activator of nuclear factor- κ B ligand (RANKL) pathway has been developed for the treatment of osteoporosis and other bone diseases. In the current study, inhibition of the RANKL pathway was evaluated to assess effects on “bone quality” and fracture incidence in an animal model of osteogenesis imperfect (OI), the *oim/oim* mouse. Juvenile *oim/oim* (~6 weeks old) and wildtype (+/+) mice were treated with either a RANKL inhibitor (RANK-Fc) or saline. After treatment, bone density increased significantly in the femurs of both genotypes. Femoral length decreased with RANK-Fc in +/+ mice. Geometric measurements at mid-diaphysis in the *oim/oim* groups showed increases in the ML periosteal and endosteal diameters and AP cortical thickness in the treated groups. Within +/+ groups, ML cortical thickness and ML femoral periosteal diameter were significantly increased with RANK-Fc. Biomechanical testing revealed increased stiffness in *oim/oim* and +/+ mice. Total strain was increased with treatment in the +/+ mice. Histologically, RANKL inhibition resulted in retained growth plate cartilage in both genotypes. The average number of fractures sustained by RANK-Fc-treated *oim/oim* mice was not significantly decreased compared to saline treated *oim/oim* mice. This preclinical study demonstrated that RANKL inhibition at the current dose improved density and some geometric and biomechanical properties of *oim/oim* bone, but it did not decrease fracture incidence. Further studies that address commencement of therapy at earlier time points are needed to determine whether this mode of therapy will be clinically useful in OI.

Keywords

Bone; Osteogenesis Imperfecta; *Oim/oim*; Mouse; RANK/RANKL

Address correspondence to Nancy Pleshko, PhD, Temple University, Dept. of Mechanical Engineering, 1947 N. 12th St., Philadelphia, PA 19122. npleshko@temple.edu.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

INTRODUCTION

Osteogenesis imperfecta (OI) is a genetic disorder characterized by bone fragility. Clinical manifestations of this disease range from perinatal lethality, to severe disability with frequent fractures, to a relatively benign condition with few fractures over a lifetime. The disorder is most often the result of a mutation in one of two genes that encode the α chains of type I collagen, COL1A1 and COL1A2, leading to a quantitative or qualitative change in type I collagen which in turn results in abnormal bone formation and high bone turnover [1]. The treatment of OI has been extremely challenging, and until the introduction of bisphosphonate therapy, mostly ineffective. Bisphosphonates are currently the mainstay of treatment and the different treatment modalities include cyclic infusions of pamidronate and neridronate, as well as oral treatment with alendronate (ALN) [2–6]. Bisphosphonates, which inhibit osteoclast activity, have been shown to ease chronic bone pain, increase bone mineral density and strength, reduce fractures, and improve final height probably due to a decreased incidence of vertebral fractures [4,7,8]. However, limitations in bisphosphonate therapy remain, including occasional respiratory distress with the first infusion [9].

In addition, bisphosphonates have half-lives of up to a decade and therefore the optimal duration of treatment is unknown [10]. There are also concerns about bone healing and remodeling in bisphosphonate-treated children with OI [11,12]. Further, these compounds are known to cross the placenta and may pose risk to a developing fetus, although the few case reports published to date do not suggest any significant ill effects for the mothers and their offspring [13].

Recently, a new class of agents directed at the receptor activator of nuclear factor- κ B (RANK) RANKL/RANK/OPG pathway has been proposed for the treatment of osteoporosis and other degenerative bone diseases. This pathway consists of three key molecules that regulate the rate of bone remodeling. RANK ligand (RANKL) is an osteoblast-secreted protein that binds to RANK, a type I membrane receptor expressed on the surfaces of macrophage precursor cells. The binding of RANKL to RANK induces differentiation of these cells into mature osteoclasts. To mitigate bone loss, molecules targeting this pathway may reduce bone resorption by blocking this differentiation process. In vivo, bone homeostasis is maintained by osteoblasts, which also express a soluble decoy receptor, osteoprotegerin (OPG). Once secreted, OPG competes with RANK and inhibits RANKL activity [14]. The role of the RANKL/RANK/OPG system has been documented in a number of pathological processes ranging from Paget's disease to osteoporosis, and several therapeutic strategies have been developed [15].

Recombinant OPG injections, anti-RANK antibodies, and RANKL vaccines are all being explored as potential treatments in clinical and preclinical studies as potential treatments of disorders of high bone turnover [16]. Two phase III studies with Denosumab, a fully human monoclonal IgG2 anti-RANKL antibody (Amgen Corp, Thousand Oaks, CA), for treatment of postmenopausal women with or at risk for osteoporosis and for metastatic bone disease in cancer patients have been published [17,18]. These agents may be a potential therapeutic modality for children with OI; however, to date, there have been no clinical studies investigating this potential application. Denosumab does not recognize murine RANKL [19], so RANKL inhibition in the current study was achieved with a murine RANK-Fc fusion protein [20] that functions in a manner analogous to that of Denosumab.

