Published in final edited form as:

Osteoporos Int. 2012 March ; 23(3): 1141–1150. doi:10.1007/s00198-011-1742-7.

Comparable outcomes in fracture reduction and bone properties with RANKL inhibition and alendronate treatment in a mouse model of osteogenesis imperfecta

R Bargman^{1,*}, R Posham², AL Boskey², E DiCarlo², C Raggio², and N Pleshko^{2,3}

R Bargman: rbargman@numc.edu; R Posham: rp1126@nyu.edu; AL Boskey: boskeya@hss.edu; E DiCarlo: dicarlo@hss.edu; C Raggio: raggio@hss.edu; N Pleshko: npleshko@temple.edu

¹NYPH-Weill Cornell Medical Center, New York, NY

²Hospital for Special Surgery, New York, NY

³Temple University, Philadelphia, PA

Abstract

We report a direct comparison of RANKL inhibition (RANK-Fc) with bisphosphonate treatment (ALN) from infancy through early adulthood in a mouse model of Osteogenesis Imperfecta. Both ALN and RANK-Fc decreased fracture incidence to the same degree with increases in metaphyseal bone volume via increased number of thinner trabeculae.

The potential therapeutic benefit of RANKL inhibitors in OI is under investigation. We report a direct comparison of RANKL inhibition (RANK-Fc) with bisphosphonate treatment (alendronate; ALN) from infancy through early adulthood in a model of OI, the *oim/oim* mouse. Two week old *oim/oim*, *oim/+* and wildtype (+/+) mice were treated with RANK-Fc 1.5 mg/kg twice per week, ALN 0.21 mg/kg/week or saline (n = 12-20 per group) for 12 weeks. ALN and RANK-Fc both decreased fracture incidence (9.0 ± 3.0 saline 4.4 ± 2.7 ALN, 4.3 ± 3.0 RANK-Fc fractures per mouse). Serum TRACP-5b activity decreased to 65% after 1 month in all treated mice, but increased to 130-200% at sacrifice with RANK-Fc. Metaphyseal density was significantly increased with ALN in +/+ and *oim/oim* mice (p < 0.05) and tended to increase with RANK-Fc in +/+ mice. No changes in *oim/oim* femur biomechanical parameters occurred with treatment. Both ALN and RANK-Fc significantly increased trabecular number (ie 3.73±0.77 1/mm for *oim/oim* saline vs 7.93±0.67ALN and 7.34±1.38 RANK-Fc) and decreased trabecular thickness (ie 0.045 mm ±0.003 for *oim/oim* saline vs 0.034±0.003 ALN and 0.032±0.002RANK-Fc) and separation in all genotypes (ie 0.28±0.08 mm for *oim/oim* saline vs 0.12±0.010 ALN and 13±0.03 RANK-Fc), with significant increase in bone volume fraction (BVF) with ALN, and a trend towards increased BVF in RANK-Fc. Treatment of *oim/oim* mice with either a bisphosphonate or a RANK-Fc causes similar decreases in fracture incidence with increases in metaphyseal bone volume via increased number of thinner trabeculae.

Keywords

RANK/RANKL; RANK-Fc; Alendronate; *oim/oim*; mouse; osteogenesis imperfecta

*Address for Correspondence: Renee Bargman, MD, 2201 Hempstead Turnpike, East Meadow, NY 11554, Telephone number (516) 572 6177, Fax number (516) 572 5483, rbargman@numc.edu.

Disclosures: The authors listed above declare no conflict of interests.

Introduction

Osteogenesis imperfecta (OI) is a genetic disorder of abnormal bone formation which leads to increased bone fragility. OI's manifestations range from extreme bone fragility leading to perinatal lethality in some cases, dozens of lifetime fractures and shortened lifespan in others, to a relatively benign condition with few lifetime fractures in mild OI [1]. Currently, moderate and severe cases of OI are treated with bisphosphonates which reduce bone turnover and increase bone density in the affected patients, leading to improvements in bone pain, strength, reduced fractures and mild improvements in height [2-9]. Although the bisphosphonates studied to date have been generally helpful in children with OI, some recent studies have shown no decrease in fracture incidence with treatment [10], and the search for other agents that may be more beneficial continues.

There has been recent interest in a new class of drugs targeting the receptor activator of nuclear factor- κ B (RANK) RANKL/RANK/OPG pathway in the treatment of osteoporosis and other disorders of excessive bone turnover and low bone mineral density (BMD) [16]. Denosumab, a fully human monoclonal IgG2 anti-RANKL antibody, has been successfully tested in Phase III clinical trials and approved by the U.S. Food and Drug Administration for clinical use for the treatment and prevention of osteoporosis in postmenopausal women and in metastatic disease in cancer patients [18,19]. To date there have not been any clinical studies evaluating the use of Denosumab in the pediatric population, or in OI. Denosumab is primate-specific and cannot be tested in most animal models except in knock-in mice expressing murine/human RANK [20]. OPG or soluble RANK-Fc, which have similar mechanisms of action, have been used as surrogates for Denosumab in most animal experiments [21-33].

