

Potential role of odanacatib in the treatment of osteoporosis

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Abstract: Cathepsin K is a key enzyme involved in the degradation of organic bone matrix by osteoclasts. Inhibition of bone resorption observed in human and animal models deficient for cathepsin K has identified this enzyme as a suitable target for intervention by small molecules with the potential to be used as therapeutic agents in the treatment of osteoporosis. Odanacatib (ODN) is a nonbasic selective cathepsin K inhibitor with good pharmacokinetic parameters such as minimal in vitro metabolism, long half-life, and oral bioavailability. In preclinical studies, ovariectomized monkeys and rabbits treated with ODN showed substantial inhibition of bone resorption markers along with increases in bone mineral density (BMD). Significant differences were observed in the effects of ODN treatment compared with those of other antiresorptive agents such as bisphosphonates and denosumab. ODN displayed compartment-specific effects on trabecular versus cortical bone formation, with treatment resulting in marked increases in periosteal bone formation and cortical thickness in ovariectomized monkeys whereas trabecular bone formation was reduced. Furthermore, osteoclasts remained viable. Phase I and II studies conducted in postmenopausal women showed ODN to be safe and well tolerated. After 5 years, women who received ODN 50 mg weekly continuously from year 1 (n = 13), showed BMD increases from baseline of 11.9% at the lumbar spine, 9.8% at the femoral neck, 10.9% at the hip trochanter, and 8.5% at the total hip. Additionally, these subjects maintained a low level of the urine bone resorption marker N-terminal telopeptide/creatinine (−67.4% from baseline) through 5 years of treatment, while levels of serum bone-specific alkaline phosphatase remained only slightly reduced relative to baseline (−15.3%). In women who were switched from ODN to placebo after 2 years, bone turnover markers were transiently increased and BMD gains reversed after 12 months off medication. Adverse experiences in the ODN-treated group were not significantly different from the placebo group. In conclusion, available data suggests that cathepsin K inhibition could be a promising intervention with which to treat osteoporosis. Ongoing studies are expected to provide information on the long-term efficacy in fracture reduction and safety of prolonged treatment with ODN.

Keywords: cathepsin K, selective cathepsin K inhibitors, bone remodeling, osteoclast, bone turnover markers

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Introduction

Major advances in the treatment of osteoporosis have occurred with the introduction of antiresorptive agents represented by bisphosphonates, estrogen receptor modulators, strontium ranelate, and the anabolic agents parathyroid hormone (PTH) 1-34 (teriparatide) and PTH 1-84. More recently, denosumab, a monoclonal antibody against receptor activator of nuclear factor kappa-B ligand (RANKL) was introduced. Nonetheless, the search for affordable, safer, and more effective forms of therapy

continues because all the therapies currently available to modify bone remodeling have limitations. There is concern that prolonged use of bisphosphonates could lead to a low bone turnover state that would be less able to repair micro-damage and allow the accumulation and coalescence of micro-cracks, which are associated with reduced mechanical properties.¹ In addition, in recent years, attention has been drawn to extremely rare complications associated with bisphosphonate use, such as osteonecrosis of the jaw and atypical subtrochanteric fractures.^{2,3} The use of teriparatide is limited to 24 months in the USA and 18 months in Europe and Australia because an animal toxicology study showed an increased incidence of osteosarcoma.⁴ Clearly, there is scope for the development of novel therapeutic agents with improved safety profiles and efficacy in fracture prevention. Osteoporosis is a common health problem and its incidence is expected to increase in the coming decades as the aged make up a greater proportion of the population.

Advances in our understanding of how osteoclasts resorb bone and communicate with osteoblasts have enabled investigators to identify potential targets in these signaling pathways where appropriate intervention can be expected to achieve substantial inhibition of bone resorption. Cathepsin K is a key enzyme involved in the degradation of organic bone matrix. The inhibition of bone resorption observed in human and animal models deficient for cathepsin K has identified this enzyme as a suitable target for intervention by small molecules with the potential to be used as therapeutic agents in the treatment of osteoporosis.