The *oim/oim* mouse is an established model of moderate to severe OI, which is characterized by limb deformities, frequent fractures, osteopenia, and small size [21]. These mice have a naturally occurring mutation leading to a deficiency in pro α 2(I) collagen [21]. This model has been previously used to assess the effects of bisphosphonates in OI [22–25]. Heterozygous *oim/+* mice have also been used to test the effect of growth hormone therapy on bone quality

in OI [26]. Other animal models of OI exist including other murine models [27–30], and a model of OI in zebra fish [28,31]. Treatment with bisphosphonates has been most extensively tested in the *oim/oim* model [22–25] although it has also been tried in the *brtl/+* mice [32]. The bone abnormalities in the *oim/oim* model are more reproducible than the genetically engineered *brtl/+* mice making it more suitable for evaluation of therapies.

In these mice, ALN was shown to decrease the incidence of fractures while maintaining calcified cartilage in *oim/oim* mice treated during a period of infancy and active growth [22, 24]. It has also been shown that ALN affects growth plate morphology [23] and increases metaphyseal bone mass [25]. A recently published paper by Delos et al. reported the effects of RANKL inhibition on fracture healing and bone strength in *oim/oim* mice, demonstrating that the mechanical properties of healing bone were not adversely affected [33]. We chose to extend this study to assess the effect of murine RANK-Fc (RANKL inhibition) on juvenile mice, testing the drug's effect on fracture frequency, bone quality, bone density, and histologic parameters.

MATERIAL AND METHODS

Animals and RANK-Fc Treatment

All procedures were approved by the IACUC committee of the Hospital for Special Surgery. Homozygous *oim/oim* and wild-type (+/+) control mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The animals were housed up to four mice per cage (according to genotype and sex) in a light controlled environment (12 hr light-dark cycles). They were given autoclaved water and fed whole and powdered rodent diet (Purina, St. Louis, MO, USA). Weaning was carried out at 3 weeks for +/+ and at 4 weeks for *oim/oim* mice. Twenty *oim/oim* mice were sacrificed at 6 weeks of age to provide a baseline fracture number. Starting at 6 weeks of age, mice were randomized into groups of 17–20 and subcutaneously injected with either soluble RANK-Fc (generously provided by Amgen Inc., Thousand Oaks, CA, USA) or saline two times per week for 8 weeks. Three different doses of RANK-Fc were used: low (0.15 mg/kg), medium (1.5 mg/kg), and high (15 mg/kg) to establish efficacy (10–15 mice per group). These doses of RANK-Fc were selected based on prior studies showing reduction in osteoclast numbers [20,34].

Radiographic Analyses

Fracture Count—At sacrifice, whole body high resolution anterior-posterior radiographs were taken via Faxitron (Faxitron X-Ray, Wheeling, PA, USA) and fractures in the femora, humeri, and tibia counted by two independent investigators. A fracture was defined as present if there was evidence of a callus or obvious bone deformity.

Bone Geometry—Isolated femora were radiographed by faxitron in the anterior-posterior (AP) and medial-lateral (ML) planes at a resolution of 20 linear pixels/mm. Each image included an aluminum alloy step density standard for calibration. Femoral length in the AP view was determined as the distance from the tip of the femoral head to the base of the condyles. Although reliance on radiographs for density and geometric measurements are no longer state-of-the-art, previous work by our group has demonstrated significant correlations between cortical measurements performed by faxitron radiograph and by micro-CT [35]. Endosteal (d_e) and periosteal (d_p) diameters were measured at mid-diaphysis in the AP and ML radiographic views (AP view yields the ML diameter and vice versa). Cortical thickness was calculated as $\frac{1}{2}(d_p - d_e)$. The femoral cross section was assumed to be elliptical, and the moment of inertia, I , a measure of the distribution of material around a given axis, was calculated as:

$$I_{\text{ellipse}} = \frac{\pi}{4} \left[\left(\frac{APd_p}{2} \right) \left(\frac{MLd_p}{2} \right)^3 - \left(\frac{APd_e}{2} \right) \left(\frac{MLd_e}{2} \right)^3 \right]$$

where d_p in the AP view corresponds to the major diameter of the ellipse, and d_p in the ML view corresponds to the minor diameter. Radiographic intensity in the metaphyseal region was calculated in the AP view. For this measurement, the average intensity was obtained through a 1 mm² box drawn 2 mm from the bottom of the condyle. Each individual image was calibrated using the density standard steps with intensity units ranging from 1 to 5. All measurements were done using Image J software (NIH, Bethesda, MD, USA).

Micro-CT Analyses—Qualitative micro-CT analysis was performed on a subset of femora. The femora were scanned at a resolution of 19 μ m voxel resolution on an EVS micro-CT system (MS-8 in vitro specimen scanner, Enhanced Vision Systems, Ontario, CA). Images were thresholded using Gems Microview's (GE Medical Systems, Ontario) autothresholding function.

Histology

Selected femora were fixed in 80% ethanol and decalcified in 10% EDTA (pH 7.2–7.4) for a ~2 weeks. Tissues were embedded in paraffin, sectioned at 7 μ thickness along the coronal plane from anterior to posterior, and stained with alcian blue for qualitative assessment of calcified cartilage.

