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Cathepsin K inhibitors and bone

Cathepsin K Inhibitors for Osteoporosis: Biology, Potential Clinical Utility, and Lessons Learned

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Cathepsin K is a cysteine protease member of the cathepsin lysosomal protease family. While cathepsin K is highly expressed in osteoclasts, lower levels of cathepsin K are also found in a variety of other tissues. Secretion of cathepsin K from the osteoclast into the sealed osteoclast-bone cell interface results in efficient degradation of type I collagen. The absence of cathepsin K activity in humans results in pycnodysostosis, characterized by increased bone mineral density and fractures. Pharmacological cathepsin K inhibition leads to continuous increases in BMD for up to five years of treatment and improves bone strength at the spine and hip. When compared to other anti-resorptive agents, cathepsin K inhibition is nearly equally efficacious for reducing biochemical markers of bone resorption, but comparatively less active for reducing bone formation markers. Despite multiple efforts to develop cathepsin K inhibitors, potential concerns related to off-target effects of the inhibitors against other cathepsins and cathepsin K inhibition at non-bone sites, including skin and perhaps cardiovascular and cerebrovascular sites, prolonged the regulatory approval process. A large multinational randomized, double-blind Phase III study of odanacatib in postmenopausal women with osteoporosis has recently been completed. While this study demonstrated significant reductions in fractures at multiple sites, odanacatib was ultimately withdrawn from the regulatory approval process after it was found to be associated with an increased risk of cerebrovascular accidents. Nonetheless, the underlying biology and clinical effects of cathepsin K inhibition remain of considerable interest and may pave the way for future therapeutic approaches for osteoporosis.

- Cathepsin K inhibitors have been in development as a new treatment for osteoporosis.
- In contrast to other drugs that inhibit bone resorption with a coupled reduction in bone formation, cathepsin K inhibitors have been shown to inhibit bone resorption with lessor effects on inhibiting bone formation.
- Despite extensive pre-clinical and clinical studies and substantial anti-fracture efficacy in a large, phase 3 trial, clinical development of the cathepsin K inhibitor, odanacatib, was terminated due to an unforeseen increase in cerebrovascular events.
- Nonetheless, the underlying biology of cathepsin K inhibitors and the lessons learned from the development of odanacatib will help inform future drug development for osteoporosis, particularly drugs that may dissociate the inhibition of bone resorption from the coupled reduction in bone formation.

I. Introduction

A. Brief overview of current osteoporosis therapies and gaps

The adult human skeleton undergoes continuous remodeling in which small packets of bone resorbed by osteoclasts are replaced with bone formed through the actions of osteoblasts at specific sites called Basic Multicellular Units (BMUs).(1,2) Collectively, the processes of bone resorption and formation replace approximately 10% of the skeleton each year; thus the entire human skeleton is replaced roughly every 10 years.(3) Within the cortical BMU, osteoclasts
form a bone resorbing edge. After osteoclasts have cut deeply into bone, osteoblasts are recruited to the resorption site where they initiate bone formation (4), where they gradually become embedded into the bone as osteocytes.(5) In contrast to cortical bone turnover, trabecular bone turnover is more rapid, with shorter periods of bone resorption followed by a reversal phase and subsequent bone and osteocyte formation.

From early to middle adult life, osteoclast-mediated bone resorption is generally well-matched both temporally and spatially by osteoblast-mediated bone formation, such that net bone mass remains approximately stable. This bone remodeling serves to replace damaged bone, to maintain calcium homeostasis, and to allow for skeletal restructuring if physical stresses on the bone are altered.(1,6-8) Both local and systemic factors regulate BMU formation and activation rates, which in turn regulate whole body bone balance. With progressive aging and across various pathologic conditions, bone remodeling becomes imbalanced, with bone resorption exceeding bone formation, a dynamic which results in net loss of bone, skeletal microarchitectural deterioration, and increased fracture risk.

The pharmacologic landscape for the management and treatment of osteoporosis has expanded markedly over the past two decades. At present, the majority of available agents function to limit bone resorption by either directly or indirectly targeting the osteoclast. Agents categorized as anti-resorptives include members of the bisphosphonate family (alendronate, risedronate, ibandronate, and zoledronate) estrogen, the selective estrogen receptor modulator raloxifene (although estrogen and raloxifene are weaker anti-resorptive drugs than bisphosphonates and may also affect bone formation) (9), and most recently denosumab - a fully humanized monoclonal antibody directed against receptor activator of nuclear factor kappa B ligand (RANKL).

In contrast to anti-resorptive agents are pharmacologic agents which can be classified as anabolic for the skeleton, a list which currently consists of full-length parathyroid hormone (PTH) 1-84 (approved in Europe) and its amino-terminal fragment PTH 1-34 (teriparatide), as well as the PTH-related peptide (PTHrP) analog, abaloparzide. Given the current limited options for bone anabolism in the setting of an expanding elderly population likely to benefit clinically from approaches to reduce fracture risk, there exists a clear need for additional pharmacologic approaches which stimulate bone formation or at least inhibit bone resorption without inhibiting bone formation (due to the coupling of bone resorption to bone formation, see section V). It is in this context that the development of caspepsin K inhibitors, which limit osteoclast activity but do not reduce osteoblast function to the same extent as other currently available anti-resorptive agents, appear to be best placed.

II. Biology of Cathepsin K

A. Role of cathepsin K in osteoclasts

Cathepsin K is a papain-like cysteine protease member of the cathepsin family of lysosomal proteases, a family categorized as consisting of cysteine (cathepsins B, C, F, H, K, L, O, S, V, X, and W), aspartate (cathepsins D and E), or serine (cathepsins A and G) proteases depending on the active site amino acid which mediates each member’s catalytic activity.(10) An important aspect of cathepsin biology is based on their cellular localization. While cathepsins have greatest activity in acidic environments such as occurs along the endosomal/lysosomal continuum, cysteine cathepsins secretion into the extracellular space has also been shown to occur under normal physiologic conditions including skeletal remodeling, wound repair, and prohormone processing.(11)
Cathepsin K is the only cathepsin expressed at high levels in osteoclasts. Within osteoclasts, cathepsin K has been shown to reside in lysosomes, cytoplasmic vesicles, or along the osteoclast-bone resorptive interface prior to release into the resorptive lacunae formed by αvβ3 integrin-mediated osteoclastic sealing to the bone surface.(12),(13) Cytochemical assays and immunostaining suggest that cathepsin K processing and activation in vivo occurs intracellularly prior to secretion into the sealed space underlying the ruffled border.(14) In addition to its expression in osteoclasts, considerably lower levels of cathepsin K have been shown to be expressed in other tissues and cells including adipose (15), skin (16), heart (17), lung (18), smooth muscle cells (19), ovary (20), placenta (17), thyroid (21), liver (17), macrophages (22), cartilage (23), osteoblasts and osteocytes,(24) and breast (25) and prostate cancers.(26)

The cathepsin K gene (CTSK), which spans approximately 12 kilobases, is located on chromosome 1q21 and consists of eight exons with seven intervening introns.(27) The codon for the translation initiator methionine is located in exon 2, with the termination codon in exon 8. The cathepsin K protein is synthesized as an inactive precursor of 329 amino acids, including a 15-amino acid pre-region, a 99-amino acid pro-region, and a mature active enzyme of 215-amino acids. Removal of the N-terminal pro-region is required for enzymatic activation, and occurs at or below pH 4.0 during a process which can be either autocatalytic or the result of catalysis by other proteases. Cathepsin K expression is mediated by RANKL-induced activation of the transcription factor nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) which targets the CTSK promoter.(28) Cathepsin K expression is also enhanced by p38 pathway activation, and the cathepsin K promoter is a transcriptional target of microphthalmia transcription factor (Mitf).(29,30) Conversely, factors such as interferon-gamma, calcitonin, and estrogen reduce cathepsin K mRNA and protein expression.(31,32)

As a protease, cathepsin K is the primary enzyme responsible for degradation of type I collagen that composes roughly 90% of the bone organic matrix. In vivo, both type I and type II collagen are composed of two α1 chains and one α2 chain within a triple helix configuration, a structure which is highly resistant to proteolysis. Although both matrix metalloproteases (MMPs) and the serine protease neutrophil elastase can cleave the collagen triple helices, neither is particularly efficient at doing so, nor is either capable of degrading the cross-linked pyridinoline–deoxypyridinoline telopeptides that form the collagen triple helix end region.(10) In contrast, cathepsin K efficiently cleaves both the collagen triple helix and the telopeptide to produce collagen monomers (33), such that in vitro, cathepsin K is able to completely dissolve human cortical bone collagen.(34)

In addition to the type I and II collagens, cathepsin K efficiently degrades other organic extracellular matrix components. While type I collagen composes approximately 90% of the organic skeletal matrix, the remaining 10% is comprised of an array of non-collagenous proteins including biglycan, bone sialoprotein, decorin, fibronectin, osteocalcin, osteonectin, and osteopontin.(35) While osteonectin is sensitive to cathepsin K degradation, other components are not efficiently degraded. However, aggrecan, type II collagen, and elastin, proteins which are present outside of the organic bone matrix including within joints and/or vascular walls, are efficiently degraded by cathepsin K, suggesting a potential role for cathepsin K in other diseases beyond osteoporosis such as osteoarthritis, rheumatoid arthritis, and lung and vascular disease.(10)

While cathepsin K is highly conserved across species, it is not identical. Thus murine cathepsin K shares 88% homology, and rabbit cathepsin K 94% homology, with human cathepsin K, making mice and rabbits useful but not ideal model systems for studies of human
cathepsin K biology. In comparison, monkey cathepsin K is identical to human cathepsin K, a fact which makes the use of non-human primates a better model for studies of human cathepsin K inhibitors in vivo.(36)