Bone remodeling, bone modeling, and coupling

Bone is a dynamic tissue that is constantly remodeled throughout life. Increased bone remodeling is a feature of postmenopausal osteoporosis and net bone loss in this condition is the result of excess bone resorption over formation. Bone remodeling is the normal physiological mechanism that ensures tissue turnover while maintaining bone mass in the adult.⁵ It provides the mechanism for adaptation to mechanical stress by resorptive removal of micro-damage and repair by bone formation. This remodeling process, which is also an integral part of the calcium homeostatic system, continues throughout adult life. Bone remodeling occurs within discrete units – known as bone remodeling units (BMUs) – throughout the skeleton. Osteoclasts and osteoblasts are active at specific times during the remodeling cycle within each BMU. There is a well-defined sequence of events that is initiated when mechanical deformation or micro-cracks and osteocyte death

in old bone generate signals that lead to osteoclasts resorbing bone over a period of about 3 weeks to create resorption cavities. Resorption by osteoclasts is followed by osteoblast activation and formation of osteoid, which fills the resorption cavities over a period of about 3 months. When this active matrix synthesis is completed, osteoblasts become embedded in the matrix to function as osteocytes. Osteocytes continue to play a principal role in initiating bone remodeling by conveying local signals to osteoblasts and osteoclasts on the bone surface via a canalicular system.

Bone modeling, as distinguished from bone remodeling, is the process associated with growth and reshaping of bones in childhood and adolescence. After the epiphyses have fused, growth in width and thickness is accomplished by formation of bone at the periosteal surface with resorption at the endosteal surface.

To preserve the shape and structural integrity of bone, the processes of bone modeling and remodeling must be tightly coordinated through the actions of systemic hormones, locally generated cytokines, and direct cell–cell communication between osteoclasts and osteoblasts. In the physiological state, signaling between osteoclasts and osteoblasts serves to “couple” the rate of bone resorption and formation, so that when resorption increases, formation does also, and when resorption decreases, formation does as well.⁶ There is intense interest in what those coupling mechanisms might be and there is evidence to implicate secreted products of osteoclasts, growth factors released from bone matrix and direct cell–cell contact between osteoclasts and osteoblasts. Mature osteoclasts secrete factors such as cardiotrophin-1, Wnt 10b, BMP-6, sphingosine-1-phosphate, and semaphorin D.^{7–9} When osteoclasts resorb bone, factors such as transforming growth factor beta, insulin-like growth factors 1 and 2, and bone morphogenetic proteins are released from bone matrix to stimulate osteoblast proliferation, differentiation and/or activity.^{10,11} Osteoclasts also regulate osteoblasts by direct cell–cell contact. Ephrin B2, expressed on osteoclast cell membranes, binds to its receptor, Eph B4, expressed on osteoblast cell surfaces to mediate cross talk between these cells through bidirectional signaling. Binding of ephrin B2 and Eph B4 between osteoclast and osteoblast and between osteoblast and osteoblast leads to enhanced osteoblastic differentiation and suppression of osteoclastic activity.^{12–14}

For bone structure to be maintained, bone formation and resorption need to be equal, except during growth and throughout aging. During growth there may be a positive BMU balance, with bone formation exceeding bone resorption. After completion of longitudinal growth and with

the attainment of peak bone strength, the need for a positive BMU balance declines. A negative BMU balance marks the onset of age-related bone loss, with osteoporosis resulting from a persistent excess of resorption over formation.

The fact that bone remodeling is an active and dynamic process enables the use of interventions in the treatment of osteoporosis that limit resorption or augment formation.¹⁵ Current antiresorptive agents significantly reduce the incidence of fractures, including both vertebral and non-vertebral fractures, particularly hip fractures.^{16–19} However, inhibition of bone resorption by the widely used antiresorptive agents does not restore bone structure or replace bone that has already been lost and is coupled with inhibition of bone formation instead of increased bone formation, which would have been more advantageous for the patient.^{20,21} In contrast, an anabolic drug such as teriparatide increases the rate of bone formation to reduce fracture risk.^{22,23}

Osteoclasts

Osteoblasts express a membrane protein, RANKL, which is regulated by osteotropic hormones such as PTH and calcitriol, as well as cytokines such as interleukin-6. RANKL plays a critical role in osteoclast differentiation, activation, and survival. The binding of RANKL to its receptor, receptor activator of nuclear factor kappa-B, expressed in mononuclear hematopoietic precursors, initiates the processes that ultimately lead to the formation of multinucleate osteoclasts. Osteoprotegerin acts as a decoy receptor for RANKL to suppress osteoclast formation.