Biomechanics

Three-point bend tests were performed as previously described [25]. One femur from each mouse was used for mechanical testing. Prior to testing of the *oim/oim* mice, a radiograph was obtained to confirm that the femur utilized for testing contained no fractures or deformities ($n = 13$ –16). Three-point bend tests were conducted at room temperature using a closed-loop servohydraulic uniaxial MTS test frame (MTS Systems, Eden Prairie, MN, USA) with Instron electronic controls (Instron, Canton, MA, USA). The anterior surface of the femur was placed on the load supports, and the load was applied posteriorly at the middiaphysis. For this configuration, the posterior surface is in compression and the anterior surface experiences tension. Since there was less than a 3% difference in average bone length among specimens (15.25 ± 0.38 vs. 15.60 ± 0.19 for the *oim/oim* versus $+/+$ femora, respectively), a set distance of 8 mm was used for the outside loading points. The tests were performed at a rate of 0.5 mm/sec, and load and displacement data were acquired at 20 Hz with a PC equipped with a 12 bit A/D data acquisition board. Specimens were kept moist throughout testing.

Both structural (whole bone) properties and intrinsic material (tissue) properties of the bone were calculated using accepted formulas for bone testing [34,36]. For structural properties, maximum load was defined as the maximum load the bone sustained during testing, and displacement was assumed to be equal to the amount of crosshead deflection during testing. Stiffness was calculated as the slope of the linear region of the load-displacement curve. For material properties, the stress was calculated as:

$$\sigma = \frac{Mc}{I_{\text{ellipse}}}$$

where M is the moment (equal to $F \cdot L/4$), c is the distance from the neutral axis to the most anterior point on the bone cross-section (equal to $1/2 APd_p$ when approximating the cross-section to be elliptical), and I_{ellipse} is the moment of inertia. Strain was calculated as:

$$\varepsilon = \frac{12cd}{L^2}$$

where c is the medial-lateral outside radius ($1/2 MLd_p$), d is the crosshead displacement, and L is the span between the loading supports. Elastic modulus (the slope of the linear elastic region of the curve, a measure of the intrinsic stiffness of the material) and yield strength were determined from the resulting stress-strain curves. Toughness (a measure of the amount of energy needed to fracture the bone tissue) was calculated as the area under the stress-strain curve. Materials that sustain very little postyield strain before fracture are termed “brittle.” Thus the “brittleness” of the cortical bone, a material property, was calculated by dividing the yield strain (point of maximum elastic deformation) by the ultimate strain.

Statistics

Statistical analyses were performed with SigmaStat software (SPSS, Chicago, IL, USA). Means and standard deviations were calculated for each measured parameter. Two-way ANOVA was performed to test for the simultaneous effects of genotype and RANK-Fc treatment on the outcome variables. Bonferroni post-hoc tests were performed for comparison of groups with values significant at $p < 0.05$. Values were considered significantly different at $p < 0.05$ and tending toward significance at $p < 0.1$.

RESULTS

Dosing Study

Clinically abnormally high density of the metaphyseal region in growing subjects is associated with poor bone quality [36,37]. Therefore the optimal dose of RANK-Fc was defined as that which would result in increased density in the metaphyseal region of the *oim/oim* mice, without the presence of sclerotic bone. Micro-CT images (Figure 1) demonstrated insufficient increase in mineralization with the low dose and features of osteoclerosis with the high dose. Therefore the medium dose was chosen for the remainder of the study.

Body Weight

All treated animals in the medium dose group gained weight consistently. The $+/+$ mice weighed significantly more than *oim/oim* mice at the start and end of the treatment period. There were no differences in weight at sacrifice between the saline and RANK-Fc groups for either the $+/+$ or *oim/oim* mice (Figure 2).

Histology

As previously described, saline-treated *oim/oim* bone differed from $+/+$ controls in that the bone was less robust with delicate trabeculae. $+/+$ RANK-Fc-treated specimens demonstrated increased central cores of growth plate cartilage with persistent chondrocytes, Erlenmeyer flask deformity (lack of remodeling of the femoral dimetaphysis with abnormal cortical thinning and lack of the concave dimetaphyseal curve resulting in an Erlenmeyer flask-like appearance [38]) and decreased marrow space. *Oim/oim* RANK-Fc-treated bones also showed persistent central cores of growth plate cartilage in addition to thin trabecular structures and the Erlenmeyer flask deformity. The marrow space was compromised less in the *oim/oim* mice compared to the controls (Figure 3).

Radiographic Results

As expected, there were no fractures in either treated or untreated *+/+* mice. At 6 weeks of age, *oim/oim* mice had already sustained an average of 1.8 fractures per mouse. At the end of the treatment period, there was no difference in fracture incidence between the saline, and RANK-Fc-treated *oim/oim* mice (Figure 4). There were no differences in the type and location of fractures in the mice that received RANK-Fc or saline (data not shown).