As noted, cathepsin localization (endosomal/lysosomal versus secretion in the extracellular space) dictates many aspects of cathepsin biology, particularly for the cysteine cathepsins, the group which includes cathepsin K. Accordingly, efforts to develop cathepsin K inhibitors which were not lysosomotropic (i.e. do not accumulate in acidic subcellular organelles such as endosomes and lysosomes which are enriched for cathepsins other than cathepsin K), was a major focus of pharmacologic efforts to limit off-target (i.e. non-cathepsin K) inhibitory activities of studied compounds.(37,38)

**B. Data from mouse cathepsin K knock-out models**

Mice with genetically targeted disruption (‘knock-out’) of the cathepsin K gene develop skeletal hypermineralization and phenotypically mimic some aspects of the human disease pycnodysostosis, as described below. Whereas mice heterozygous for cathepsin K deletion are phenotypically normal, mice homozygous deficient for cathepsin K have significant increases in bone mass at both trabecular and cortical sites.(39,40) As originally described, osteoclasts are histologically normal, and demineralization of bone adjacent to the osteoclast-bone interface appears to occur normally. When compared to wild-type osteoclasts, however, cathepsin K-deficient osteoclasts exhibit a poorly defined resorptive surface in which a demineralized bone matrix fringe occurs in the presence of abundant undigested collagen fibrils. In addition, cathepsin K-deficient osteoclasts lack the collagen-fibril-containing vacuoles normally present within the osteoclast cytoplasm during active bone resorption, consistent with their failure to resorb bone organic matrix. Interestingly, whereas bones which undergo endochondral ossification, including both vertebrae and long bones, are hypermineralized in cathepsin K-deficient mice, skeletal sites at which intramembranous ossification occurs, such as the skull and clavicles, are comparatively unaffected.(40)

Whereas the initial studies noted above documented normal osteoclast numbers in cathepsin K-null mice, other murine models in which cathepsin K deficiency was studied in different mouse strains suggests that genetic background may play a modifying role in both the osteoclast and overall skeletal phenotypes.(41-43) In these models, overall osteoclast numbers were significantly increased, with cathepsin K-deficient osteoclasts failing to demonstrate both normal apoptosis and senescence.

Although pycnodysostosis in humans is associated with increased fracture risk, a study which evaluated bone strength in 19-week-old homozygous cathepsin K-null mice at both the femur and lumbar vertebrae failed to demonstrate increased bone fragility.(44) Thus, cathepsin K-null mice had both higher bone mineral content (BMC) at the femur (a primarily cortical site) and lumbar vertebrae (a primarily trabecular site), with enhanced ultimate load strength compared to wild-type mice at both sites when assessed biomechanically, strongly suggesting that at least in this murine model, targeted disruption of the cathepsin K gene does not impact bone tissue quality. Consistent with this, a study which examined a mid-shaft femoral fracture healing model in 8-10-week-old cathepsin K null mice found that both fracture callus mineralization and healing were accelerated, and callus mechanical strength was increased, in cathepsin K-deficient versus wild-type animals.(45)

Beyond the effects of cathepsin K deletion on the skeleton, murine cathepsin K knock-out models have also been used to examine the role of cathepsin K in other tissues. Due to the presence of cathepsin K within lung bronchial epithelial cells and the importance of the
extracellular matrix for maintenance of lung function, Buhling et al. examined chemotherapy-induced pulmonary fibrosis in mice with either normal or absent cathepsin K activity. When compared to wild-type mice, cathepsin K-deficient mice showed significantly more extracellular matrix deposition, while *in vitro* lung fibroblasts from null mice had decreased collagenolytic activity. In comparison, wild-type mice showed increased cathepsin K activity in regions of pulmonary fibrosis, findings also seen in lung specimens from humans with pulmonary fibrosis, suggesting a potential protective role for cathepsin K in modulating excessive collagen matrix deposition in conditions marked by fibrotic lung disease. Consistent with this hypothesis, cathepsin K overexpression was associated with decreased collagen deposition and pulmonary fibrosis in another mouse model of bleomycin-induced pulmonary injury. More recently, the effects of cathepsin K deletion in neonatal lung development and response to hyperoxic challenge were examined. Although mice with cathepsin K deletion initially showed thinner alveolar walls, these differences did not persist to postnatal day 14. In response to hyperoxic challenge, wild-type mice rapidly increased cathepsin K expression, which was associated with increased survival in comparison to cathepsin K null littermates. Collectively, these findings suggest that cathepsin K impacts neonatal lung development, and may also play a role in mediating the response to hyperoxia-induced neonatal lung damage.

Evidence from cathepsin K-null mice suggests that cathepsin K may also play an important role in lipid metabolism and/or atherosclerosis. When compared to wild-type controls, cathepsin K-knockout mice treated with a high fat diet for 12 weeks demonstrated significantly less weight gain, had a lower liver mass and body fat percentage, had lower circulating triglycerides, cholesterol, and leptin levels, while also having increased rates of adipocyte lipolysis. Collectively, these data suggest that cathepsin K deficiency may be partially protective against the development of dyslipidemia. Consistent with these findings are data from studies of the effect of cathepsin K gene disruption on atherosclerosis development, in which cathepsin K-null mice were crossed with atherosclerosis-prone apolipoprotein E (apoE) deficient mice. After maintenance on a high fat atherogenic diet, plaque area in the cathepsin K/apoE double-null mice was decreased by 42% when compared to apoE null mice alone due to a decrease in both the number of advanced lesions and the individual advanced plaque area. Advanced plaques of double-null mice also had an increase in collagen content consistent with plaque fibrosis which would be expected increase plaque stability. However, macrophage foam cell formation in the double knock-out animals was also increased, which might decrease atheroma stability. As a consequence, it remains unclear whether deletion of cathepsin K has positive or negative effects on atherosclerosis. Finally, recent work examining cardiac function in cathepsin K-deficient mice demonstrates that the absence of cathepsin K significantly attenuates cardiac hypertrophy and contractile dysfunction in both pressure overload- and high-fat induced murine models of cardiac dysfunction.

Finally, multiple cathepsins appear to have fundamental roles in skin biology, including cysteine (cathepsins B, H, K, L, S, and V), aspartate (cathepsins D and E), and serine (cathepsins A and G) family members, where they regulate aspects of both normal and pathologic dermal physiology. As will be noted later for human studies of the cathepsin K inhibitors balacatib and odanacatib, the development of skin hardening (morphea-like reactions) in a small subset of subjects treated under clinical trial conditions with each compound may reflect either off-targets effects of these compounds on other cathepsin family members, or alternatively direct effects on cathepsin K expressed in dermal fibroblasts.

**C. Cathepsin K deficiency in humans: pycnodysostosis**
The hypermineralization phenotype found in mice homozygous null for the cathepsin K gene (62) is recapitulated in a very rare human disease. Pycnodysostosis is an autosomal recessive lysosomal storage disorder (OMIM 265800) which, due to CTSK gene mutations, causes cathepsin K deficiency and osteosclerosis.(63) It is believed that the famed French painter Henri de Toulouse-Lautrec may have been affected by pycnodysostosis.(64) Since first reported in 1962, fewer than 200 patients with pycnodysostosis have been described.(65) The incidence of pycnodysostosis is estimated to be 1.0-1.7 per million live births, with an equal sex distribution(66). While parental consanguinity increases the risk for pycnodysostosis, consanguinity is reported to occur in less than 30% of patients. Most reports to date have come from Europe or the United States, although pycnodysostosis has also been described in Japan, China, Thailand, Israel, Indonesia, India, and Africa.(67)

Osteoclasts in individuals with pycnodysostosis are unable to degrade type I collagen or other non-collagenous proteins which form the bone matrix. Thus, patients have a bone matrix which is highly mineralized and associated with increased BMD. Despite this increased BMD, however, bone is of poor quality and affected individuals at an increased risk of fragility fractures, particularly in the lower extremities, as also occurs in osteopetrosis.

Pathogenesis
Loss of function mutations in the cathepsin K gene were reported to cause pycnodysostosis by genetic linkage analysis and positional cloning in 1996.(68,69) At least 44 different mutations have been reported to date (70), with most resulting in total loss or inactivity of the CTSK protein due a variety of mutations including missense or nonsense mutations, duplications, deletions, insertions, or splicing mutations (Table 1). Of the reported mutations, approximately 70% occur in the mature domain of CTSK, 24% in the pro-region, and 6% in the pre-region.(65) Mutational hot spots have been reported in exons 6 and 7.

Clinical and laboratory manifestations
The diagnosis of pycnodysostosis is typically made in infancy or young childhood as a result of characteristic effects on the skeleton during growth and development. Affected persons have disproportionately short stature and a comparatively large skull, with fronto-occipital prominence, mid-facial hypoplasia, mandibular hypoplasia resulting in a small chin, obtuse mandibular angle, high-arched palate, dental malocclusion with retained deciduous teeth, enamel hypoplasia, proptosis, and a beaked and pointed nose.(71,72) The anterior fontanel and other cranial sutures may remain open, but craniosynostosis of some sutures has been reported to occur in some patients.(73) Fingers are short and clubbed from acro-osteolysis or aplasia of terminal phalanges, and the hands are small and square. The thorax is narrow and pectus excavatum, or rarely pectus carinatum may be present. The spine typically shows kyphoscoliosis or increased lumbar lordosis. Low- or minimal-trauma fractures usually affect the lower extremities, including femoral shafts and patellae, and may cause genu valgum. Rickets has been described. Adult height usually varies between 130 cm (4’3”) and 150 cm (4’11”). Mental retardation affects fewer than 10% of cases.(71)

Recurrent respiratory infections and right heart failure may result from chronic upper airway obstruction caused by micrognathia, and severe obstructive sleep apnea has been described.(74,75) Craniosynostosis may cause papilledema (76), and hypoacusia has been rarely reported.(77) Seizures associated with porencephalic cysts have been reported.(78) A single case of chondroblastic osteosarcoma has been reported in a 22 year-old male with pycnodysostosis.(79)
Examination of teeth from a single patient with pycnodysostosis (21) due to novel compound heterozygous mutations in the CTSK gene showed extensive periradicular high-density clumps, with unclear periodontal space by orthopantomography examination and micro-computed tomography scanning analysis. Hematoxylin/eosin and toluidine blue staining and atomic force microscopy analysis showed that the cementum was significantly thickened, softened, and full of cementocytes. Disorganized bone structure was the main characteristic of the alveolar bone.