The *oim/oim* mouse is an established model of moderate to severe OI that contains a naturally occurring mutation leading to deficiency of proc α 2(I) collagen chains. These mice are characterized by frequent fractures, small size, osteopenia and bone deformities [34]. Heterozygous *oim/+* mice have osteopenia but typically no spontaneous fractures and have been used as a model of mild OI [35]. Homozygous *oim/oim* mice have been used in a number of studies to evaluate the effect of bisphosphonates [36-38] and RANKL inhibition in OI [24,39]. The bisphosphonate alendronate (ALN) has been shown to increase BMD, alter geometric and biomechanical properties of *oim/oim* bone and reduce fractures in these mice [36-38] whereas RANKL inhibition was also found to increase BMD and alter geometric and biomechanical properties [24,40] but to have no discernible effect on fracture incidence [39]. It was hypothesized that the lack of fracture reduction with RANKL inhibition was due to a relatively late start (6 weeks of age) of treatment and a high baseline number of fractures in the previous study [39,40]. In the current study, we directly compare bisphosphonate therapy and RANKL inhibition in neonatal *oim/oim*, *oim/+* and wildtype (+/+) mice to assess whether they are equally effective in decreasing fracture incidence and improving bone properties.

Materials and Methods

Animals and treatment

All procedures were approved by the IACUC committee of the Hospital for Special Surgery. Homozygous *oim/oim*, heterozygous *oim/+* and wildtype (+/+) control mice were obtained from Jackson Laboratories (Bar Harbor, ME). The animals were housed up to six mice per cage (according to genotype and sex) in a light controlled environment (12h light-dark cycles). They were given autoclaved water and fed whole and powdered Rodent diet mixture (Purina, St. Louis, MO). Starting at 2 weeks of age, mice were randomized by cage into groups of 6-16 and subcutaneously injected with either soluble RANK-Fc (generously

provided by Amgen Inc., Thousand Oaks, CA) at 1.5mg/kg twice a week, ALN 0.21mg/kg once a week and saline once a week, or saline twice per week for 12 weeks. Doses of ALN and RANK-Fc were based on results of previous studies that showed increased BMD without side-effects when the same doses were used [24,36,38]. Weaning was carried out at 3 weeks, and animals were weighed weekly. All animals were sacrificed by CO inhalation 1 week after the last injection.

Radiographic Analyses

Fracture Count—At sacrifice, whole body high resolution AP radiographs were obtained via Faxitron (Lincolnshire, IL) and fractures in the femora, tibiae, humeri, radii and individual tail bones counted by two independent investigators. Given that the majority of fractures seen in *oim/oim* mice occur in the long bones and in the tail, and the difficulty of assessing vertebral and rib fractures on the faxitrons, only long bone and tail fractures were counted. A fracture was defined based on 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) views 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. Endosteal (d_e) and periosteal (d_p) diameters were measured at mid-diaphysis in the AP and ML radiographic views. Cortical thickness was calculated as $\frac{1}{2}(d_p - d_e)$ and average cortical thickness was derived from the AP and ML views. 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 condyles. Each individual image was calibrated used a density standard with intensity units ranging from 1 to 5. All measurements were done using Image J software (NIH, Bethesda, MD).

Biomechanics—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.

From the measured geometry at the mid-diaphysis, the femoral cross-section was assumed to be elliptical. The area moment of inertia was calculated as:

$$I = \frac{\pi}{64} [D_{MLp} \times D_{APp}^3 - D_{MLE} \times D_{APE}^3]$$

where D_{MLp} and D_{APp} are the periosteal diameters in the medial-lateral and anterior-posterior views, and D_{MLE} and D_{APE} are endosteal diameters in the medial-lateral and anterior-posterior view.

Three-point bending tests of the femurs were performed as previously described [41]. The femur was positioned on two supports with a span width (L) between supports of 7.0mm. The central load was applied midway between the supports on the mid-diaphysis and anterior surface at a displacement rate of 0.05 mm/s using a materials test system (ELF3200, Bose Corp, Eden Prairie, MN). Whole bone structural properties determined included maximum load (F_{max}), structural stiffness (k), and bending stiffness (EI). Structural stiffness was calculated as the slope of the linear ascending portion of the load-displacement curve. Bending stiffness was calculated as:

$$EI = \frac{kL^3}{48}$$

The material properties obtained were: Young's modulus (E); total strain, brittleness, yield stress (σ_y); and total energy to failure. Stress was calculated as:

$$\sigma = \frac{D_{AP_p} FL}{8I}$$

where F is force during loading. Strain was calculated as:

$$\varepsilon = \frac{6D_{AP_p} d}{L^2}$$

where d is displacement during loading. Young's modulus was calculated as the slope of the linear ascending region of the stress-strain curve. By plotting a line parallel to the linear region with a 0.2% strain offset, yield was determined as the intersection point of the offset line with the stress-strain curve. Total strain was determined to be strain at failure, and post-yield strain was strain after yield until failure. Yield strain divided by ultimate strain * 100 was calculated and termed "brittleness". Total energy to failure was calculated as the area under the stress-strain curve.

Serum chemistry—Osteoclast (TRACP-5b) activity was evaluated at 4 and 8 weeks after the initiation of treatment in $+/+$ and $oim/+$ mice, and in all mice at sacrifice. To evaluate short versus long term activity of the RANK-Fc, in 7 mice, the 4 and 8 week specimens were obtained 5 hours after RANK-Fc administration while in the rest bleeds were done without regard for the timing of the last injection (random). Blood was obtained via retro-orbital bleeds under isoflurane anesthesia in the live mice and by cardiac puncture at sacrifice. Blood was collected into serum separator tubes, centrifuged (speed, time) after 30 minutes and frozen at -80 degrees. The specimens were analyzed with the TRACP-5b assay (IDS inc, Fountain Hills, Az).