Within *+/+* groups, femoral length decreased with RANKL inhibition, while it was not significantly decreased for the *oim/oim* mice (Table 1). Within both the *+/+* group and the *oim/oim* group, the radiographic metaphyseal density was significantly higher in the treated mice. Geometric measurements at middiaphysis in the *oim/oim* groups showed significant increases in the ML view of AP periosteal and endosteal diameters, as well as AP view ML cortical thickness, and a trend toward increased ML view AP cortical thickness and AP view ML femoral periosteal diameter in the RANK-Fc-treated group compared to the saline (Table 2). Within *+/+* groups, ML view AP cortical thickness and ML view AP femoral periosteal diameter were significantly increased with RANK-Fc while there was a trend to increased AP view ML femoral endosteal diameter (Table 2). Within saline, and RANK-Fc-treated groups, *oim/oim* mice had significantly lower metaphyseal density, smaller AP and ML femoral periosteal diameters, as well as AP and ML cortical thickness (Table 2). Within RANK-Fc-treated mice AP view ML femoral endosteal diameter was lower for *oim/oim* mice (Table 2).

Biomechanical Results

Extrinsic (Structural) Properties—Within the *oim/oim* groups, no treatment-related difference was observed for yield displacement, total displacement, or moment of inertia. However, stiffness was significantly greater for the RANK-Fc-treated compared to saline-treated mice and maximum load tended to increase. For the *+/+* groups, stiffness was also greater for the RANK-Fc-treated mice, and total displacement tended to increase while yield displacement, maximum load, and moment of inertia were unchanged (Table 3).

Intrinsic (Material) Properties—RANKL inhibition did not affect yield strength, yield strain, brittleness, or Young's modulus for either *+/+* or *oim/oim* mice. Additionally, there were no significant differences in total strain or toughness for the *oim/oim* mice. Within the *+/+* mice, total strain increased with treatment and toughness tended to increase (Table 3).

All extrinsic and intrinsic properties measured except for Young's modulus were significantly different between *+/+* and *oim/oim* mice for both saline and RANK-Fc (Table 3).

DISCUSSION

Antiresorptives are the most frequently used therapeutics for OI patients, in particular for children with OI [7]. In the current preclinical study, we examined the safety and efficacy of a RANKL inhibitor, RANK-Fc, in a growing mouse model of OI.

RANKL inhibition did not adversely affect weight gain or long bone growth over the 8-week treatment period in the *oim/oim* mice but it did lead to shortened femora in the *+/+* mice. A similar study with ALN performed in 6-week-old *oim/oim* mice led to decreased femoral length, but in a follow-up study of *oim/oim* mice treated starting at 2 weeks of age, this adverse affect was not found [22,24]. In the *+/+* mice the femoral periosteal diameter in the ML view increased significantly in the current study as it did in the *+/+* mice treated with ALN starting at 6 weeks of age, and as it tended to increase in the mice who were treated with ALN starting at 2 weeks of age [22,24]. In addition, *+/+* mice had significantly increased ML cortical thickness, similar to ALN mice in the earlier study [22]. Other similarities to ALN treatment

were found in the increased metaphyseal density and increased stiffness in *oim/oim* bones [24].

We had expected a decrease in the endosteal diameter because of osteoclast inhibition with decreased resorption, similarly to what happens with ALN [24], but this was not observed. We also did not observe the *oim/oim* bone becoming less brittle and the *+/+* bone more brittle with treatment as has been seen in studies with ALN [24]. In contrast to the ALN-treated mice which showed a reduction in fracture number, no significant differences were seen in the number of fractures in the *oim/oim* mice when treated with RANK-Fc. Thus, whereas RANKL inhibition improved some biomechanical and geometric properties of bone in the *oim/oim* mice in the current study, it did not decrease the fracture incidence.

Interestingly, a study by Delos et al. [33] that evaluated RANKL inhibition in fracture healing found that work to failure increased in the intact femora of treated *+/+* mice compared to saline controls. We did not find comparable results in the current study, but the same type of testing was not performed. Delos et al. [33] performed 4-point bend tests, while 3-point bend tests were performed in the current study. The 4-point bend test in the Delos et al. study resulted in a completely elastic fracture of the bones, with no region of plasticity evident. However, since this is not the case for the 3-point bend tests, the results of the two cannot be considered comparable. In the Delos study, no biomechanical differences were found in the *oim/oim* mice with treatment [33], and in the current study only one biomechanical property was affected by treatment. Nonetheless, both studies show that RANKL inhibition did not have any detrimental effects with respect to mechanical strength of *oim/oim* bones, which is important for OI patients who may suffer many fractures even with the best treatments.

RANKL inhibition resulted in increased calcified cartilage under the growth plate, similar to the effect seen with ALN treatment [22]. This is likely the effect of suppression of osteoclasts that results in the primary spongiosa not being resorbed, and could in fact be beneficial for bone strength in patients with OI [39], although some recent studies suggest that this may not be the case [40]. Similar findings were observed in iliac crest biopsies from children with OI treated with ALN. These areas of unresorbed cartilage seem to correspond to the white lines commonly seen in radiographs of long bones in patients who have received bisphosphonate treatment [39].