To resorb bone, osteoclasts first attach themselves to the bone surface to seal off an extracellular compartment, termed a “resorption lacuna,” in which mineralized bone matrix can be resorbed. The attachment of the osteoclast to bone is facilitated by podosomes containing filamentous actin and the alpha-v beta-3 integrin. The osteoclast becomes morphologically and functionally polarized to form a ruffled border at the apical membrane of the osteoclast facing the resorption lacuna. To achieve acidification of the resorption lacuna and begin the process of bone demineralization, carbonic anhydrase II within the osteoclast generates a proton and bicarbonate from carbon dioxide and water. The proton is actively transported across the membrane of the ruffled border through the action of the osteoclast-specific vacuolar-type H⁺-ATPase “proton pump” to reduce the pH to about 4. Passive transport of chloride through a chloride channel coupled to the proton pump facilitates balancing the charge of ions across the membrane. The acid pH removes the bone mineral, exposing matrix proteins like type I collagen to be degraded by

enzymes such as cathepsins, a family of cysteine proteinases that operate under acidic conditions^{24,25} (Figure 1).

New therapeutic agents to treat osteoporosis

The aims of drug discovery are to develop:

- drugs that reduce fracture risk without long-term deleterious effects on the skeleton or other side effects;
- drugs whose effects cease with cessation of therapy;
- bone resorption inhibitors that do not inhibit bone formation;
- anabolic agents; and
- drugs that are safe, cost-effective, and that act specifically on osteoclasts and/or osteoblasts.

A single drug or combination of drugs that dissociates bone resorption from bone formation could decrease the rate of bone resorption while increasing the rate of bone formation, with the potential for improved clinical outcomes compared with current drug therapy.²¹ In mice deficient for either *c-src* or the chloride-7 channel, bone resorption is inhibited without any inhibition of the rate or extent of formation.^{26,27} In each of these mouse mutations, osteoclast numbers are maintained, but the osteoclasts are unable to resorb bone. This is also the case in human subjects with inactivating mutations either of chloride-7 channel or the vacuolar H⁺ATPase.^{28,29} These findings suggest that the development of an agent that inhibits bone resorption but permits bone formation could be possible.

This article focuses on the development of specific cathepsin K inhibitors as potential therapeutic agents in the treatment of osteoporosis, with in vivo evidence suggesting that cathepsin K inhibitors not only reduce bone resorption but are also capable of dissociating bone resorption from bone formation.

Cathepsin K

Cathepsins are homeostatic enzymes critically involved in the proteolytic processing of specific substrates. Thus, cathepsins contribute to distinct physiological processes such as antigen presentation in the immune system, collagen turnover in bone and cartilage, and neuropeptide and hormone processing.³⁰ Cathepsins are lysosomal proteases that belong to the papain-like cysteine protease family. Eleven different types have been described (B, C, F, H, K, L, O, S, V, X, and W), with cathepsin K being the most important with respect to bone remodeling, since it is a protease with intense collagenase activity. It is a protein of 329 amino acids that consists of an amino-terminal region of 15 amino acids, a pro-peptide of 99 amino acids, and a catalytic unit of 215 amino acids.^{31–33}