Radiographically, pycnodysostosis is characterized by uniform osteosclerosis throughout the skeleton which becomes apparent in childhood, and increases with age. Patients may be misdiagnosed with intermediate osteopetrosis based on similar phenotypes, but exome sequencing for CTSK mutations will distinguish between these two disorders.(80) Fractures tend to occur repeatedly over time. Some femoral fractures may have the radiological appearance and characteristics of atypical femoral fractures.(81) The skeletal sclerosis affects the skull (particularly the base) and orbital ridges, but abnormalities in modeling do not occur as with other forms of osteosclerosis. Long bone plain films show narrowing of the medullary canals.(82) The cranial sutures and fontanels typically close late, especially the anterior fontanel. Wormian bones, slender clavicles with hypoplastic lateral ends, partial hyoid bone absence, and hypoplasia of the distal phalanges and ribs are characteristic.(83)

In terms of laboratory findings, serum calcium, phosphorus, and alkaline phosphatase are usually normal, without anemia. Affected children have been reported to have decreased circulating growth hormone and insulin-like growth factor 1 levels.(84) In a small study in which biochemical markers of bone metabolism were assayed in serum and urine from seven patients with pycnodysostosis (85), two markers of bone formation, type I collagen carboxy-terminal propeptide (PICP) and osteocalcin, were normal in all patients. Tartrate-resistant acid phosphatase (TRAP), a marker of osteoclast numbers, was also normal in these patients. Two markers that detect type I collagen telopeptide cross-links from the N- and C-termini, NTX and CTX, respectively, were low in these patients. A third marker which detects a more proximal portion of the C-terminus of type I collagen in serum, ICTP, was increased, a seemingly paradoxical result. The finding of decreased osteoclast-mediated type I collagen degradation, as well as the use of alternative collagen cleavage sites by other proteases, and the accumulation of larger C-terminal fragments containing the ICTP epitope, established a unique biochemical phenotype for this disorder.

Only a few trans-iliac bone biopsies from patients with pycnodysostosis have been published. An example of bone cell morphology from two patients with pycnodysostosis is shown in Figure 1. In general, when compared to age and sex-matched control subjects, biopsies from patients with pycnodysostosis have shown increased bone mass, decreased bone remodeling, and severely decreased dynamic parameters of bone formation. Decreased bone turnover with quantitative decreases in static and dynamic parameters of bone formation is thought to explain the increased degree of mineralization and increased risk of fragility fracture.(86) Multinucleated osteoclasts adjacent to areas of demineralized matrix, as well as bone lining cells adjacent to undigested collagen, have also been described (87), as has increased inhomogeneity of the mineralized matrix resulting from large inclusions of mineralized cartilage residues. At the nanostructural level, marked increases in the mean thickness of mineral particles reflecting decreased bone remodeling has been noted.(87) Examination of the trabecular structure revealed that the lamellae were highly disordered, with poor alignment of mineral crystals oriented along the longitudinal axis of collagen fibrils.
Taken together, results from these biopsies in humans with naturally occurring cathepsin K deficiencies strongly suggest that functional cathepsin K is important for balanced bone turnover, and that enzyme deficiency results in a profound deterioration of bone quality with respect to trabecular architecture and lamellar arrangement, factors which likely underlie the bone fragility seen in this disorder.

Treatment
To date, no medical therapy has been shown to improve pycnodysostosis. Because patients with pycnodysostosis can reach near-normal stature and skeletal proportions with personalized growth hormone treatment targeted at appropriate insulin-like-growth factor levels, some have proposed that this be offered to affected children.(88) Although teriparatide treatment for six months has been described in one patient, no changes were seen in structure, microarchitecture, or bone turnover, as assessed by high-resolution peripheral quantitative CT scanning (HRpQCT), bone histology, or bone turnover markers, strongly implying that functional osteoclasts are required for an anabolic effect of teriparatide on bone.(89) While bone marrow transplantation has been evaluated in osteopetrosis, there are no reports of its use in patients with pycnodysostosis.

Long bone fractures are usually transverse, and may occur contralaterally either sequentially or simultaneously similar to atypical femoral fractures reported after long-term bisphosphonate therapy. However in contrast to atypical femoral fractures, long bone fractures appear to heal at a relatively normal rate in patients with pycnodysostosis. In some cases, delayed union may occur, and huge calluses may develop during healing. Internal fixation of long bones with intramedullary nails may be used to repair fractures.(90,91) Plate and screw fixation may also be used, although the hardness of the bone makes this more difficult.(92) Tooth extraction may also be difficult due to hardness of the bone. Mandibular osteomyelitis may require surgery and antibiotics.(93) The treatment of severe obstructive sleep apnea by adenotonsillectomy and palatoplasty has been described.(74,75)

III. Pre-clinical data
As noted previously, murine cathepsin K is 88% homologous and rabbit cathepsin K 94% homologous, to human cathepsin K. This homology renders both mice and rabbits useful but not ideal model systems of study due to enzymatic differences between species which limit the potency of compounds developed as specific inhibitors of human cathepsin K. Monkey cathepsin K, however, is identical to human cathepsin K, and accordingly makes the use of non-human primates better models for pharmacologic studies of cathepsin K inhibitors in vivo.(36) Pre-clinical studies have primarily focused on the impact of cathepsin K inhibition on skeletal outcomes.

A. Mouse studies

ONO-5334
ONO-5334, an orally active low-molecular-weight synthetic cathepsin K inhibitor, was compared to alendronate for skeletal effects in ovariectomized rats. When compared to untreated animals, daily treatment with ONO-5334 for 8 weeks lead to dose-dependent restoration of total body BMC and BMD at the proximal tibia, while also decreasing markers of bone resorption, urinary deoxypyridinoline and plasma CTX. When compared to alendronate, ONO at the highest dose studied of 15 mg/kg was less effective than alendronate at preserving trabecular BMD and BMC, but was more potent than alendronate at increasing cortical BMD and BMC.
Interestingly, this increase in cortical thickness observed was primarily the result of a decrease in endosteal circumference without an effect on periosteal circumference.\(^{(94)}\)

**Odanacatib**

Odanacatib, an oral, non-lysosomotropic, highly-selective, reversible cathepsin K inhibitor studied in phase III clinical trials in postmenopausal women (as described below), has also been studied in murine models of oral cavity bone loss.\(^{(95,96)}\) In these models, administration of odanacatib limited the development of periodontic and endodontic disease, bone erosion, and dampened local periapical inflammation.

**B. Rabbit studies**

*L-235 and odanacatib*

In an ovariectomized rabbit model, the orally available cathepsin K inhibitors L-235 and odanacatib were compared with alendronate.\(^{(97)}\) While vehicle-treated rabbits exhibited 9.8% to 12.8% BMD loss at the lumbar spine 13-weeks after ovariectomy, treatment with L-235 at a dose of 10 mg/kg completely prevented this BMD loss, a result comparable to treatment with alendronate. However, whereas alendronate decreased trabecular mineralizing surface at the spine by 70%, L-235 had no effect, nor was the rate of endocortical bone formation rate or the number of double-labeled Haversian canals in the femoral diaphysis affected by treatment with L-235. Consistent with these findings, treatment of ovariectomized rabbits with the closely related cathepsin K inhibitor odanacatib, at average daily doses of 4 or 9 µM, prevented lumbar spine BMD loss while also increasing BMD at the proximal femur and femoral neck. Like L-235, no reduction in bone formation at any sites was seen with odanacatib treatment. Further, biomechanical testing of the lumbar vertebrae and central femur demonstrated that the increased BMC found at both sites provided a biomechanical advantage which correlated closely with BMC, consistent with preservation of normal biomechanical properties. Thus although both cathepsin K inhibitors had similar efficacy to alendronate for bone mass preservation, their relative ability to preserve bone formation while inhibiting bone resorption was consistent with other studies of cathepsin K inhibitors, and again suggestive of a novel mode of action for agents in this pharmacologic class.

More recently, work from another group which examined the effects of daily odanacatib treatment at a dose of 9 µM on bone quality using a similar ovariectomy rabbit model suggested that odanacatib reduced ductility and enhanced brittleness at the femur, perhaps due to higher crystallinity and tissue mineralization.\(^{(98)}\) Reasons for the discordant results between these two studies were not immediately evident.

The effect of odanacatib has also been assessed in a growing rabbits where it was shown to significantly increase BMD at the distal femur compared to vehicle \(^{(99)}\), as well as in an ulnar osteotomy fracture healing model in skeletally mature rabbits where odanacatib was found to markedly enhance mineralized callus formation during the early phase of fracture repair, while also improving biomechanical integrity via increases in callus yield load (by 20%) and stiffness (by 26%) when compared to treatment with vehicle alone.\(^{(100)}\)

**C. Primate studies**

*Relacatib*

Relacatib (SB-462795) is an orally bioavailable small molecule inhibitor with equal potency for cathepsins K, L, and V. In normal and ovariectomized cynomolgus monkeys, relacatib rapidly reduced both serum and urinary markers of bone resorption, an effect maintained for up to 48
hours. (101) Treatment of ovariectomized cynomolgus monkeys for nine months with relacatib reduced bone resorption and formation at sites of cancellous bone when assessed by histomorphometry, while simultaneously preserving osteonal bone formation rates in cortical bone and increasing periosteal bone formation. (102)

Balicatib

Balicatib (AAE-581) is an orally available reversible cathepsin K inhibitor. In \textit{in vitro} whole cell assays, it was shown to accumulate in lysosomes (lysosomotropic) where it was determined to also inhibit cathepsin S, thereby reducing its functional selectivity for cathepsin K. (103) When tested in ovariectomized cynomolgus monkeys, balicatib was able to partially prevent ovariectomy-induced bone loss while preventing increased bone turnover at both the vertebra and femoral neck. However, periosteal bone formation rates and cortical thickness, particularly at the mid-femur, were increased. (104) Ultimately, this lysosomotropic effect resulted in the non-selective inhibition of cathepsin S and led to discontinuation of further clinical development efforts with balicatib.