In contrast to the previous ALN studies in the *oim/oim* mouse model, the current study did not find reduced fracture incidence in *oim/oim* mice treated with RANK-Fc, despite increased metaphyseal density and bone strength. This could be due to inadequate power to determine a fracture difference given the large standard deviations. For a power of 0.8 to detect a 50 % difference in fracture incidence 21 mice per group were required, and to detect a 25 % difference in fracture incidence, 79 mice per group were required. It is well known from a number of preclinical and clinical studies that bone mineral density (BMD) changes are not predictive of fracture risk. A clinical study of two different bisphosphonates, ALN and risedronate, showed that although the former produced a greater increase in BMD, the two therapies were similar in reducing fracture risk in a population of over 1000 postmenopausal women [41,42]. A meta-analysis of trials of antiresorptives in osteoporosis showed that the magnitude of reductions in nonvertebral fracture risk was not associated with the magnitude of increased in BMD after 1 year of therapy [43].

There are a few recent studies that indicate that in addition to osteoclast inhibition, bisphosphonates may have other effects beneficial to bone such as prevention of osteocyte and osteoblast apoptosis [44,45]. Although we did not investigate cell function in the current study, the absence of an effect on fracture reduction by RANKL blockade possibly could be explained by a lack of effect on osteoblast and osteocyte apoptosis. That being said, one of the few studies

directly comparing the effects of a RANKL inhibitor versus a bisphosphonate on bone strength reported improvements in femur bending strength with OPG-Fc but not with ALN [46]. There are no published data suggesting significant differences in bone material properties with RANKL inhibitors versus bisphosphonates.

An alternative explanation of the lack of therapeutic efficacy with respect to fracture incidence concerns the number of fractures prior to the start of treatment. The mice in the current study accumulated most of their fractures prior to the start of the treatment period, which is clearly a limitation of the study. The increase in fracture number over the 8 week period among both treated and untreated mice was small compared to the number of fractures they already sustained by 6 weeks (1.8 fractures per mouse at baseline and 2.5 fractures per mouse in the RANK-Fc and saline-treated mice after 8 weeks of treatment). It is known that patients with OI, especially those with more severe disease, fracture more frequently as children than they do in later life [47]. This may be due to naturally increasing bone density with age, and especially with puberty. This phenomenon has also been confirmed in the Brittle IV mouse model of OI [29]. We did not treat our mice during the period of earliest infancy when they may be most susceptible to fractures because of uncertainty regarding how they would tolerate RANK-Fc at that early age.

Another important aspect with respect to fracture incidence concerns the fact that RANKL inhibition delays callus resorption [48]. Therefore, calluses in RANK-Fc-treated mice may still be apparent on a faxitron, whereas those fractures in their saline-treated counterparts may have healed with full callus resolution by the time of sacrifice. Consequently, the data presented here do not give us the definitive answer as to the potential of RANKL inhibitors to reduce fractures. Further studies should be performed where RANKL inhibitors are administered to younger animals, e.g., 2 weeks of age, similar to the McCarthy et al. ALN study [24]. Nonetheless, this initial study has demonstrated no obvious adverse effects of RANK-Fc in OI mice at the administered dose. Increments in BMD with RANK-Fc were numerically similar to increases found previously with ALN treatment using a similar study design. RANK-Fc improved certain femoral geometry parameters and improved femur bending strength and certain bone material properties. These factors together would support the further investigation of RANKL inhibitors as therapeutic agents for OI patients.

Acknowledgments

The authors thank Ram Posham for help with analysis and statistics. This study utilized the facilities of the NIH sponsored Musculoskeletal Repair and Regeneration Core Center, AR046121. Dr. Pleshko received support from NIH AR48337. The first two authors contributed equally to the work on this project.

References

1. Rauch F, Glorieux FH. Osteogenesis imperfecta. *Lancet* 2004;363:1377–1385. [PubMed: 15110498]
2. Gatti D, Antoniazzi F, Prizzi R, et al. Intravenous neridronate in children with osteogenesis imperfecta: a randomized controlled study. *J Bone Miner Res* 2005;20:758–763. [PubMed: 15824848]
3. Glorieux FH, Bishop NJ, Plotkin H, et al. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. *N Engl J Med* 1998;339:947–952. [PubMed: 9753709]
4. Letocha AD, Cintas HL, Troendle JF, et al. Controlled trial of pamidronate in children with types III and IV osteogenesis imperfecta confirms vertebral gains but not short-term functional improvement. *J Bone Miner Res* 2005;20:977–986. [PubMed: 15883638]
5. Madenci E, Yilmaz K, Yilmaz M, et al. Alendronate treatment in osteogenesis imperfecta. *J Clin Rheumatol* 2006;12:53–56. [PubMed: 16601536]
6. Sakkers R, Kok D, Engelbert R, et al. Skeletal effects and functional outcome with olpadronate in children with osteogenesis imperfecta: a 2-year randomised placebo-controlled study. *Lancet* 2004;363:1427–1431. [PubMed: 15121405]