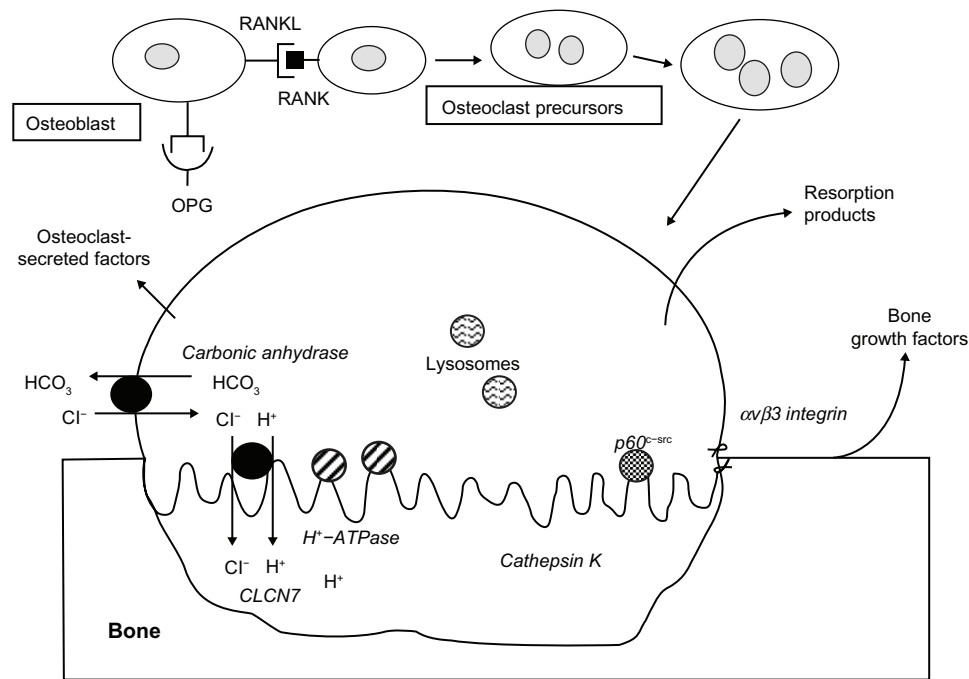


Figure 1 Osteoblasts express a membrane protein, receptor activator of nuclear factor kappa-B ligand (RANKL), which plays a critical role in osteoclast differentiation, activation, and survival.

Notes: The binding of RANKL to its receptor, receptor activator of nuclear factor kappa-B, expressed in mononuclear hematopoietic precursors, initiates the processes that ultimately lead to the formation of multinucleate osteoclasts. Mature osteoclasts attach to bone surface to seal off an extracellular compartment, termed a “resorption lacuna,” in which mineralized bone matrix can be resorbed. The attachment of the osteoclast to bone is facilitated by podosomes containing filamentous actin and the alpha-v beta-3 integrin. The osteoclast forms a ruffled border at the apical membrane of the osteoclast, facing the resorption lacuna. Carbonic anhydrase II within the osteoclast generates a proton and bicarbonate from carbon dioxide and water. The proton is actively transported across the membrane of the ruffled border through the action of the osteoclast-specific vacuolar-type H⁺-ATPase “proton pump” to reduce the pH to about 4. Passive transport of chloride through a chloride channel coupled to the proton pump facilitates balancing the charge of ions across the membrane. The acid pH removes the bone mineral, exposing matrix proteins like type I collagen to be degraded by lysosomal enzymes such as cathepsin K that are secreted into the resorption lacuna. Osteoclast-secreted factors and growth factors released from bone matrix during resorption stimulate osteoblast proliferation, differentiation, and/or activity (see text). Bone resorption products are released into the systemic circulation.

It is predominantly expressed in osteoclasts and is the most abundant cysteine protease in these cells, accumulating in specific subcellular compartments, possibly lysosomal vesicles. In actively resorbing osteoclasts, cathepsin K is localized at the ruffled border and discharged into the extracellular space when the lysosomal vesicles fuse with the cell membrane, to degrade the two main types of collagen, I and II within the acidic microenvironment of resorption lacunae.^{34–36} Cathepsin K cleaves the N-telopeptide of collagen to generate N-terminal telopeptide (NTX) and degrades serum C-terminal telopeptide of type I collagen (ICTP), to generate C-terminal telopeptide (CTX).³⁷

The expression of cathepsin K is downregulated by estrogen and upregulated by RANKL, tumor necrosis factor, and many other agents capable of increasing osteoclast formation and differentiation, such as vitamin D, PTH, and interleukins.^{38–40}