ONO-5334

A recent study compared the effects of treatment for eight months with ONO-5334 on bone turnover, BMD, biomechanical strength and microstructure in ovariectomized cynomolgus monkeys versus alendronate. (105) Consistent with human Phase II clinical trial data (described below), ONO-5334 treatment resulted in dose-dependent decrease of bone resorption markers, prevented loss of vertebral BMD, improved biomechanical bone strength, and increased total and cortical BMD at the femoral neck. Whereas alendronate treatment decreased the ovariectomy-induced increase in femoral mid-shaft osteonal bone formation rate, ONO-5334 at a dose of 30 mg/kg showed no reduction of periosteal, osteonal or endocortical rates of bone formation. Collectively, these results are consistent with a significant effect of ONO-5334 on both cortical BMD and mechanical strength at the femoral neck.

Odanacatib

Odanacatib has also been evaluated in ovariectomized skeletally mature rhesus monkeys in a study in which animals were treated for 21 months with either vehicle or daily oral odanacatib (6 mg/kg or 30 mg/kg). Consistent with previous work in rabbits, odanacatib treatment significantly reduced biochemical markers of bone resorption (urinary NTX by 75% to 90%; serum CTX by 40% to 55%) compared to vehicle-treated animals, yet did not reduce tartrate-resistant acid phosphatase 5b (TRAP5b levels), indicating that odanacatib treatment impaired osteoclast function without a reduction in osteoclast numbers. (106) In contrast to its effects on bone resorption markers, odanacatib at both concentrations reduced the bone formation marker P1NP to levels comparable to those seen in intact animals treated only with vehicle. Both the 6 mg/kg and 30 mg/kg doses of odanacatib increased lumbar spine BMD from baseline (by +7% and +15%, respectively), while biomechanical strength at the lumbar spine trended toward, but did not reach, statistical significance. Histomorphometric analysis showed that osteoclast numbers were maintained or increased with odanacatib treatment. (107) Analyses at the hip found similar dose dependent results, with increases in femoral neck BMD of 11% to 15% and ultimate load increases of 25% to 30% when compared to baseline, with the ultimate load changes correlating closely with the observed increases in femoral neck BMD, BMC, and cortical thickness. (108)

As demonstrated by histomorphometry, odanacatib treatment at the dose of 30 mg/kg stimulated femoral neck and proximal femur periosteal bone formation rates by 3.5-fold and 6-
fold, respectively, compared to vehicle treatment. Additional studies of the effects of odanacatib treatment in ovariectomized adult monkeys using methods to assess bone microstructure, estimates of bone strength from finite element analyses, bone mineralization density distribution, and dynamic histomorphometric changes have reinforced these findings.(109-113) In contrast, however, results at the lumbar spine, a primarily trabecular site, showed that odanacatib treatment led to a reduction in bone formation, a result in contradistinction to earlier findings in trabecular bone with odanacatib treatment in rabbits. Reasons for these noted differences both between species and site-specific effects on bone formation at cortical versus trabecular sites remain uncertain.

IV. Clinical Studies

A. Phase I clinical trials

Relacatib
Relacatib was studied in a Phase I trial of 32 subjects to determine its effects on the metabolism of ibuprofen, acetaminophen, and atorvastatin.(114) Despite promising preclinical data, further development was discontinued following this phase I trial due to concerns raised for potential drug-drug interactions.

Balicatib
In a Phase I study of 675 postmenopausal women (mean age 62 years) with lumbar spine T-scores <-2 randomized to either placebo or increasing doses of balicatib for 12 months, balicatib treatment resulted in a dose-related increase in BMD at both the lumbar spine (+4.4%) and hip (+2.2%), as well as a dose dependent decrease in markers of bone resorption (serum CTX by 61% and urinary NTX by 55%) with the highest two doses studied. Notably, at these doses, serum markers of bone formation were not statistically different from placebo at 12 months.(115) Due to the development of morphea-like skin hardening in nine of 709 subjects treated with the higher doses of balicatib, however, the trial was stopped due to safety concerns. Lesions in eight patients resolved completely with balicatib cessation, with partial resolution occurring in one patient. (60,61) The reason for morphea development is unclear, but may reflect the fact that cathepsin K is normally expressed in dermal fibroblasts where it mediates extracellular matrix degradation, particularly associated with scar tissue.

Odanacatib
Odanacatib was studied in three phase I trials designed to examine safety, tolerability, pharmacokinetic, and pharmacodynamic endpoints. In the first study, 49 postmenopausal subjects were randomized in a blinded manner to either once weekly odanacatib at doses of 5, 25, 50, or 100 mg versus placebo for 21 days.(116) The second study included 30 postmenopausal women and randomized subjects to 0.5, 2.5, or 10 mg odanacatib provided daily for 21 days. Pharmacokinetic analyses demonstrated a half-life of 66-93 hours which would permit once weekly dosing. Following 21 days of treatment, serum CTX and urinary NTX levels declined by approximately 62% from baseline in women treated with once-weekly odanacatib doses of either 50 mg or 100 mg, with even greater reductions in CTX and NTX levels seen in women treated with the highest daily odanacatib dose of 10 mg. In these short-term studies, no serious adverse events were reported, and odanacatib was well tolerated. A more recent report which examined the safety, tolerability, pharmacokinetics, and pharmacodynamics of odanacatib in 44 healthy volunteers (36 men and 8 postmenopausal women) at doses of up to 600 mg in men and 100 mg in women again showed relatively good
tolerability with the exception of one subject who developed gastroenteritis, but who tolerated subsequent higher odanacatib doses without gastroenteritis recurrence.\(^{(117)}\) This study also demonstrated that odanacatib administration with a high-fat meal increased plasma odanacatib concentrations by approximately two-fold versus the fasted state.

B. Phase II clinical trials

ONO-5334
ONO-5334 was evaluated in 285 postmenopausal women with either osteoporosis (defined as a T-score \(\leq -2.5\)), or with osteopenia (defined by a BMD T-score of \(< -1\) and \(> -2.5\)) and a history of prior fragility fracture at the lumbar or total hip. All subjects were studied in a twelve month, randomized, blinded, placebo- and active-comparator (alendronate) controlled trial performed in Europe.\(^{(118)}\) Over twelve months, ONO-5334 was effective at increasing lumbar spine BMD (+3.1\% to +5.1\%), as was alendronate (+5.2\%). At the highest dose of 300 mg once daily, ONO-5334 showed a 3.0\% increase in total hip BMD and a 2.6\% increase in femoral neck BMD, results similar to those seen with alendronate. As also seen in the Phase I study of balicatib, both ONO-5334 and alendronate decreased biochemical markers of bone resorption by 50-70\%, with only ONO-5334 at the highest dose of 300 mg daily showing modest reduction of bone formation markers. These findings again highlight the differences in mechanism of action between bisphosphonates which decrease biochemical markers of both bone resorption and formation, and cathepsin K inhibitors which appear to dissociate these effects.

Extension of the study to 24 months in 197 of the study subjects revealed continued increases in BMD (+6.7\% at the lumbar spine; +3.4\% at the total hip; and +3.7\% at the femoral neck) with the 300 mg/day ONO-5334 dose, continued reduction of serum CTX and urinary NTX levels, but no significant decreases of bone formation markers.\(^{(119)}\) As described below, this apparent cathepsin K inhibition-mediated dissociation of bone resorption and formation in favor of bone formation is likely a critical determinant of the continued increases in BMD that occur with prolonged cathepsin K inhibitor therapy. Notably, treatment discontinuation following 24 months of treatment lead to increases in both urinary NTX and serum TRAP5b levels above baseline, demonstrating rapid reversibility of cathepsin K inhibition. More recently, a small study examining the effects of a sustained release ONO-5334 formulation has been reported.\(^{(120)}\) Review of the clinical trials registry available at clinicaltrials.gov, however, does not show any ongoing clinical trials which involve ONO-5334.

Odanacatib
Odanacatib was evaluated in a double-blind, randomized, placebo-controlled phase II study in which 399 postmenopausal women with low BMD (T-score \(< -2\) but \(\geq -3.5\) at the lumbar spine, femoral neck, total hip, or trochanter) but no history of fragility fracture were treated with either placebo or 3, 10, 25, or 50 mg of odanacatib once weekly for twelve months with a planned twelve month extension.\(^{(121)}\) Endpoints evaluated were percentage change from baseline in BMD at all measured sites, and percentage change from baseline in biochemical indices of bone resorption and formation. All subjects received vitamin D supplementation, and supplemental calcium was provided to those subjects whose average daily calcium intake from all sources was \(< 1000\) mg. At the end of twelve months, 331 of 399 subjects (83\%) remained on study. Of these, 320 subjects continued on the extension study, with 270 (70\%) completing the full pre-planned 24 months. Odanacatib treatment at doses of 10 mg and higher induced dose dependent BMD increases at the lumbar spine and all femoral sites at twelve months, which further increased by 24 months. At the 50 mg once weekly dose, BMD increases were 5.7\% at the
lumbar spine, 4.1% at the total hip, and 4.7% at the femoral neck compared to placebo at 24 months (Figure 2).

At doses of 10 mg and higher, odanacatib treatment decreased markers of bone resorption. Mean urinary NTX values decreased by -60.2% at 12 months and -51.8% at 24 months (Figure 3). In comparison, mean serum CTX values in the three highest odanacatib treatment groups decreased rapidly following treatment initiation, but then increased progressively over the remaining 24 months of study, eventually approaching baseline values, but remaining below values seen in the placebo-treated group. Intriguingly, although there was an initial dose-related decline in TRAP5b levels at week 1, this decrease diminished by the end of the first month and resolved by the end of the third month. When assessed at months 18 and 24, TRAP5b levels were similar across all odanacatib treatment groups and up to 15% higher when compared to placebo treatment. In comparison, odanacatib at a weekly dose of 10 mg or more resulted in a decrease in serum markers of bone formation (bone specific alkaline phosphatase and P1NP) in the first six months of treatment, with gradual increases seen thereafter, again approaching baseline for all but the 50 mg dose which remained reduced below baseline, albeit not quite to the level seen in the bone resorption marker level analyses (Figure 3).