7. Glorieux FH. Experience with bisphosphonates in osteogenesis imperfecta. *Pediatrics* 2007;119(suppl 2):S163–S165. [PubMed: 17332237]
8. Vallo A, Rodriguez-Leyva F, Rodriguez Soriano J. Osteogenesis imperfecta: anthropometric, skeletal and mineral metabolic effects of long-term intravenous pamidronate therapy. *Acta Paediatr* 2006;95:332–339. [PubMed: 16497645]
9. Munns CF, Rauch F, Mier RJ, et al. Respiratory distress with pamidronate treatment in infants with severe osteogenesis imperfecta. *Bone* 2004;35:231–234. [PubMed: 15207762]
10. Rauch F, Munns C, Land C, et al. Pamidronate in children and adolescents with osteogenesis imperfecta: effect of treatment discontinuation. *J Clin Endocrinol Metab* 2006;91:1268–1274. [PubMed: 16434452]
11. Land C, Rauch F, Glorieux FH. Cyclical intravenous pamidronate treatment affects metaphyseal modeling in growing patients with osteogenesis imperfecta. *J Bone Miner Res* 2006;21:374–379. [PubMed: 16491284]
12. Pizones J, Plotkin H, Parra-Garcia JI, et al. Bone healing in children with osteogenesis imperfecta treated with bisphosphonates. *J Pediatr Orthop* 2005;25:332–335. [PubMed: 15832149]
13. Chan B, Zacharin M. Maternal and infant outcome after pamidronate treatment of polyostotic fibrous dysplasia and osteogenesis imperfecta before conception: a report of four cases. *J Clin Endocrinol Metab* 2006;91:2017–2020. [PubMed: 16551739]
14. Teitelbaum SL. Osteoclasts: what do they do and how do they do it? *Am J Pathol* 2007;170:427–435. [PubMed: 17255310]
15. de Vernejoul MC. Sclerosing bone disorders. *Best Prac Res Clin Rheumatol* 2008;22:71–83.
16. Tanaka S, Nakamura K, Takahasi N, et al. Role of RANKL in physiological and pathological bone resorption and therapeutics targeting the RANKL-RANK signaling system. *Immunol Rev* 2005;208:30–49. [PubMed: 16313339]
17. Bone HG, Bolognese MA, Yuen CK, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. *J Clin Endocrinol Metab* 2008;93:2149–2157. [PubMed: 18381571]
18. Brown JP, Prince RL, Deal C, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. *J Bone Miner Res* 2009;24:153–161. [PubMed: 18767928]
19. Kostenuik PJNH, McCabe J, Warmington K, Kurahara C, Sun N, Chen C, Li L, Cattley RC, Van G, Scully S, Elliott R, Grisanti M, Morony S, Tan HL, Asuncion F, Li X, Ominsky MS, Stolina M, Dwyer D, Dougall WC, Hawkins N, Boyle WJ, Simonet WS, Sullivan JK. Denosumab, a fully human monoclonal antibody to RANKL, inhibits bone resorption and increases bone density in knock-in mice that express chimeric (human/murine) RANKL. *J Bone Miner Res* 2009;24:182–195. [PubMed: 19016581]
20. Miller RE, Branstetter D, Armstrong A, et al. Receptor activator of NF-kappa B ligand inhibition suppresses bone resorption and hypercalcemia but does not affect host immune responses to influenza infection. *J Immunol* 2007;179:266–274. [PubMed: 17579046]
21. Chipman SD, Sweet HO, McBride DJ Jr, et al. Defective pro alpha 2(I) collagen synthesis in a recessive mutation in mice: a model of human osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1993;90:1701–1705. [PubMed: 8446583]
22. Camacho NP, Raggio CL, Doty SB, et al. A controlled study of the effects of alendronate in a growing mouse model of osteogenesis imperfecta. *Calcif Tissue Int* 2001;69:94–101. [PubMed: 11683430]
23. Evans KD, Lau ST, Oberbauer AM, et al. Alendronate affects long bone length and growth plate morphology in the oim mouse model for osteogenesis Imperfecta. *Bone* 2003;32:268–274. [PubMed: 12667554]
24. McCarthy EA, Raggio CL, Hossack MD, et al. Alendronate treatment for infants with osteogenesis imperfecta: demonstration of efficacy in a mouse model. *Pediatr Res* 2002;52:660–670. [PubMed: 12409511]
25. Misof BM, Roschger P, Baldini T, et al. Differential effects of alendronate treatment on bone from growing osteogenesis imperfecta and wild-type mouse. *Bone* 2005;36:150–158. [PubMed: 15664013]