Genetic models of cathepsin K deficiency

Pycnodysostosis, derived from the Greek “pycnos” (dense), “dys” (defective), and “ostosis” (bone), was first

described in 1962 by Maroteaux and Lamy.⁴¹ It is a rare osteochondral dysplasia linked to defects in the gene encoding cathepsin K.⁴² The renowned French impressionist painter Henri de Toulouse-Lautrec (1864–1901), was thought to suffer from pycnodysostosis. The disorder is characterized by osteosclerosis and bone fragility with a high fracture risk, reduced stature, acro-osteolysis of the distal phalanges, clavicular dysplasia, hypoplasia of the mandible and skull deformities with delayed suture closure and open fontanelles. The characteristic facial appearance includes calvarial bossing, proptosis, overhanging nasal tip, micrognathia, and dental abnormalities.⁴³ NTX and CTX levels are low in patients with pycnodysostosis, consistent with decreased bone turnover but no abnormality is seen in markers of bone formation, osteocalcin, or carboxy-terminal pro-peptide of type I collagen, suggesting normal osteoblast activity.⁴⁴

Similar findings were demonstrated in genetically modified mouse models. Cathepsin K-deficient mice exhibit an osteopetrotic phenotype characterized by increased bone volume and in the number and thickness of bone trabeculae.

Histomorphometric analysis of cancellous bone reveals increased bone formation rates (BFRs). Osteoclast number and bone demineralization are normal but osteoclasts fail to resorb the organic bone matrix.⁴⁵⁻⁴⁸ In contrast, transgenic mice that overexpress cathepsin K have reduced trabecular bone volume as a result of accelerated bone turnover.⁴⁹

Specific cathepsin K inhibitors

The catalytic unit of cathepsin K is arranged in two domains separated by a cleft that contains the substrate binding site. Peptide inhibitors are designed to inhibit the action of cathepsin K by binding at this substrate site, mimicking a natural cathepsin K-substrate complex.^{50,51} Ideally, cathepsin K inhibitors should be of low molecular weight, exhibiting minimal peptide character, and should bind reversibly and highly selectively without affecting other major cysteine cathepsin family members, particularly the closely related cathepsins L, S, and V. The major challenge of the inhibitor design also requires standard drug-like properties such as oral bioavailability with high pharmacological profiles (high membrane permeability, long plasma half-lives, slow elimination rates, no or low toxicity) for acute and chronic use. Cathepsin K inhibitors have to be delivered into the lysosomes, but, in contrast to bisphosphonates, they do not need to bind to bone in order to exert their action.⁵² It is the concentration of these drugs in the resorption lacunae that is most relevant to their activity.⁵³

Because cathepsin K and most other cathepsins are lysosomal enzymes, early inhibitors were designed to contain lipophilic and basic moieties to allow cell permeability and localization to lysosomes (lysosomotropism). However, their increased accumulation in acidic lysosomes resulted in the inhibition of other cysteine proteases such as B and L, causing undesired effects.⁵⁴⁻⁵⁶ The strategy subsequently shifted to the design of nonbasic inhibitors, which are non-lysosomotropic but still maintain their potency and selectivity against individual cathepsins and *in vivo* efficacy in animal studies.^{57,58} Rodent and rabbit cathepsin K has 88% and 94% homology with human cathepsin K, respectively, while monkey and human cathepsin K are identical, making the use of nonhuman primate models more appropriate for studying these inhibitors *in vivo*.⁵⁹⁻⁶¹

L-006235

L-006235, a selective, basic cathepsin K inhibitor, administered orally to ovariectomized (OVX) rhesus monkeys once daily for 7 days, resulted in a dose-dependent decrease in bone resorption.⁶² When given once daily for 27 weeks to

adult newly OVX rabbits, L-006235 blocked bone loss with efficacy similar to alendronate. However, unlike alendronate, L-006235 had no effect on cancellous and endocortical mineralizing surface or on Haversian bone formation.