Due to the potential for skin and pulmonary adverse events based upon the previous study of the cathepsin K inhibitor balicatib (60), particular attention was paid to these potential side effects. However, clinical and laboratory adverse events rates (including all reported skin reactions and upper respiratory tract infections) were not different between treatment groups. Finally, iliac crest bone biopsies obtained for histomorphometric analysis near the end of the second year of treatment in 32 subjects showed no significant abnormalities in measured indices including bone formation rate, activation frequency, or osteoclast surface/bone surface ratio between placebo and odanacatib-treated subjects, although the authors noted that small sample sizes limited power to determine the potential significance of small differences noted.

In an extension of this clinical trial, women who had completed 24 months of treatment in the parent Phase II dose-ranging study were invited to continue for an additional 12 months. 189 subjects underwent re-randomization in a 1:1 ratio to continue odanacatib treatment at a fixed dose of 50 mg once weekly, or were switched to placebo.(122) Women who were switched to placebo after two years of odanacatib therapy experienced rapid bone loss at the at the lumbar spine, total hip and femoral neck, with BMD levels returning to near baseline levels within 12 months of odanacatib discontinuation. Further, biochemical markers of both bone resorption and formation increased markedly within one month of placebo initiation before returning to baseline by 36 months (Figure 4). In contrast, subjects continuously treated with odanacatib 50 mg for 36 months showed BMD increases from baseline at the lumbar spine of +7.9%, total hip of +5.8%, and femoral neck of +5.0%. Continued treatment with odanacatib at a dose of 50 mg for the full 36 month time period resulted in a decrease of urinary NTX levels by approximately 50% from baseline, while P1NP levels had returned to baseline and bone specific alkaline phosphatase levels were 18% above baseline (Figure 4). Consistent with the 24 month data, TRAP5b levels remained increased above baseline at 36 months in women who received continuous treatment with odanacatib 50 mg once weekly, but declined to placebo levels in subjects switched to placebo. Overall adverse event rates were again similar between both treatment groups, with the exception that odanacatib-treated subjects had a greater number of uncomplicated urinary tract infections (n=12) compared to placebo (n=3) in the third year, a finding which was not seen during the initial two years of the study. No serious skin disorders were noted, and morphea was not observed in any study subject.
In the pre-specified extension which prolonged this Phase II study to five years, women who had received placebo or odanacatib at a weekly dose of 3 mg in the first two years and placebo for the third year were treated with odanacatib 50 mg per week starting at year 4, with the other included subjects continuing with the same treatment as per year three.(123) Subjects who received weekly odanacatib 50 mg weekly for five years demonstrated near linear increases in BMD when compared to baseline at the lumbar spine (+11.9%), femoral neck (+9.5%), and total hip (+8.5%) (Figure 5). In women who had received any dose of odanacatib continuously for five year, urinary NTX and serum CTX levels remained significantly reduced, whereas they had returned to near baseline at five years in subjects treated with odanacatib for two years followed by placebo for three years. In comparison, at five years the bone formation marker serum bone specific alkaline phosphatase was slightly lower than baseline in women who had received five consecutive years of odanacatib treatment and in women who were switched to placebo after two years. P1NP levels, however, were not different from baseline for either group at the five year endpoint. Consistent with previous results, TRAP5b levels, a biochemical surrogate of osteoclast numbers, were higher at five years in women who received odanacatib for the final two years than in subjects who received placebo during the final two years. For safety endpoints, no significant differences were again seen between subjects treated with placebo versus odanacatib 50 mg in years 4-5 of the trial, although uncomplicated urinary tract infections were again higher in subjects who received odanacatib. Finally, an additional extension of this Phase II study in which postmenopausal women received continuous treatment with odanacatib for up to eight years has recently been reported.(124) Consistent with the earlier data, continuous odanacatib treatment for 8 years resulted in continued increases in BMD at the lumbar spine of +14.8% versus baseline, with a similar pattern of increases noted at the femoral neck and total hip. Whereas bone resorption markers remained decreased relative to baseline, bone formation markers remained near baseline levels.

A recently reported study of odanacatib treatment for 12 months in Japanese female and male patients with osteoporosis has described similar changes in BMD and bone turnover markers to results previously reported in predominantly Caucasian populations.(125) In addition, studies which evaluated the effect of odanacatib in older men have confirmed that pharmacokinetic and pharmacodynamic parameters in older men are comparable to those of older women.(126)

The effects of treatment with odanacatib 50 mg once weekly on changes from baseline in BMD and bone turnover markers has also been evaluated in a randomized, double-blind, placebo-controlled study of 24 months in 243 postmenopausal women previously treated with alendronate for at least three years.(127) In comparison to placebo, odanacatib treatment for 24 months lead to significant but incremental gains from baseline at the lumbar spine (2.3%), femoral neck (1.7%), and total hip (0.8%).

Odanacatib has also been studied in women with breast cancer and metastatic bone disease. In a small study of 43 patients in women with breast cancer complicated by bone metastases, daily treatment for four weeks with odanacatib at a dose of 5 mg reduced urinary NTX levels by -77% from baseline (versus -73% in women treated with a single dose of the potent intravenous bisphosphonate zoledronic acid).(128) In both the odanacatib and zoledronic acid groups, two subjects had progression of disease. Rash and pruritus were documented in two patients treated with odanacatib, but resolved after treatment discontinuation.

C. Phase III clinical trials

Odanacatib
Among the cathepsin K inhibitors, only odanacatib has been studied in the setting of a Phase III clinical trial. In a randomized, double-blind study of 214 postmenopausal women with osteoporosis defined by BMD as determined by dual energy x-ray absorptiometry (DXA) imaging, treatment with odanacatib 50 mg versus placebo once weekly for 24 months increased lumbar spine areal BMD by 3.5% and induced changes in serum CTX and P1NP levels similar to previously reports.(129) Quantitative computed tomography (QCT) imaging coupled with finite element analyses found that odanacatib produced significant increases in both trabecular volumetric BMD and estimates of compressive strength at both the spine and hip when compared to placebo. In addition, at the cortical envelope of the femoral neck, BMD, thickness, volume, and cross-sectional area were also significantly increased compared to baseline with odanacatib treatment. Exploratory analyses using high-resolution peripheral QCT (HRpQCT) to evaluate cortical geometry and bone strength at the distal radius and tibia demonstrated that odanacatib treatment resulted in significantly greater improvements in total, trabecular, and cortical volumetric BMD, cortical thickness, and estimated strength (failure load) at the distal radius.(130) In addition, odanacatib treatment reduced the increase in cortical porosity seen in placebo-treated women. Similar changes in volumetric BMD and cortical thickness were seen at the distal tibia. From QCT analyses, both trabecular and cortical bone compartments at the proximal femur were also determined to have been affected by similar gains in BMC, with increases in cortical volume and BMC paralleling the increase in cortical volumetric BMD.(131)

The Long-Term Odanacatib Fracture Trial (LOFT) was a large international, randomized, blinded, placebo-controlled study which included 16,713 postmenopausal women age ≥ 65 years with a BMD T-score of ≤ -2.5 at the total hip or femoral neck, or a prior history of vertebral fracture and a T-score at the total hip or femoral neck of ≤ -1.5.(132) In this event-driven trial, subjects were randomly assigned in a 1:1 ratio to weekly treatment with either odanacatib 50 mg or placebo. Primary endpoints were radiographically determined vertebral, hip, and clinical nonvertebral fractures, with pre-planned interim analyses included to permit early study termination if significant fracture reduction was demonstrated; secondary endpoints were clinical vertebral fractures, change from baseline in BMD, biochemical markers of bone turnover, and safety and tolerability including bone histomorphometry. All subjects received 5600 IU supplemental vitamin D once weekly and daily calcium supplementation as needed for total daily calcium intake of approximately 1200 mg. Due to robust efficacy for the primary endpoints, an independent data monitoring committee recommended early study termination following a planned interim analysis. Following closure of the primary study, 8256 study subjects were enrolled in the study extension. The results of the study remain unpublished, but have been presented in abstract form at recent annual meetings of the American Society for Bone and Mineral Research.

Consistent with the Phase II and smaller Phase III studies described above, when compared to placebo, treatment with odanacatib 50 mg once weekly for three years was associated with relative risk reductions of 54% for new and worsening morphometric vertebral fractures; 47% for clinical hip fractures; 23% for clinical nonvertebral fractures; and 72% for clinical vertebral fractures (133). Subsequent subgroup analyses showed that these relative risk reductions were generally consistent across subgroups assessed by baseline age, race, bisphosphonate intolerance, prior history of radiographic vertebral fracture, and baseline BMD.(134) A second subgroup analysis of 164 women (78 treated with odanacatib; 84 treated with placebo) demonstrated that odanacatib treatment significantly increased trabecular, cortical, and integral volumetric BMD at both the spine and total hip versus placebo, changes which were associated with increases in
whole bone estimated strength at both sites as assessed by finite element analyses. In the pre-planned double-blinded LOFT extension study which included 8257 subjects for a mean follow-up of 44 months, odanacatib treatment resulted in relative risk reductions of 52% for morphometric vertebral fractures, 48% for hip fractures, 26% for non-vertebral fractures, and 67% for clinical vertebral fractures with mean increases in BMD at the lumbar spine of 10.9% and 10.3 at the total hip.