Micro CT Bone Architecture Analyses—Microcomputed tomographic analysis (micro CT) was performed on a subset of femora. The femora were scanned at a resolution of 6 μm using a Scanco-35 microCT (Scanco USA, Inc, Southeastern PA, USA). Each scan included a phantom containing air, saline and a bone reference material (1.18 g/cm^3) for conversion of Hounsfield units to mineral density in g/cm^3 . Reconstruction of the individual projections to computed tomography volume data was performed using instrument software. Specimen-specific thresholds were determined by first selecting a volume of interest, generating the attenuation histogram, and determining the threshold that segments mineralized tissue from background. Analyses of the reconstructed scans was also performed using instrument software. Properties determined included trabecular and cortical bone mineral density (BMD), cortical diameter, trabecular number, trabecular and cortical thickness, trabecular separation, bone surface to bone volume ratio (BS/BV), and bone volume fraction (bone volume to total volume ratio, BVF) [42].

Histology—Selected femora for histology were fixed in 90% formalin and decalcified in 10% EDTA (pH 7.2-7.4) for approximately 2 weeks. Tissues were embedded in paraffin, sectioned at 7 microns thickness along the coronal plate from anterior to posterior and stained with Hematoxylin and Eosin, Alcian blue for calcified cartilage visualization, and TRAP for osteoclasts [43].

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 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 towards significance at $p < 0.08 > 0.05$.

Results

Body weight

The $+/+$ and the $oim/+$ mice weighed significantly more than oim/oim mice at 2 weeks of age. There were no significant differences in weight gain among the saline, ALN or RANK-Fc groups for any genotype for the duration of the study (data not shown). However, at sacrifice (~15 weeks of age), the saline and ALN oim/oim mice, but not the RANK-Fc oim/oim mice, weighed significantly less than the $+/+$ or $oim/+$ mice (Figure 1).

Radiographic results

As expected, there were no fractures in any $+/+$ or $oim/+$ mice with or without treatment. In the oim/oim mice, the number of fractures was significantly decreased to the same degree with both ALN and RANK-Fc treatment; the saline group sustained 9.0 ± 3.0 fractures per mouse, and the ALN and RANK-Fc treated mice had 4.4 ± 2.7 and 4.3 ± 3.0 fractures per mouse respectively (Figure 2). There were no differences in the type and location of fractures in the mice that received RANK-Fc, ALN or saline (data not shown).

Serum TRACP-5b analysis

There was no difference in TRACP-5b values in mice whose blood was drawn 5 hours after injection versus those whose blood was drawn at random timepoints at either 1 or 2 months after the start of treatment. After 1 month of treatment, TRACP-5b levels were significantly reduced in $+/+$ and $oim/+$ mice treated with RANK-Fc compared to $+/+$ mice treated with saline (Figure 3A). At sacrifice (1 week after the last treatment), $oim/+$ and oim/oim mice treated with RANK-Fc had significantly higher TRACP-5b levels than those who were treated with either saline or ALN (Figure 3B). In addition, oim/oim mice treated with RANK-Fc had higher TRACP-5b levels than $+/+$ mice treated with the same agent, and ALN-treated oim/oim mice had significantly higher values than ALN treated $+/+$ or $oim/+$ mice (Figure 3B).

Histology

Oim/oim mice had thin cortices, fewer than normal thin trabeculae in the metaphysis, and increased osteocytic density compared to $+/+$ mice (Figure 4G1). *Oim/+* mice had histologic features that were not appreciably different from those of $+/+$ animals (Figure 4D1). TRAP staining did not appear to be diminished with treatment for any genotype (Figure 4 A2-I2). ALN treatment led to a retention of primary spongiosa for up to one third of the length of the diaphysis and fusiform deformity in all genotypes (Figure 4B1, 4E1 and 4H1). RANK-Fc treatment also caused retention of primary spongiosa for approximately one half of the

length of the diaphysis, and caused a more pronounced fusiform deformity (Figure 4C1, 4F1 and 4I1).

Tissue Density and Bone Geometry

Saline-treated *oim/oim* femurs were shorter than saline treated *+/+* and *oim/+* femurs, and ALN-treated *oim/oim* femurs were shorter than ALN-treated *+/+* femurs (Table 1). Metaphyseal and cortical densities were greater in *+/+* compared to *oim/oim* femurs for all treatment groups. Metaphyseal density significantly increased with ALN treatment in *+/+* and *oim/oim* mice while it tended to increase with RANK-Fc treatment in *+/+* animals (Table 1).

Biomechanics

Maximum load, stiffness and Young's modulus were greater in *+/+* and *oim/+* animals compared to *oim/oim* mice with all three treatments (Table 2). Brittleness was greater in *oim/oim* mice that received saline and ALN compared to *+/+* and *oim/+* animals, and in saline treated *oim/+* mice compared to *+/+* controls. RANK-Fc treatment also increased brittleness in *oim/+* compared to those treated with saline or ALN. Finally, energy to failure was lower in *oim/oim* than *+/+* in all treatment groups (Table 2).

MicroCT Bone Architecture Parameters

Trabecular bone—BVF was lower in *oim/oim* compared to *+/+* and *oim/+* femurs within all treatment groups (Table 3). Trabecular architecture also differed, with *oim/oim* mice having thinner trabeculae relative to *+/+* mice with all treatments (Figure 5, Table 3). ALN and RANK-Fc treatment increased BVF and trabecular number, and decreased trabecular thickness and trabecular separation, for most genotypes (Figure 5, Table 3). However, RANK-Fc treatment resulted in *oim/oim* mice having fewer trabeculae (trabecular number) compared to *+/+* and *oim/+* mice treated with RANK-Fc (Table 3).

Cortical bone—There were no significant differences in BMD among the genotypes within any of the treatment groups. *Oim/oim* saline and ALN-treated mice had thinner cortices compared to saline and ALN-treated *+/+* and *oim/+* mice (Table 3).