26. King D, Jarjoura D, McEwen HA, et al. Growth hormone injections improve bone quality in a mouse model of osteogenesis imperfecta. *J Bone Miner Res* 2005;20:987–993. [PubMed: 15883639]
27. Bonadio J, Jepsen KJ, Mansoura MK, et al. A murine skeletal adaptation that significantly increases cortical bone mechanical properties. Implications for human skeletal fragility. *J Clin Invest* 1993;92:1697–1705. [PubMed: 8408623]
28. Kamoun-Goldrat AS, Le Merrer MF. Animal models of osteogenesis imperfecta and related syndromes. *J Bone Miner Metab* 2007;25:211–218. [PubMed: 17593490]
29. Kozloff KM, Carden A, Bergwitz C, et al. Brittle IV mouse model for osteogenesis imperfecta IV demonstrates postpubertal adaptations to improve whole bone strength. *J Bone Miner Res* 2004;19:614–622. [PubMed: 15005849]
30. Sillence DO, Ritchie HE, Dibbayawan T, et al. Fragilitas ossium (fro/fro) in the mouse: a model for a recessively inherited type of osteogenesis imperfecta. *Am J Med Genet* 1993;45:276–283. [PubMed: 8456819]
31. Fisher S, Jagadeeswaran P, Halpern ME. Radiographic analysis of zebrafish skeletal defects. *Dev Biol* 2003;264:64–76. [PubMed: 14623232]
32. Uveges TE, Kozloff KM, Ty JM, Ledgard F, Raggio CL, Gronowicz G, Goldstein SA, Marini JC. Alendronate treatment of Brl osteogenesis imperfecta mouse improves geometry and load response before fracture but decreases predicted material properties and has detrimental effects on osteoblasts and bone formation. *J Bone Miner Res* 2009;24(5):849–59. [PubMed: 19113917]
33. Delos D, Yang X, Ricciardi BF, et al. The effects of RANKL inhibition on fracture healing and bone strength in a mouse model of osteogenesis imperfecta. *J Orthop Res* 2008;26:153–164. [PubMed: 17729310]
34. Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. *Bone* 1993;14:595–608. [PubMed: 8274302]
35. Huang, ARC.; Fritton, J.; Camacho, N. Comparison of radiographic and micro-CT determined parameters in mouse bone specimens. 51st annual meeting of the Orthopaedic Research Society; 2005.
36. Van Der Meulen MC, Jepsen KJ, Miki B. Understanding bone strength: size isn't everything. *Bone* 2001;29:101–104. [PubMed: 11502469]
37. Van Slyke MA, Marks SC Jr. Failure of normal osteoclasts to resorb calcified cartilage from osteosclerotic (oc/oc) mice in vitro. *Bone* 1987;8:39–44. [PubMed: 3580216]
38. Faden MA, Krakow D, Ezgu F, et al. The Erlenmeyer flask bone deformity in the skeletal dysplasias. *Am J Med Genet* 2009;149A(6):1334–45. [PubMed: 19444897]
39. Cho TJ, Choi IH, Chung CY, et al. Efficacy of oral alendronate in children with osteogenesis imperfecta. *J Pediatr Orthop* 2005;25:607–612. [PubMed: 16199940]
40. Rauch F, Cornibert S, Cheung M, et al. Long-bone changes after pamidronate discontinuation in children and adolescents with osteogenesis imperfecta. *Bone* 2007;40:821–827. [PubMed: 17223617]
41. Bonnick S, Saag KG, Kiel DP, et al. Comparison of weekly treatment of postmenopausal osteoporosis with alendronate versus risedronate over two years. *J Clin Endocrinol Metab* 2006;91:2631–2637. [PubMed: 16636120]
42. Rosen CJ, Hochberg MC, Bonnick SL, et al. Treatment with once-weekly alendronate 70 mg compared with once-weekly rise-dronate 35 mg in women with postmenopausal osteoporosis: a randomized double-blind study. [see comment]. *J Bone Miner Res* 2005;20:141–151. [PubMed: 15619680]
43. Delmas PD, Seeman E. Changes in bone mineral density explain little of the reduction in vertebral or nonvertebral fracture risk with anti-resorptive therapy. [see comment]. *Bone* 2004;34:599–604. [PubMed: 15050889]
44. Plotkin LI, Aguirre JI, Kousteni S, et al. Bisphosphonates and estrogens inhibit osteocyte apoptosis via distinct molecular mechanisms downstream of extracellular signal-regulated kinase activation. *J Biol Chem* 2005;280:7317–7325. [PubMed: 15590626]
45. Plotkin LI, Weinstein RS, Parfitt AM, et al. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *J Clin Invest* 1999;104:1363–1374. [PubMed: 10562298]

46. Samadfam R, Xia Q, Goltzman D. Co-treatment of PTH with osteoprotegerin or alendronate increases its anabolic effect on the skeleton of oophorectomized mice. *J Bone Miner Res* 2007;22:55–63. [PubMed: 17014384]
47. Albright JA, Grunt JA. Studies of patients with osteogenesis imperfecta. *J Bone Joint Surg Amer* 1971;53:1415–1425. [PubMed: 5114704]
48. Flick LM, Weaver JM, Ulrich-Vinther M, et al. Effects of receptor activator of NFkappaB (RANK) signaling blockade on fracture healing. *J Orthop Res* 2003;21:676–684. [PubMed: 12798068]

FIG. 1.

Micro-CT images of femora at the end of 8 weeks of treatment. Insufficient increase in density of the distal femur is seen with the low dose RANK-Fc (0.15 mg/kg), moderate increase in density with medium dose RANK-Fc (1.5 mg/kg), and sclerotic-like changes with the high dose of RANK-Fc (15 mg/kg). In both $+/+$ [WT] and oim/oim [oim] mice.

* Significantly different compared to same treatment +/+ ($p < 0.05$)

FIG. 2.

Average weights in +/+ and *oim/oim* mice at the end of treatment at 15 weeks of age. There were no significant differences in adult weight of +/+ or *oim/oim* mice with RANK-Fc treatment.