Due to potential safety concerns raised in the Phase II studies, multiple distinct categories of specific adverse events were designated for adjudication by an external independent clinical adjudication committee. Specific categories included dermatologic (morphea-like skin lesions and systemic sclerosis), serious respiratory infections, skeletal (delayed fracture union, osteonecrosis of the jaw, atypical femoral shaft fractures), and major adverse cardiovascular events. Dermatologic and respiratory adverse events were included for adjudication based on adverse event signals identified in the earlier Phase II studies of balicatib. Bone-related adverse events were included due to adverse events that have been reported in association with other classes of anti-resorptive agents. Major adverse cardiovascular events were included based on earlier reports of excess atrial fibrillation in placebo controlled trials of the anti-resorptive zoledronic acid, and observations of atheroma stabilization in a murine genetic cathepsin K null model of dyslipidemia.

Although adverse events in LOFT were reported to be generally similar between the odanacatib and placebo-treated groups, final adjudication of safety endpoints demonstrated that some adverse events were more common in subjects treated with odanacatib. Thus, both diarrhea and extremity pain were both seen more frequently with odanacatib treatment, as was morphea (thirteen adjudicated cases of morphea-like lesions in subjects treated with odanacatib (0.1% incidence) compared to three in subjects treated with placebo). While no cases of osteonecrosis of the jaw were noted in either group, atypical femoral fractures were observed in ten patients treated with odanacatib (0.1% incidence) versus none in the placebo group. There were no differences in serious respiratory infections, systemic sclerosis, or delayed fracture unions noted between the groups.

Ultimately of greater concern were differences between odanacatib and placebo treated patients as determined by the independent Thrombosis in Myocardial Infarction (TIMI) adjudication committee which was tasked specifically with evaluating for differences in cardiovascular and cerebrovascular events based upon earlier, statistically non-significant signals for increases in cerebrovascular accidents as well as atrial fibrillation and atrial flutter in subjects treated with odanacatib. As reported at the 2016 American Society for Bone and Mineral annual meeting, whereas adjudicated atrial fibrillation and atrial flutter events were more common in patients treated with odanacatib, the difference did not reach statistical significance (hazard ratio (HR) 1.22; 95% confidence interval (CI) 0.99-1.50). Likewise, when considered in aggregate, major adverse cardiovascular events were also higher in the odanacatib group versus the placebo group, but this also did not reach statistical significance. However, relatively to treatment with placebo, odanacatib treatment was associated with a significantly increased risk for cerebrovascular accidents group (HR 1.37; 95% CI 1.10-1.71; P < 0.01), the majority of which were ischemic rather than hemorrhagic. Based on this independent analysis which demonstrated that the earlier trend towards an increased risk for cerebrovascular accidents appeared to be further increased during the extension phase of LOFT, the study sponsor ultimately withdrew odanacatib from regulatory consideration by the United States Food and Drug Administration (US FDA).
V. Preclinical and Clinical Studies Providing Novel Insights into the Biology of Osteoclast-Osteoblast Coupling

Collectively, the preclinical and clinical data presented provide strong evidence for a novel mechanism of action for cathepsin K inhibitors when compared to other classes of anti-resorptive agents such as the bisphosphonates or denosumab. Based on these data, this fundamental difference is the result of comparatively greater decreases of bone resorption when compared to bone formation. Although the molecular and cellular bases for how this relative uncoupling of bone resorption from bone formation remain incompletely understood, recent insights into the biology and importance of osteoclast-osteoblast coupling may provide some insight into the novel skeletal effects seen with cathepsin K inhibition.

As first proposed by Harold Frost and more recently extensively reviewed (141-148), the dynamics of bone remodeling require that bone resorption and subsequent bone formation be coupled. Evidence that coupling of bone formation subsequent to bone resorption occurs has come from rat, mouse, and human studies. In rodents, a “lag phase” between bone resorption and the initiation of new bone formation has been observed, with elevated bone formation measured within two to four weeks following removal of sex steroids.(149,150) Studies have revealed that this lag phase and coupling also exists in humans. Post-menopausal women treated for 24 weeks with estrogen exhibited an immediate decrease in bone resorption markers, while bone formation markers did not decrease until 4 weeks post-treatment.(151) In addition, suppression of sex steroids in men resulted in immediate increases in serum markers for bone resorption, while a delay (12 weeks) occurred prior to an increase in bone formation markers.(149,152) These data support the concepts that changes in bone resorption and, by inference, in the number of osteoclasts, alter bone formation rates and that a lag phase between bone resorption and formation exists in both sexes.

Control of the initiation and progress of this sequence of events is subject to both systemic and local modulators. Osteocyte apoptosis resulting from micro-cracks or damage to bone may serve as a stimulus to initiate remodeling, and immune cells may also promote this process.(153-155) Bone remodeling can be initiated by bone damage, a change in the load experienced by bone, or the necessity to remove old bone. An early event in the initiation of bone remodeling is the formation of a bone resorption compartment (BRC) by bone lining cells, closely associated with capillaries (156-158) (Figure 6). The signals that initiate the formation of the BRC are not well-understood, although it has been postulated that osteocytes, with their interconnections throughout bone, may somehow recognize the need for bone replacement and convey a stimulus to the bone surface to initiate canopy formation.(159) It is also possible that bone lining cells themselves may sense the need for remodeling and thereby release paracrine factors to drive BRC formation.

Osteoclasts, which originate from hematopoietic precursors, can be recruited from the local marrow population or arrive at resorption sites through the local capillary blood supply. Osteoblast precursors can likewise arise from the local bone marrow environment (e.g., from perivascular precursor cells) or arrive at the BMU through the local capillary system.(160,161) Bone lining cells can become bone forming osteoblasts if stimulated mechanically or by PTH, so these cells may also represent an osteoprogenitor pool.(162-164) Local endothelial cells and osteoblast lineage cells provide M-CSF and RANKL to drive osteoclast differentiation.(165-168) While it is recognized that osteoclast precursor differentiation and the initiation of bone resorption require M-CSF and RANKL, how osteoblast progenitors are recruited and induced to differentiate remains an active area of research.
**In vitro** studies indicate that osteoprogenitor cells can differentiate into mature osteoblasts within a week.(169) Expression of osteocyte marker genes follows with a 2 to 3 week further delay.(170,171) However, the reversal lag phase between bone resorption and bone formation is estimated to be between 5 and 8 weeks (144), prompting interest and speculation as to what is occurring at the bone surface during the reversal phase. Within the BRC, osteoclasts actively resorbing bone are separated from osteoblasts replacing that bone by the recently resorbed surface, about 80% of which is covered with flat cells termed reversal cells (reviewed in (148)). Subsequent to resorption, the resorbed surface is further processed by both catabolic and anabolic activities of these flat cells, leaving a smoother bone surface.(4)

During bone remodeling, minute areas of bone are resorbed prior to osteoblast replacement of the resorbed bone, usually with great precision in both location and amount. This requires recruitment of osteoprogenitor cells, stimulation of their differentiation, and control of the amount of new bone formed. Two crucial aspects for coupling bone resorption to formation are correctly targeting the site of bone formation and regulating the amount of new bone formed. Regulation of the site of bone formation could be controlled by highly localized chemokine production, while control of the amount of bone formed may be modulated by regulating the number of osteoblasts that differentiate once recruited to the resorption site, in addition to mechanisms which cause cessation of bone formation once the required amount of bone has been deposited.

A number of candidate factors have been proposed to initiate and drive bone formation, including factors released from the bone matrix itself during the resorption process and factors produced by osteoclast lineage cells themselves. Bone is a significant storage compartment for transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1).(172-174) Osteoclasts release and activate bone-bound TGF-β, which has been documented to participate in the recruitment of osteoblast lineage cells.(173,175,176) Resorption-released IGF-1 has also been shown to stimulate the differentiation of osteoblasts.(177) Thus, TGF-β and IGF-1 released by osteoclast activity have been implicated in osteoclast-osteoblast coupling.

However, there is also evidence that bone resorption may not be essential for coupling. Both humans and genetically altered mice with reduced osteoclast function or defective osteoclast differentiation have provided important insights into the role of bone resorption in coupling (reviewed in (142,178)). In mice in which osteoclasts are present but unable to degrade bone, such as c-Src or chloride-7 channel null mice, bone formation appears normal.(179-181) Similarly, in humans with an inactivated chloride-7 channel gene, osteoclasts are present but there is inhibition of bone resorption with no reduction in bone formation.(180,182,183) Inhibition of bone resorption achieved via targeting of acid secretion effectively blocked bone degradation and partially blocked anabolic bone formation, further supporting a role for both bone resorption and for osteoclasts, independent of resorption, in coupling.(184) In contrast, mice with gene defects that reduce osteoclast differentiation, such as mice lacking c-Fos or M-CSF also have defects in bone formation.(185-187)

Osteopetrosis is a syndrome in which there is an immense excess of bone. Osteopetrosis can be divided into two types: osteoclast-rich and osteoclast-poor.(188,189) In osteoclast-rich osteopetrosis, nonfunctional osteoclasts are present.(180,182,190-192) In these patients, osteoblast formation appears comparatively normal or elevated despite a lack of ongoing bone resorption.(182,193) Indeed, there is a direct correlation between osteoclast and osteoblast numbers.(182) In comparison, in osteoclast-poor osteopetrosis, there is a marked reduction in osteoblast numbers (194), which likely contributes to the fact that osteoclast-poor osteopetrosis
produces a milder phenotype. These observations support the concept that the presence of osteoclasts, whether or not actively engaged in bone resorption, is needed for normal bone formation. Thus, release of bone-bound factors may not be required to couple bone resorption to bone formation during bone turnover.