Discussion

This study demonstrates that treatment of a mouse model of OI from infancy through early adulthood (~15 weeks of age) with either a bisphosphonate (ALN) or with a RANKL inhibitor (RANK-Fc) cause similar decreases in fracture incidence, from 9 fractures per mouse in untreated animals to 4 in both treatment groups, with minimally distinct biochemical, histologic, geometric, architectural and biomechanical changes in the bone in the two treatment groups. In both treatment groups, however, an increase in metaphyseal bone volume via increased number of thinner trabeculae is apparent.

RANK-Fc treatment was effective in decreasing fracture incidence even though TRACP-5b levels, a surrogate for osteoclast activity, were not suppressed to the degree typical for RANKL inhibition, to approximately 75% of baseline [44]. In the mice in the current study, after 4 weeks of treatment, TRACP-5b values were decreased to 65%, while at 15 weeks of age (1 week after the last injection), TRACP-5b values were actually 130-200% higher than in saline-treated mice. This suggests the possibility of an immune response that may have inactivated RANK-Fc in the mice, but unfortunately, we did not measure antibodies to RANK-Fc. Thus it is not absolutely clear whether the bone changes and fracture reduction seen with RANK-Fc treatment were the result of its effects in the first few weeks of treatment, whether RANK-Fc acted only immediately after injection, or whether RANK-Fc

continued to be effective throughout the greater part of the study. In a subset of mice, TRACP-5b levels assessed 5 hours after RANK-Fc injection were not different from TRACP-5b levels assessed at random time points, data that supports an immune response, as opposed to activity in a short timeframe post-injection. The fact that histological evidence of TRAP staining was not different among all three genotypes and treatment groups also implies that osteoclasts were not eliminated or diminished by RANK-Fc treatment and supports the serum TRAP-5b findings. In spite of the possible immune response, at the end of the study, the weights of the RANK-Fc treated *oim/oim* mice were statistically equivalent to those of the *+/+* mice treated with RANK-Fc, and increased metaphyseal mineralization occurred in all treatment groups. The increased body weight in RANK-Fc treated *oim/oim* mice could possibly be attributed to increased bone mass and commensurate increases in associated soft tissues and muscle mass, although these features was not directly measured.

Similar to previous studies in bisphosphonate-treated *oim/oim* mice [36-38], in the current study, fracture reduction was achieved in both treatment groups in conjunction with changes in bone density and geometry. Here, ALN treatment increased metaphyseal density in *+/+* and *oim/oim* bones as it did in the previous studies [36-38]. However, no significant changes in biomechanical parameters were noted with ALN treatment in our current study while previously, when treatment started at a later age, structural stiffness and failure load were increased in ALN treated *+/+* bone, and structural stiffness increased in *oim/oim* bone [38]. RANK-Fc treatment increased energy to failure in the *+/+* mice and increased brittleness in *oim/+* mice compared to either their saline and ALN treated counterparts. The increase in brittleness in *oim/+* bone can be viewed as a negative consequence of RANK-Fc treatment in mice that normally do not have brittle bone, and likely arises from hypermineralization of the cortical bone. A study of RANKL inhibition in 6 week old *oim/oim* mice previously carried out by our group showed some changes in bone properties but no reduction in fracture incidence [39]. In that study, RANK-Fc treatment significantly increased metaphyseal density of *+/+* and *oim/oim* bone [39] while in the present study it only tended to increase in the *+/+* animals. The discrepancies among the biomechanical and geometric results of the different studies involving similar but not identical experimental design suggest that the detection of changes in specific bone parameters may require increased numbers, in particular when comparing more than one treatment regime. Nonetheless, the results of the current study implies equivalence of RANK-FC and ALN treatment and indicate that, as discussed below, fracture reduction occurs with slight but clinically significant modifications in the bone architecture.

Unlike the previous studies, the current one includes a three dimensional analysis of bone architecture parameters by micro CT, in addition to the other methods. This analysis revealed the geometric features that underlie the increased BVF that occurred with ALN and RANK-Fc treatment; namely, we can now see that an increase in trabecular number is the primary contributor to the increased trabecular BMD. Interestingly, our study demonstrated decreased trabecular thickness with both ALN and RANK-Fc whereas previous studies in adult rats and humans showed no significant change in trabecular thickness with bisphosphonate treatment [45,46]. A study on ALN in 6 week old mice showed an increase in trabecular thickness in the *+/+* and no change in *oim/oim* mice [41]; however, that earlier study evaluated the average trabecular thickness throughout the entire longitudinal bone section, whereas we evaluated trabecular thickness in one consistently defined area in the cross-section of the metaphyseal bone. It is possible that the decrease in thickness that we observed in the current study was due to the treated mice having retained primary spongiosa in the cross-sectional area, which is generally comprised of thinner trabeculae compared to secondary spongiosa, whereas in control mice secondary spongiosa was present[47,48].

This study demonstrates similar bone property changes and fracture reduction with ALN and RANK-Fc treatment. Although there were some subtle differences between the two treatments, one did not appear to be superior to the other. Complete inhibition of osteoclasts was not achieved with RANK-Fc, and based on another study, is likely undesirable in young rapidly growing animals [40] during the time when bone remodeling is essential for normal growth. The rate of growth in mammals slows as they age, with the highest rates prior to birth and decreasing rates all through childhood, until growth ceases entirely when growth plates fuse [49]. Bone remodeling, a process in which osteoclasts are essential, is very active during the periods of rapid growth [49]. Finding an optimal dose of Denosumab that would be beneficial but not excessive in pediatrics is the challenge that must be achieved clinically. Bisphosphonates have the disadvantage of persisting in the bone for decades [11] as well as other side-effects [14], but have been used successfully in the OI population for over a decade [4]. Denosumab is a newer, more expensive treatment option [50], but may be attractive to some families and clinicians as it is not deposited in the bone matrix. Based on the findings in the current study, RANKL inhibition is a possibility for reducing fracture incidence and increasing bone density in young children with OI, but clearly studies to optimize the dose and the age of onset of therapy in the pediatric population should be pursued.