Relacatib

Relacatib is an equipotent inhibitor of cathepsins K, L, and V while exhibiting some selectivity against cathepsins S and B.^{63,64} It is a nonbasic 5-, 6-, and 7-methyl-substituted azepanone analog (SB-462795; GlaxoSmithKline, London, UK). A decrease in bone resorption is observed when administered to OVX monkeys. Relacatib also reduces bone formation at cancellous sites while increasing femur periosteal bone – possibly due to transiently increased plasma PTH levels.^{65,66} Relacatib was discontinued following a Phase I study that showed possible drug–drug interactions with the commonly prescribed medications paracetamol, ibuprofen, and atorvastatin.⁶⁰

Balicatib

Balicatib (AAE-581) is a highly selective nitrogen-containing inhibitor of cathepsin K with properties of lysosomotropism.⁶⁷ It prevented OVX-induced loss of vertebral and femoral bone mineral density (BMD), increased bone strength, and suppressed the cancellous BFR, while increasing periosteal bone formation and cortical thickness in monkeys.⁶⁸ In a 1-year dose-ranging study in postmenopausal women, balicatib at 25 and 50 mg reduced serum CTX by 61% and urinary NTX by 55%. However, serum bone formation markers (osteocalcin, BSAP, and PINP) were not reduced by the 10, 25, and 50 mg doses, and were increased in the 5 mg dose group. There were dose-dependent BMD increases in lumbar spine and hip of 4.5% and 2.2%, respectively.⁶⁹ Balicatib trials were discontinued due to dermatologic adverse effects, including a morphea-like syndrome.⁷⁰ It is possible that the skin toxicities were caused by increased accumulation of balicatib in the lysosomes of human skin fibroblasts, in which cathepsins B and L are highly expressed and localized along with cathepsin K.^{33,54}

ONO-5334

ONO-5334 (Ono Pharmaceutical Company, Ltd, Osaka, Japan) is a low-molecular-weight synthetic inhibitor of cathepsin K being developed as a therapeutic agent for bone metabolic diseases including osteoporosis. ONO-5334 has shown improvement in both BMD and bone strength in the ovariectomized monkey osteoporosis model.⁷⁰⁻⁷²

In the Phase II OCEAN study, 295 postmenopausal women with osteoporosis were randomly assigned to one

of five treatment arms: (1) 50 mg twice daily, (2) 100 mg daily, or (3) 300 mg daily, (4) alendronate, or (5) placebo for 12 months with a 12-month extension. The primary outcome measure was BMD at the lumbar spine. At month 24, 300 mg ONO-5334 suppressed bone resorption markers as potently as alendronate. While alendronate maintained suppression of bone formation markers, ONO-5334 did not show suppression even at the highest dose. The 300 mg dose of ONO-5334 had no effect on TRAP5b, while this was significantly suppressed by alendronate. All doses of ONO-5334 resulted in significant and dose-dependent increases in spine and hip BMD versus placebo. At month 24, 300 mg ONO-5334 increased lumbar spine BMD by 6.7%, total hip BMD by 3.4%, and femoral neck BMD by 3.7%. Alendronate BMD results for lumbar spine, total hip, and femoral hip were 6.3%, 4.2%, and 2.9%, respectively.⁷³

Studies with L-006235, relacatib, and balicatib have been discontinued, while the results of a Phase II study of ONO-5334 were published last year. Odanacatib (ODN) is the only specific cathepsin K inhibitor currently undergoing Phase III studies.

ODN

ODN (MK-0822; Merck and Co, Inc, Whitehouse Station, NJ) is a nonbasic and non-lysosomotropic nitrile-based molecule displaying high potency for cathepsin K ($IC_{50} = 0.2$ nM) and increased selectivity versus cathepsins B, L, and S when compared with balicatib and relacatib. In preclinical studies, ODN presented good pharmacokinetic parameters such as minimal in vitro metabolism and long half-life, and oral bioavailability.^{32,60} In OVX rabbits, ODN inhibited bone resorption without decreasing bone formation.⁷⁴