A number of groups have examined whether osteoclasts produce coupling factors independent of bone resorption. Recent work from our group used an unbiased gene array survey to identify candidate osteoclast coupling factors. We found that osteoclasts express Wnt10b and BMP6, as well as produce the chemokine sphingosine-1-phosphate (S1P). Wnt10b was primarily involved in promoting differentiation of osteogenic precursors with little influence on their recruitment. In vitro studies of conditioned medium from non-resorbing osteoclasts revealed that while BMP6 and Wnt10b concentrations were individually too low to stimulate osteoblast differentiation, in combination they were capable of promoting differentiation, confirming roles for these candidate coupling factors acting in concert to promote bone formation. Additional evidence supports a role for S1P as a candidate coupling factor that recruits osteoblast lineage cells. In addition, S1P may be involved in osteoclast recruitment through activation of the S1P receptor S1PR2, whereas activation of S1PR1 leads to a chemorepulsion response, reducing bone resorption by maintaining osteoclast precursors in the circulatory system. Thus the roles of S1P in osteoclast-osteoblast coupling appear complex. Cathepsin K-null mouse osteoclasts studies have lent further support for a role for S1P in coupling. In these mice, osteoclasts are formed, but bone resorption is compromised. Osteoclasts were generated in vitro from Cathepsin K-null mice and the conditioned medium examined for effects on osteoblastic cells. The osteoclast conditioned medium exhibited enhanced promotion of bone formation, which was blocked by a S1P receptor antagonist.

In our studies, pharmacological blockade of BMP6, Wnt10b, and S1P separately reduced the ability of osteoclast conditioned medium to promote mineralization by mesenchymal cells. Inhibition of all three factors in combination did not completely block induction, suggesting that other osteoclast-osteoblast coupling factors likely contribute by mechanisms independent of these stimulatory factors. Several other groups have documented that other locally generated factors produced at sites of bone resorption are likely to function in both recruitment and differentiation of osteoprogenitor cells as well. For example platelet derived growth factor (PDGF)-BB is produced by osteoclasts independent of bone resorbing activity and can induce migration of mesenchymal stem cells and mouse pre-osteoblasts. However, another study reported that PDGF-BB inhibited osteoprogenitor differentiation; thus the potential role of PDGF-BB on coupling requires further clarification.

In global cardiotrophin-1 (CT-1) knockout mice, osteoclasts formed but their activity was impaired, and bone formation was reduced. While a reduction in matrix released factors may have contributed to the observed phenotype, further studies documented that CT-1 can directly stimulate bone formation in vivo as well as osteoblast differentiation in vitro. Thus the relative contribution of CT-1 to osteoclast-osteoblast coupling remains unresolved. Afamin, a vitamin D/albumin binding protein family member, is produced by osteoclasts and has been shown to recruit osteoblast progenitors in vitro. Collagen triple helix repeat containing 1 (CTHRC1) is produced by osteoclasts during the bone resorptive process and can stimulate bone formation in vivo and in vitro. Semaphorin 3A (Sema3A) is a secreted protein that has been shown to inhibit osteoclast recruitment and to promote osteoblast recruitment. However, studies by other groups have provided conflicting results, raising questions as to whether Sema3A has a direct role in osteoclast-osteoblast coupling.
Several studies have examined the extent to which coupling factors tethered to membranes may be involved in recruiting osteoprogenitor cells. The Ephs form a family of tyrosine kinase receptors, and ephrins are ligands for Ephs and are similarly membrane-bound, thus requiring physical contact between cell types for receptor activation. Importantly, signaling between ligand and receptor is bi-directional. Ligand binding not only stimulates receptor signaling, but receptor binding to the ligand causes rapid phosphorylation of tyrosine residues on the ephrins. Osteoclasts express ephrin B2, and in vivo and in vitro evidence indicates that osteoclast lineage ephrinB2 binds to osteoblast lineage EphB4 to promote osteoblast differentiation, while ephrinB2 reverse signaling inhibits osteoclast differentiation. However, targeting ephrinB2 in osteoclast lineage cells did not support that reverse signaling was a major contributor for control of osteoclast differentiation in vivo. Semaphorin4D (Sema4D) can exist as either a secreted protein or as a membrane-associated regulator of cell differentiation. Sema4D is an osteoclast-produced inhibitor of bone formation, which suggests a mechanism by which osteoclasts could limit the amount of bone formed following resorption to thereby provide required fine tuning of bone turnover. To fully resolve the impact of each of these candidate coupling factors on the bone remodeling process will require complex in vivo studies.

Currently available anti-resorptive therapies target osteoclasts, either by inhibiting their differentiation (estrogen, the selective estrogen receptor modulator raloxifene, and the RANKL inhibitor denosumab) or by disrupting osteoclast viability to the extent that apoptosis is induced (bisphosphonates). Importantly, all currently approved anti-resorptive drugs cause a parallel reduction in bone formation. In contrast to anti-resorptive agents that reduce osteoclast numbers, cathepsin K inhibitors decrease osteoclast function but maintain or even increase osteoclast numbers. As shown for odanacatib and other cathepsin K inhibitors in primate studies, cathepsin K inhibition reduces both bone resorption and bone formation in trabecular bone, similar to other antiresorptive therapies. By contrast, in the femur, cathepsin K inhibition treatment reduces bone resorption yet increases histologically measured bone formation rates on periosteal surfaces.

These findings suggest that the effects of cathepsin K inhibition on bone are likely complex, and Figure 7 provides a conceptual model for how cathepsin K inhibitors may differ from current anti-resorptive drugs. Panel A depicts both the direct effects of osteoclasts on bone formation as well as the indirect effects mediated by release of growth factors from bone. As shown in Panel B, current anti-resorptive drugs, which markedly reduce osteoclast numbers, result in a profound reduction in bone formation on all surfaces. In contrast, the effects of cathepsin K inhibitors are likely both surface and time dependent. As depicted in Panel C, as with all anti-resorptive agents, the reduction in bone resorption following cathepsin K inhibitor treatment will lead to a reduction in growth factor release from bone matrix, leading to a reduction in bone formation. However, in contrast to other antiresorptive drugs, cathepsin K inhibition leads to an accumulation of relatively normal (but non-resorbing) osteoclasts. As such, the cell-cell and secreted coupling mechanisms described above would be expected to remain intact during cathepsin K inhibitor treatment. Indeed, as noted earlier, deletion of cathepsin K in mice leads to increased production of S1P by the mutant osteoclasts, leading to a stimulation of bone formation. Thus, the net effect on bone formation would depend on the offsetting effects of the loss of growth factor release from the bone matrix leading to a reduction in bone formation, versus the ongoing, perhaps enhanced effects of increased numbers of relatively healthy osteoclasts on directly stimulating bone formation. In trabecular
bone, with its high remodeling rate (219), the release of growth factors from the bone matrix may be particularly important and here odanacatib reduces bone formation, as shown in the primate studies. (107,108) By contrast, on periosteal surfaces, where the remodeling rate is much lower (219), the loss of growth factor release from the bone matrix may have only a minor inhibitory effect on bone formation, with the major effect being the direct stimulatory effects of osteoclasts [which are present on periosteal surfaces (220)] on osteoblasts, leading to a net increase in bone formation.

Of interest, this model for cathepsin K inhibitor effects on bone formation may also explain why, in the phase II study with odanacatib, bone formation markers decreased significantly in postmenopausal women during the first 6 months of therapy but then returned to baseline by 24 months despite a persistent reduction in bone resorption markers. (121) As depicted in Panel C, the initial decrease in bone formation following the initiation of odanacatib therapy likely reflects the dominant effects, in these women with high bone turnover, of reducing bone resorption and coupling factor release from the bone matrix. Over time, however, the accumulation of relatively normal osteoclasts on bone surfaces would be expected to counteract through direct mechanisms (cell-cell contact and osteoclast secreted factors) this initial inhibitory effect, leading by 24 months to near baseline levels of bone formation.

VI. Potential clinical role of cathepsin K inhibitors

Given ongoing concerns regarding the use of pharmacologic agents with potent anti-resorptive properties (bisphosphonates and denosumab) and prolonged biologic half-lives (bisphosphonates) and their potential relationship to very rare, but widely reported, complications of prolonged therapy including osteonecrosis of the jaw (221) and subtrochanteric femoral fractures (222), the development of another class of potent anti-resorptive agents with a different mechanism of action warrants careful consideration. With the final results of the Phase III study of odanacatib now publicly reported, including the independently adjudicated safety endpoints, odanacatib has been withdrawn from the US FDA regulatory approval process. Thus, although odanacatib may not proceed to clinical use, the lessons learned about the underlying biology and clinical efficacy of cathepsin K inhibitors, as well as understanding the adverse events that ultimately led to the demise of odanacatib as a therapeutic agent, are useful in terms of informing future drug development efforts in osteoporosis, particularly with regard to the development of agents that may inhibit bone resorption without inhibiting bone formation.

Summary and future directions

Cathepsin K inhibition represents a novel approach for the treatment of osteoporosis. Whereas currently available anti-resorptive agents diminish both osteoclast activity and numbers thereby resulting in both decreased bone resorption but also a secondary decrease in bone formation, cathepsin K inhibition results in increased numbers of osteoclasts which while impaired in their ability to resorb bone matrix, remain on the bone surface where they appear capable of local signaling to adjacent cells, including osteoblasts and osteoblast-lineage cells, to thereby maintain osteoblast function and activity. Thus although cathepsin K inhibition effectively limits osteoclast-mediated bone resorption, it also permits a relative preservation of bone formation. Further, these differential effects on osteoclast and osteoblast activity appear to most profoundly affect cortical bone sites such the femur, where increases in both cortical thickness and bone strength have been observed in both pre-clinical models and clinical studies. Such effects might
be anticipated to reduce non-vertebral fracture risk more than currently available anti-resorptive therapies.

While it is uncertain if the significant suppression of bone remodeling that occurs in patients treated with either bisphosphonates or denosumab leads to impaired skeletal fragility (223), the introduction of cathepsin K inhibitors as a new pharmacologic class with a potentially different safety profile and a novel mechanism of action was anticipated to have been timely. However, unlike both bisphosphonates and denosumab whose primary biologic target is the osteoclast, cathepsin K inhibitors have the potential to affect other tissues given that cathepsin K expression is not limited solely to the osteoclast. Such potential concerns limited the development of all cathepsin K inhibitors except odanacatib, which as noted was recently withdrawn from US FDA consideration due to safety concerns related to an increased risk for cerebrovascular accidents.