Acknowledgments

The authors thank Lyudmila Lukashova for assistance with microCT and Dr Stephen Doty and the Analytical Microscopy core for assistance with histology. The funding for this study was provided by the fellow grant of the Osteogenesis Foundation (2007-08), a research grant from Amgen (2008-2009) and NIH Grant AR48337. The Micro-CT and Analytical Microscopy Cores are components of the NIH supported Musculoskeletal Repair and Regeneration Core Center (AR046121).

References

1. Rauch F, Glorieux FH. Osteogenesis imperfecta. *Lancet*. 2004; 363:1377–85. [PubMed: 15110498]
2. Gatti D, Antoniazzi F, Prizzi R, Braga V, Rossini M, Tato L, Viapiana O, Adami S. Intravenous neridronate in children with osteogenesis imperfecta: a randomized controlled study. *J Bone Miner Res*. 2005; 20:758–63. [PubMed: 15824848]
3. Gatti D, Viapiana O, Lippolis I, Braga V, Prizzi R, Rossini M, Adami S. Intravenous bisphosphonate therapy increases radial width in adults with osteogenesis imperfecta. *J Bone Miner Res*. 2005; 20:1323–6. [PubMed: 16007328]
4. Glorieux FH. Experience with bisphosphonates in osteogenesis imperfecta. *Pediatrics*. 2007; 119(2):S163–5. [PubMed: 17332237]
5. Letocha AD, Cintas HL, Troendle JF, Reynolds JC, Cann CE, Chernoff EJ, Hill SC, Gerber LH, Marini JC. 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; 20:977–86. [PubMed: 15883638]
6. Madenci E, Yilmaz K, Yilmaz M, Coskun Y. Alendronate treatment in osteogenesis imperfecta. *J Clin Rheumatol*. 2006; 12:53–6. [PubMed: 16601536]
7. Sakkars R, Kok D, Engelbert R, van Dongen A, Jansen M, Pruijs H, Verbout A, Schweitzer D, Uiterwaal C. Skeletal effects and functional outcome with olpadronate in children with osteogenesis imperfecta: a 2-year randomised placebo-controlled study.[see comment]. *Lancet*. 2004; 363:1427–31. [PubMed: 15121405]
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; 95:332–9. [PubMed: 16497645]
9. Cheung MS, Glorieux FH. Osteogenesis Imperfecta: update on presentation and management. *Rev Endocr Metab Disord*. 2008; 9:153–60. [PubMed: 18404382]

10. Ward, LmFau; Rauch, F.; Rauch, FFau; Whyte, MP.; Whyte, MpFau; D'Astous, J.; D'Astous, JFau; Gates, PE.; Gates, PeFau; Grogan, D.; Grogan, DFau; Lester, EL.; Lester, ElFau; McCall, RE.; McCall, ReFau; Pressly, TA.; Pressly, TaFau; Sanders, JO.; Sanders, JoFau; Smith, PA.; Smith, PaFau; Steiner, RD.; Steiner, RdFau; Sullivan, E.; Sullivan, EFau; Tyerman, G.; Tyerman, GFau; Smith-Wright, DL.; Smith-Wright, DiFau; Verbruggen, N.; Verbruggen, NFau; Heyden, N.; Heyden, NFau; Lombardi, A.; Lombardi, AFau; Glorieux, FH.; Glorieux, FH. Alendronate for the treatment of pediatric osteogenesis imperfecta: a randomized placebo-controlled study.
11. Rauch F, Munns C, Land C, Glorieux FH. Pamidronate in children and adolescents with osteogenesis imperfecta: effect of treatment discontinuation. *J Clin Endocrinol Metab.* 2006; 91:1268–74. [PubMed: 16434452]
12. Pizones J, Plotkin H, Parra-Garcia JI, Alvarez P, Gutierrez P, Bueno A, Fernandez-Arroyo A. Bone healing in children with osteogenesis imperfecta treated with bisphosphonates. *J Pediatr Orthop.* 2005; 25:332–5. [PubMed: 15832149]
13. Rauch F, Cornibert S, Cheung M, Glorieux FH. Long-bone changes after pamidronate discontinuation in children and adolescents with osteogenesis imperfecta. *Bone.* 2007; 40:821–7. [PubMed: 17223617]
14. Papapetrou PD. Bisphosphonate-associated adverse events. *Hormones (Athens).* 2009; 8:96–110. [PubMed: 19570737]
15. Djokanovic N, Klieger-Grossmann C, Koren G. Does treatment with bisphosphonates endanger the human pregnancy? *J Obstet Gynaecol Can.* 2008; 30:1146–8. [PubMed: 19175968]
16. Teitelbaum SL. Osteoclasts: what do they do and how do they do it? *Am J Pathol.* 2007; 170:427–35. [PubMed: 17255310]
17. de Vernejoul MC. Sclerosing bone disorders. *Best Prac Res Clin Rheumatol.* 2008; 22:71–83.
18. Bone HG, Bolognese MA, Yuen CK, Kendler DL, Wang H, Liu Y, San Martin J. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. *J Clin Endocrinol Metab.* 2008; 93:2149–57. [PubMed: 18381571]
19. Brown JP, Prince RL, Deal C, Recker RR, Kiel DP, de Gregorio LH, Hadji P, Hofbauer LC, Alvaro-Gracia JM, Wang H, Austin M, Wagman RB, Newmark R, Libanati C, San Martin J, Bone HG. 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; 24:153–61. [PubMed: 18767928]
20. 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]
21. Armstrong AP, Miller RE, Jones JC, Zhang J, Keller ET, Dougall WC. RANKL acts directly on RANK-expressing prostate tumor cells and mediates migration and expression of tumor metastasis genes. *Prostate.* 2008; 68:92–104. [PubMed: 18008334]
22. Canon JR, Roudier M, Bryant R, Morony S, Stolina M, Kostenuik PJ, Dougall WC. Inhibition of RANKL blocks skeletal tumor progression and improves survival in a mouse model of breast cancer bone metastasis. *Clin Exp Metastasis.* 2008; 25:119–29. [PubMed: 18064531]
23. Childs LM, Paschalis EP, Xing L, Dougall WC, Anderson D, Boskey AL, Puzas JE, Rosier RN, O'Keefe RJ, Boyce BF, Schwarz EM. In vivo RANK signaling blockade using the receptor activator of NF-kappaB:Fc effectively prevents and ameliorates wear debris-induced osteolysis via osteoclast depletion without inhibiting osteogenesis. *J Bone Miner Res.* 2002; 17:192–9. [PubMed: 11811549]
24. Delos D, Yang X, Ricciardi BF, Myers ER, Bostrom MP, Camacho NP. The effects of RANKL inhibition on fracture healing and bone strength in a mouse model of osteogenesis imperfecta. *J Orthop Res.* 2008; 26:153–64. [PubMed: 17729310]
25. Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M Jr, Kostenuik PJ, Giannobile WV. RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. *J Periodontol.* 2007; 78:1300–8. [PubMed: 17608585]