FIG. 3. RANKL inhibition results in retained cartilage in $+/+$ and *oim/oim* animals, Erlenmeyer flask deformity, and decreased marrow space. Hematoxylin and eosin staining. 5x and 25x Magnification. A = $+/+$ saline, B = $+/+$ RANK-Fc, C = *oim/oim* saline, D = *oim/oim* RANK-Fc.

FIG. 4.

Average number of fractures per mouse (*oim/oim*) at the end of treatment. There were no significant differences in the number of fractures sustained by mice in the treatment versus the control group. Of note is the relatively high number of fractures at baseline.

TABLE 1

Average femur length after 8 weeks of treatment

Genotype	Treatment	Femur Length (mm)	No. of Animals
+/+	Saline	15.54 ± 0.27	20
+/+	RANK-Fc	15.19 ± 0.35*	19
<i>oim/oim</i>	Saline	14.48 ± 0.28◇	18
<i>oim/oim</i>	RANK-Fc	14.42 ± 0.56◇	17

* Significantly different compared to same genotype saline ($p < 0.05$).

◇ Significantly different compared to same treatment +/+ ($p < 0.05$).

TABLE 2Geometrical properties of bone after 8 weeks of treatment (mean $\pm$ SD)

	+/+ Saline	+/+ RANK-Fc	<i>oim/oim</i> Saline	<i>oim/oim</i> RANK-Fc
Metaphyseal density	1.44 $\pm$ 0.15	2.68 $\pm$ 0.40 *	1.08 $\pm$ 0.05 *	1.87 $\pm$ 0.29 *,•
ML femoral endosteal diameter, AP view(mm)	1.05 $\pm$ 0.12	1.11 $\pm$ 0.11 ◇	0.99 $\pm$ 0.11	1.02 $\pm$ 0.1 *
ML femoral periosteal diameter, AP view(mm)	1.69 $\pm$ 0.16	1.73 $\pm$ 0.14	1.41 $\pm$ 0.11 *	1.49 $\pm$ 0.15 ◇
ML cortical thickness AP view(mm)	0.32 $\pm$ 0.04	0.31 $\pm$ 0.04	0.21 $\pm$ 0.02 *	0.24 $\pm$ 0.05 *,•
AP femoral endosteal diameter, ML view(mm)	0.65 $\pm$ 0.07	0.66 $\pm$ 0.08	0.61 $\pm$ 0.07	0.65 $\pm$ 0.05 *
AP femoral periosteal diameter, ML view(mm)	1.22 $\pm$ 0.09	1.27 $\pm$ 0.09 *	1.03 $\pm$ 0.06 *	1.13 $\pm$ 0.07 *,•
AP cortical thickness, ML view(mm)	0.28 $\pm$ 0.03	0.31 $\pm$ 0.03 *	0.21 $\pm$ 0.03 *	0.24 $\pm$ 0.04 ◇

* Significantly different compared to same genotype saline ($p < 0.05$).

◇ Trending to significance compared to same genotype saline ($p = 0.05-0.1$).

• Significantly different compared to same treatment +/+ ($p < 0.05$).

TABLE 3

Biomechanical data: structural and material properties of femora after 8 week of treatment

	+/+ Saline	+/+ RANK-Fc	<i>oim/oim</i> Saline	<i>oim/oim</i> RANK-Fc
Number of samples	20	19	16	13
Yield strength (kPa)	172.17 ± 25.95	168.91 ± 30.64	121.86 ± 26.35*	124.32 ± 27.66*
Yield strain	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.01*	0.02 ± 0.01*
Yield displacement (mm)	0.24 ± 0.03	0.24 ± 0.06	0.16 ± 0.05*	0.15 ± 0.04*
Total displacement (mm)	0.5 ± 0.12	0.62 ± 0.28◇	0.18 ± 0.08*	0.15 ± 0.04*
Total strain	0.09 ± 0.02	0.12 ± 0.05*	0.03 ± 0.01*	0.03 ± 0.01*
Max load (N)	27.01 ± 6.18	29.83 ± 7.26	11.60 ± 2.33*	15.50 ± 3.21◇
Moment of inertia (mm ⁴)	0.14 ± 0.05	0.16 ± 0.05	0.07 ± 0.01*	0.09 ± 0.03*
Young's modulus (GPa)	6678 ± 1446	6849 ± 1620	6257 ± 1786	6773 ± 1430
Brittleness (%elasticity)	54.27 ± 16.96	47.85 ± 26.37	96.86 ± 10.14*	99.76 ± 0.87*
Toughness (N*mm)	11.59 ± 3.22	13.57 ± 5.44◇	1.94 ± 0.88*	1.91 ± 0.77*
Stiffness (N/mm)	182.22 ± 41.61	220.87 ± 43.54*	85.88 ± 19.55*	130.15 ± 23.63*,*

* Significantly different compared to same genotype saline ($p < 0.05$).◇ Trending to significance compared to same genotype saline ($p = 0.05-0.1$).* Significantly different compared to same treatment +/+ ($p < 0.05$).