Notably, odanacatib was only tested in Phase III trials against placebo rather than against an active comparator such as alendronate. As such, it might have been challenging for clinicians to know where to place odanacatib within the current range of pharmacologic options. Given the well-documented benefits of other anti-resorptive agents for limiting bone loss and fracture risk, it was unclear that cathepsin K inhibition would have been considered as first-line therapy in all eligible patients, but rather likely that it might have been of particular benefit for those patients in whom other established agents are either contraindicated or deemed unsuitable. Accordingly, the loss of this novel agent as an anticipated treatment option in our osteoporosis pharmacologic armamentarium was a setback to the bone health community. Nonetheless, the lessons learned about the underlying biology and clinical efficacy, as well as adverse events, of cathepsin K inhibitors should continue to guide future drug development efforts for osteoporosis.

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**References**
1. Frost HM. Treatment of osteoporoses by manipulation of coherent bone cell populations. Clin Orthop Relat Res 1979;227-244


Cleutjens KB. Disruption of the cathepsin K gene reduces atherosclerosis progression and induces plaque fibrosis but accelerates macrophage foam cell formation. Circulation 2006; 113:98-107


69. Gelb BD, Spencer E, Obad S, Edelson GJ, Faure S, Weissenbach J, Desnick RJ. Pycnodysostosis: refined linkage and radiation hybrid analyses reduce the critical region to 2 cM at 1q21 and map two candidate genes. Human genetics 1996; 98:141-144


86. Chavassieux P, Seeman E, Delmas PD. Insights into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biomechanical properties of bone are compromised by disease. Endocrine reviews 2007; 28:151-164


102. Stroup GB, Kumar S, Jerome CP. Treatment with a potent cathepsin K inhibitor preserves cortical and trabecular bone mass in ovariectomized monkeys. Calcified tissue international 2009; 85:344-355
104. Jerome C, Missbach M, Game R. Balicatib, a cathepsin K inhibitor, stimulates periosteal bone formation in monkeys. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2011; 22:3001-3011


Evaluation of high-resolution peripheral quantitative computed tomography, finite element analysis and biomechanical testing in a pre-clinical model of osteoporosis: a study with odanacatib treatment in the ovariectomized adult rhesus monkey. Bone 2012; 50:1379-1388

Odanacatib on bone mineralization density distribution in thoracic spine and femora of ovariectomized adult rhesus monkeys: a quantitative backscattered electron imaging study. Calcified tissue international 2013; 92:261-269

Effect of odanacatib on bone turnover markers, bone density and geometry of the spine and hip of ovariectomized monkeys: a head-to-head comparison with alendronate. Bone 2013; 56:497-505

Effect of odanacatib on bone turnover markers, bone density and geometry of the spine and hip of ovariectomized monkeys: a quantitative backscattered electron imaging study. Calcified tissue international 2013; 92:261-269

Effect of odanacatib on bone turnover markers, bone density and geometry of the spine and hip of ovariectomized monkeys: a head-to-head comparison with alendronate. Bone 2013; 56:497-505


Effect of One Year Treatment with the Cathepsin-K Inhibitor, Balicatib, on Bone Mineral Density (BMD) in Postmenopausal Women with Osteopenia/Osteoporosis. J Bone Miner Res 2006; 21(Suppl 1):Abstract 1085


Women with Osteoporosis: 5-Year Data from the Extension of the Phase 3 Long-Term Odanacatib Fracture Trial (LOFT). American Society for Bone and Mineral Research Annual Meeting; 2016; Atlanta, Georgia.


143. Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. Bonekey Rep 2014; 3:481

144. Sims NA, Martin TJ. Coupling Signals between the Osteoclast and Osteoblast: How are Messages Transmitted between These Temporary Visitors to the Bone Surface? Front Endocrinol (Lausanne) 2015; 6:41


146. Martin TJ. Coupling factors: how many candidates can there be? J Bone Miner Res 2014; 29:1519-1521


156. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. J Bone Miner Res 2001; 16:1575-1582


164. Dobnig H, Turner RT. Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. Endocrinology 1995; 136:3632-3638


Khosla S. Odanacatib: location and timing are everying. J Bone Miner Res 2012; 27:506-508

Cusick T, Chen CM, Pennypacker BL, Pickarski M, Kimmel DB, Scott BB, Duong le T. Odanacatib treatment increases hip bone mass and cortical thickness by preserving endocortical bone formation and stimulating periosteal bone formation in the ovariectomized adult rhesus monkey. J Bone Miner Res 2012; 27:524-537


**Figure 1.** Bone cell morphology by light microscopy on 5-µm-thick undecalcified bone biopsy sections from patients with pycnodysostosis. The top four rows (A, B, D, and E) correspond to patient A, and the bottom row corresponds to patient B (C and F). Left column: Goldner’s
Trichrome stain showing osteoclasts. Right column: Giemsa staining showing lining cells and osteoclasts. A-C, defective multinucleated osteoclasts adjacent to demineralized collagen fringes (pink) and mineralized bone matrix (green). Note the comparatively deeper resorption lacunae in patient A (A and B) versus patient B (C). D-F, Bone-lining cells (dark blue) in a resorption area (light pink, arrows) after osteoclast detachment from the bone surface (D) or in orphan resorption pits (E and F). The mineralized bone surface is dark pink. Used with permission.(87)

Figure 2. Changes in BMD. Mean percentage change from baseline over 24 months in BMD at the lumbar spine (A), total hip (B), femoral neck (C), and distal one-third radius (D) in subjects treated once weekly with either placebo (open circles) or odanacatib 50 mg (solid circles). Adapted from (121). Data from John Wiley and Sons, with permission.

Figure 3. Changes in biochemical markers of bone resorption and formation. Mean percentage change from baseline over 24 months for markers of bone resorption [(A) urinary NTX/Cr and (B) serum CTX] and bone formation [(C) serum bone specific alkaline phosphatase and (D) serum P1NP] in subjects treated once weekly with either placebo (open circles) or odanacatib 50 mg (solid circles). Adapted from (121). Data from John Wiley and Sons, with permission.

Figure 4. Changes in biochemical markers of bone resorption and formation. Mean percentage change from baseline over 36 months for markers of bone resorption [(A) urinary NTX/Cr and (B) serum CTX] and bone formation [(C) serum bone specific alkaline phosphatase and (D) serum P1NP] in subjects treated once weekly with placebo/placebo (dark squares), odanacatib 50 mg/placebo (open circles), or odanacatib 50 mg/odanacatib 50 mg (solid circles). Adapted from (122).

Figure 5. Changes in BMD. Mean percentage change from baseline over 60 months in BMD at the lumbar spine (A) and total hip (B) in subjects treated once weekly with placebo/placebo (dark squares), odanacatib 50 mg/placebo/placebo (open circles), or odanacatib 50 mg/odanacatib 50 mg/odanacatib 50 mg (solid circles). Adapted from (123).

Figure 6. Histology (A-C, magnification 40x) and composite schematic (D) of the BRC, which comprises the cells constituting the BMU — specifically osteoclasts (OCs), osteoblasts (OBs), and osteocytes — as well as the canopy of bone-lining cells and the associated capillary. Panel A shows a BRC in trabecular bone, demonstrating the location of the OBs along the bone-forming surface. The osteocytes are shown embedded in the bone matrix and the canopy of cells consists of bone-lining cells. Panel B shows a BRC in cortical bone (outer demarcation by the broken line) that is filled with erythrocyte ghosts (EG) and OBs; a few OCs are also seen. CV denotes the central vessel of the Haversian system, which forms the basic structural unit in cortical bone. Panel C shows a BRC stained with an antibody specific for CD34, which demonstrates staining of endothelial cells in the marrow capillary adjacent to the BRC. Panel D is a composite schematic of the BRC, showing connections between the osteocyte network, surface bone-lining cells, and the BRC. All cells in this network are connected with gap junctions, which might provide a pathway (block arrows) by which signals generated by osteocytes deep within the bone reach the surface and elicit remodeling events by OCs and OBs. Note also the potential direct physical contact between OCs and OBs, which would allow for signaling between these cells. Reproduced from Khosla et al. (224), with permission.
Figure 7. (A) Working model for mechanisms by which osteoclasts regulate osteoblasts and bone formation; (B) Proposed changes in osteoclast-osteoblast coupling following treatment with conventional anti-resorptive agents, including bisphosphonates and denosumab; and (C) Proposed, more complex changes in osteoclast-osteoblast coupling following treatment with cathepsin K inhibitors. Adapted from Khosla (216).

Table 1: Reported CTSK Mutations Causing Pycnodysostosis

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<th>Coding DNA Sequence Variants</th>
<th>Effect on Amino Acid</th>
<th>Location in Protein Sequence</th>
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<td>g.9169G&gt;A</td>
<td>c.890G&gt;A;785_890del</td>
<td>p.Gly262AlafsX70</td>
<td>Mature</td>
<td>(226)</td>
</tr>
</tbody>
</table>

### Stop Codon

| Exon 8  | g.11538A>G | c.990A>G | p.X330TrpextX19 | Mature | (69) |

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![Graphs showing changes in bone mineral density (BMD) over time.](image)

- **A**: LS BMD
- **B**: Total hip BMD
- **C**: FN BMD
- **D**: One-third radius BMD

**Legend:**
- ○ Placebo
- ● ODN 50 mg

**Axes:**
- X-axis: Month
- Y-axis: Weighted LS mean % change from baseline

**Data Points:**
- N = 399
- N = 320
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**Figure Legend**

**A** Urine NTx/Cr

**B** Serum CTx

**C** Serum BSAP

**D** Serum P1NP

- **PBO/PBO**
- **50 mg/PBO**
- **50mg/50mg**

**Geometric mean percent change from baseline**

**Month**

0 3 18 24 27 36
A. Proposed mechanisms for osteoclast – osteoblast coupling

- Release of growth factors from bone matrix
- Net effect on bone formation
- Direct effects

B. Therapy with bisphosphonates, denosumab

- Release of growth factors from bone matrix
- Net effect on bone formation
- Direct effects

C. Therapy with cathepsin K inhibitors

- Release of growth factors from bone matrix
- Net effect on bone formation
- Direct effects
- or no change
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