26. Kim HK, Morgan-Bagley S, Kostenuik P. RANKL inhibition: a novel strategy to decrease femoral head deformity after ischemic osteonecrosis. *J Bone Miner Res.* 2006; 21:1946–54. [PubMed: 17002576]
27. Miller RE, Branstetter D, Armstrong A, Kennedy B, Jones J, Cowan L, Bussiere J, Dougall WC. 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; 179:266–74. [PubMed: 17579046]
28. Ominsky MS, Kostenuik PJ, Cranmer P, Smith SY, Atkinson JE. The RANKL inhibitor OPG-Fc increases cortical and trabecular bone mass in young gonad-intact cynomolgus monkeys. *Osteoporos Int.* 2007; 18:1073–82. [PubMed: 17384898]
29. Ominsky MS, Li X, Asuncion FJ, Barrero M, Warmington KS, Dwyer D, Stolina M, Geng Z, Grisanti M, Tan HL, Corbin T, McCabe J, Simonet WS, Ke HZ, Kostenuik PJ. RANKL inhibition with osteoprotegerin increases bone strength by improving cortical and trabecular bone architecture in ovariectomized rats. *J Bone Miner Res.* 2008; 23:672–82. [PubMed: 18433301]
30. Padagas J, Colloton M, Shalhoub V, Kostenuik P, Morony S, Munyakazi L, Guo M, Gianneschi D, Shatzen E, Geng Z, Tan HL, Dunstan C, Lacey D, Martin D. The receptor activator of nuclear factor-kappaB ligand inhibitor osteoprotegerin is a bone-protective agent in a rat model of chronic renal insufficiency and hyperparathyroidism. *Calcif Tissue Int.* 2006; 78:35–44. [PubMed: 16362459]
31. Saidenberg-Kermanac'h N, Corrado A, Lemeiter D, deVernejoul MC, Boissier MC, Cohen-Solal ME. TNF-alpha antibodies and osteoprotegerin decrease systemic bone loss associated with inflammation through distinct mechanisms in collagen-induced arthritis. *Bone.* 2004; 35:1200–7. [PubMed: 15542046]
32. Stolina M, Adamu S, Ominsky M, Dwyer D, Asuncion F, Geng Z, Middleton S, Brown H, Pretorius J, Schett G, Bolon B, Feige U, Zack D, Kostenuik PJ. RANKL is a marker and mediator of local and systemic bone loss in two rat models of inflammatory arthritis. *J Bone Miner Res.* 2005; 20:1756–65. [PubMed: 16160733]
33. Zwerina J, Hayer S, Tohidast-Akrad M, Bergmeister H, Redlich K, Feige U, Dunstan C, Kollias G, Steiner G, Smolen J, Schett G. Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. *Arthritis Rheum.* 2004; 50:277–90. [PubMed: 14730626]
34. Chipman SD, Sweet HO, McBride DJ Jr, Davisson MT, Marks SC Jr, Shuldiner AR, Wenstrup RJ, Rowe DW, Shapiro JR. Defective pro alpha 2(I) collagen synthesis in a recessive mutation in mice: a model of human osteogenesis imperfecta. *Proc Natl Acad Sci U S A.* 1993; 90:1701–5. [PubMed: 8446583]
35. King D, Jarjoura D, McEwen HA, Askew MJ. Growth hormone injections improve bone quality in a mouse model of osteogenesis imperfecta. *J Bone Miner Res.* 2005; 20:987–93. [PubMed: 15883639]
36. Camacho NP, Raggio CL, Doty SB, Root L, Zraick V, Ilg WA, Toledano TR, Boskey 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]
37. Evans KD, Lau ST, Oberbauer AM, Martin RB. Alendronate affects long bone length and growth plate morphology in the oim mouse model for Osteogenesis Imperfecta. *Bone.* 2003; 32:268–74. [PubMed: 12667554]
38. McCarthy EA, Raggio CL, Hossack MD, Miller EA, Jain S, Boskey AL, Camacho NP. Alendronate treatment for infants with osteogenesis imperfecta: demonstration of efficacy in a mouse model. *Pediatr Res.* 2002; 52:660–70. [PubMed: 12409511]
39. Bargman R, Huang A, Boskey A, Raggio C, Pleshko N. RANKL Inhibition Improves Bone Properties in a Mouse Model of Osteogenesis Imperfecta. *Connect Tissue Res.* 2010; 51:123–31. [PubMed: 20053133]
40. Bargman, R.; Boskey, A.; DiCarlo, E.; Posham, R.; Raggio, C.; Verdelis, K.; Pleshko, N. Osteopetrosis-like changes after treatment with high and low dose OPG-Fc in the diaphyseal regions of infant mice. The Endocrine Society's 91st Annual Meeting; 2009. p. P2-196. Abstract

41. Misof BM, Roschger P, Baldini T, Raggio CL, Zraick V, Root L, Boskey AL, Klaushofer K, Fratzl P, Camacho NP. Differential effects of alendronate treatment on bone from growing osteogenesis imperfecta and wild-type mouse. *Bone*. 2005; 36:150–8. [PubMed: 15664013]
42. Bouxsein, MIFau; Boyd, SK.; Boyd, SkFau; Christiansen, BA.; Christiansen, BaFau; Guldberg, RE.; Guldberg, ReFau; Jepsen, KJ.; Jepsen, KjFau; Muller, R.; Muller, R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography.
43. Filgueira L. Fluorescence-based staining for tartrate-resistant acidic phosphatase (TRAP) in osteoclasts combined with other fluorescent dyes and protocols. *J Histochem Cytochem*. 2004; 52:411–4. [PubMed: 14966208]
44. Ominsky MS, Stolina M, Li X, Corbin TJ, Asuncion FJ, Barrero M, Niu QT, Dwyer D, Adamu S, Warmington KS, Grisanti M, Tan HL, Ke HZ, Simonet WS, Kostenuik PJ. One year of transgenic overexpression of osteoprotegerin in rats suppressed bone resorption and increased vertebral bone volume, density, and strength. *J Bone Miner Res*. 2009; 24:1234–46. [PubMed: 19257823]
45. Gasser JA, Ingold P, Venturiere A, Shen V, Green JR. Long-term protective effects of zoledronic acid on cancellous and cortical bone in the ovariectomized rat. *J Bone Miner Res*. 2008; 23:544–51. [PubMed: 18072878]
46. Recker RR, Delmas PD, Halse J, Reid IR, Boonen S, Garcia-Hernandez PA, Supronik J, Lewiecki EM, Ochoa L, Miller P, Hu H, Mesenbrink P, Hartl F, Gasser J, Eriksen EF. Effects of intravenous zoledronic acid once yearly on bone remodeling and bone structure. *J Bone Miner Res*. 2008; 23:6–16. [PubMed: 17892374]
47. Blouin S, Moreau MF, Basle MF, Chappard D. Relations between radiograph texture analysis and microcomputed tomography in two rat models of bone metastases. *Cells Tissues Organs*. 2006; 182:182–92. [PubMed: 16914920]
48. Kimmel, DbFau; Jee, WS.; Jee, WS. A quantitative histologic analysis of the growing long bone metaphysis.
49. Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC, Sheffield-Moore M, Mauras N, Bowers CY. Endocrine control of body composition in infancy, childhood, and puberty. *Endocr Rev*. 2005; 26:114–46. [PubMed: 15689575]
50. Jonsson B, Strom O, Eisman JA, Papaioannou A, Siris ES, Tosteson A, Kanis JA. Cost-effectiveness of Denosumab for the treatment of postmenopausal osteoporosis. *Osteoporos Int*. 2010

Weight of mice at the end of treatment

Figure 1.

Weight of mice at 15 weeks of age after 12 weeks of treatment with either saline, ALN or RANK-Fc.

* $p < 0.05$ compared to +/+ and oim/+, same treatment

Figure 2.

Fractures sustained at 15 weeks of age by *oim/oim* mice after 12 weeks of treatment with either saline, ALN or RANK-Fc.

* $p < 0.05$

Figure 3.

(A) Serum TRACP-5b levels were significantly reduced in +/+ and *oim/+* mice (combined) treated with RANK-Fc compared to +/+ mice treated with saline after 1 month of treatment (A). At sacrifice, *oim/+* and *oim/oim* mice treated with RANK-Fc had significantly higher TRACP-5b levels than those treated with either saline or ALN (B).

$p < 0.05$, * $p < 0.05$ compared to +/+, • $p < 0.05$ compared to +/+ and *oim/+*

Figure 4.

Histologic evaluation of all mice and treatments. H&E staining demonstrates that ALN (B1, E1, H1) and RANK-Fc treatment (C1, F1, I1) cause retention of primary spongiosa and fusiform deformity in all genotypes compared to saline +/+, oim/+ and oim/oim (A1, D1, H1). TRAP staining remains qualitatively unchanged with either treatment with no diminution of staining (A2-I2).

Figure 5.

Micro CT findings demonstrate decreased trabecular number and thickness with increased trabecular space in oim/oim (B) compared to +/+ saline treated animals. (A). Treatment of oim/oim with both ALN and RANK-Fc causes increased trabecular number and decreased trabecular thickness and separation (C and D).

Table 1
Density and geometrical properties of bone after 12 weeks of treatment

	Number	Femoral length	Metaphyseal density	Cortical density	Average cortical thickness (mm)
+/+	Saline	15	15.57±0.41	0.80±0.14	0.78±0.11
	ALN	13	15.28±0.38	0.91±0.13 *	0.79±0.10
	RANK-Fc	12	15.56±0.37	0.90±0.09 ■	0.82±0.08
oim/+	Saline	11	15.60±0.42	0.74±0.08	0.67±0.11
	ALN	9	14.95±0.37	0.80±0.05	0.70±0.09
	RANK-Fc	6	15.05±0.84	0.82±0.05	0.69±0.05
oim/oim	Saline	13	14.8±1.14 ●▲	0.53±0.19 ●▲	0.64±0.21 ●
	ALN	16	14.59±0.72 ●	0.74±0.13 * ●	0.68±0.09 ●
	RANK-Fc	16	15.05±0.71	0.60±0.09 ●▲	0.65±0.14 ●

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

■ Trending to significance compared to same genotype saline (p < 0.08)

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

▲ Significantly different compared to same treatment oim/+ (p < 0.05)

Tables 2
Biomechanical properties of bone after 12 weeks of treatment

	Number	Moment of Inertia (mm ⁴)	Maximum Load (N)	Stiffness (N/mm)	Youngs Modulus (Gpa)	Total Strain	Brittleness (%)	Yield Stress (Mpa)	Energy (Mpa)
+/+	Saline	12	0.14±0.06	20.7±4.20	148±27	8.17±2.32	0.07±0.02	150±36	8.62±3.05
	ALN	13	0.14±0.06	22.54±4.80	157±26	9.57±3.59	0.07±0.03	157±45	9.8±3.57
	RANK-Fc	12	0.12±0.04	22.17±2.95	153±27	9.83±2.68	0.08±0.03	175±61	11.83±3.59*
oim/+	Saline	11	0.13±0.04	18.24±4.52	120±37	7.21±2.29	0.05±0.02	153±49	5.90±2.38●
	ALN	9	0.13±0.06	19.64±5.65	135±34	8.06±2.57	0.05±0.01	145±32	6.00±1.72●
	RANK-Fc	6	0.12±0.03	20.77±2.96	146±16	8.82±1.54	0.04±0.003	189±29	3.50±0.83●
oim/oim	Saline	13	0.22±0.23	11.50±4.59●▲	86±35●▲	4.51±2.77●▲	0.03±0.01●▲	104±55	1.85±1.47●▲
	ALN	15	0.18±0.14	14.12±13.44●▲	96±23●▲	5.28±2.39●▲	0.04±0.01●	118±58	2.41±1.45●▲
	RANK-Fc	16	0.16±0.07	13.44±2.95●▲	96±23●▲	5.28±2.39●▲	0.04±0.01●▲	117±58●▲	2.41±1.45●▲

* Significantly different compared to same genotype saline (p < 0.05)
 ▲ Significantly different compared to same genotype ALN (p < 0.05)
 ● Significantly different compared to same treatment +/+ (p < 0.05)
 ▲ Significantly different compared to same treatment oim/+ (p < 0.05)

MicroCT properties of bone after 12 weeks of treatment

Tables 3

		Trabecular Bone						Cortical Bone			
	Number	BVF	BMD (mg/cc)	Trabecular number (1/mm)	Trabecular thickness (mm)	Trabecular Separation (mm)	Bone Surface/Bone Volume (1/mm)	BVF	BMD (mg/cc)	Cortical Thickness (mm)	Outer Perimeter (mm)
+/+	Saline	0.23±0.02	923.9±14.6	4.91±0.48	0.054±0.006	0.19±0.02	48.04±4.25	0.94±0.004	1184.6±21.5	0.23±0.02	3.59±1.27
	ALN	0.34±0.05*	924.6±12.2	8.49±0.87*	0.042±0.003*	0.10±0.01*	54.42±5.19	0.93±0.01	1162.4±25.8	0.23±0.03	3.51±1.38
	RANK-Fc	0.31±0.07*	930.1±14.6	9.18±1.99*	0.038±0.004*	0.10±0.02*	62.54±7.50*	0.93±0.01	1177.7±19.9	0.22±0.02	3.50±1.11
oim/+	Saline	0.19±0.05	939.2±16.0	4.94±0.38	0.050±0.008	0.20±0.02	55.35±10.41	0.93±0.01	1177.8±12.0	0.22±0.02	4.91±0.17
	ALN	0.30±0.03*	929.6±30.6	8.36±0.52*	0.039±0.002*	0.11±0.01*	61.43±4.67	0.94±0.01	1187.8±18.3	0.24±0.02	4.98±0.49●
	RANK-Fc	0.25±0.07	922.7±17.9	9.05±1.80*	0.033±.0005*	0.10±0.02*	75.63±3.47*●	0.88±0.14	1186.6±7.6	0.20±0.02	4.62±0.11
oim/oim	Saline	0.11±0.03●▲	944.4±18.4	3.73±0.77	0.045±0.003●	0.28±0.08●▲	62.94±4.96●	0.91±0.03	1166.1±30.5	0.18±0.04●▲	3.77±1.02
	ALN	0.23±0.05*●▲	921.0±20.3	7.93±0.67*	0.034±0.003*●▲	0.12±0.01*	75.83±9.71*●▲	0.90±0.05	1146.5±41.5▲	0.17±0.05●▲	3.84±1.14
	RANK-Fc	0.17±0.05●●▲	912.1±15.8	7.34±1.38*●▲	0.032±0.002*●	0.13±0.03*	82.29±7.43*●	0.93±0.01	1160.1±24.9	0.20±0.03	3.55±1.22

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

■ Trending to significance compared to same genotype saline ($p < 0.08$)

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

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

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