Two related studies in OVX monkeys that evaluated the effects of ODN on bone turnover, BMD, and bone strength had findings that were common with conventional antiresorptives and other findings that were different.^{75,76} OVX monkeys were treated for 21 months with either vehicle, ODN 6 mg/kg, or ODN 30 mg/kg (orally, once daily), and compared with intact animals. ODN treatment persistently suppressed bone resorption markers (urinary NTX by 75% to 90% and serum CTX by 40% to 55%) and serum bone formation markers (BSAP by 30% to 35% and P1NP by 60% to 70%) versus vehicle-treated OVX monkeys. In the lumbar vertebrae and iliac crest, both doses of ODN prevented bone loss and maintained bone mass at a level comparable to intact animals. BFRs in trabecular bone at the iliac crest and lumbar vertebrae decreased by comparable amounts. However, in the femoral neck and proximal femur, there was no effect on endocortical BFR,

while trabecular and intracortical BFR were reduced. Furthermore, ODN stimulated long-term femoral neck and proximal femur periosteal BFR by 3.5-fold and 6-fold, respectively, with the 30 mg/kg dose versus vehicle, resulting in a 21% and 19% increase in cortical thickness in the femoral neck and proximal femur, respectively. Thus, unlike conventional antiresorptives, ODN displayed compartment-specific effects on trabecular versus cortical bone formation, with treatment resulting in marked increases in periosteal bone formation and cortical thickness in OVX monkeys, whereas trabecular bone formation was reduced. This compartment-specific effect of ODN in OVX monkeys is similar to that previously reported for balicatib and relacatib. Another point of difference from current antiresorptives is the maintenance of osteoclast numbers in the ODN-treated groups compared with the vehicle controls. If the osteoclasts remain viable, they could still be functional, even though they could no longer resorb bone. Serum level of TRAP5b, an indicator of osteoclast cell number and biomarker of osteoclast viability was maintained, providing support for the distinct mechanism of cathepsin K inhibition in effectively suppressing bone resorption without reducing osteoclast numbers.^{77,78}

These findings, suggesting a compartment-specific effect of bone resorption and bone formation through cathepsin K inhibition, may represent a novel therapeutic approach for the treatment of osteoporosis.

A preclinical study showed that ODN can be effectively combined with an anabolic agent such as PTH. This study with estrogen-deficient rabbits compared the effect of five different treatment regimens on BMD, bone histology, and bone strength: the combinations of human PTH 1-34 and ODN, PTH and alendronate, and the monotherapies including ODN, alendronate, or PTH. Treatment with PTH+ODN resulted in the fastest rate of BMD gain. Bone histology showed that although both the PTH+ODN and PTH+alendronate combinations resulted in increases in BMD, the PTH+alendronate combination eventually halted bone formation in the trabecular lumbar vertebrae, whereas the PTH+ODN combination increased BMD and BFR.⁷⁹

Cathepsin K inhibition by ODN in human studies

Phase I trials

Two Phase I trials were conducted to determine safety, tolerability, pharmacokinetics, and pharmacodynamics of ODN based on biomarkers of bone turnover.^{33,80-82}

Three doses of ODN (0.5, 2.5, and 10 mg) were administered once daily to 30 postmenopausal female subjects

($n = 10$ per group [eight active, two placebo]) for 21 days in the first Phase I study.⁸¹ In the second Phase I study, 48 postmenopausal female subjects were divided into four groups (nine active, three placebo) and administered 5, 25, 50, and 100 mg ODN or placebo once weekly for 3 weeks.⁸¹

Dose-dependent decreases in CTX and NTX/creatinine (Cr) were observed, reaching a maximal effect following once-weekly doses of ≥ 50 mg or once-daily doses of ≥ 2.5 mg. At these doses, maximal suppression (CTX, 62%; NTX/Cr, 62% at day 21 with 50 mg) was evident by day 2 in the first week of weekly dosing with 50 mg ODN, and maintained at steady state over the dosing interval. Similarly, maximal suppression (CTX, 69%; NTX/Cr, 79% at day 21 with 2.5 mg) was evident by days 3–5 with once-daily dosing and also sustained throughout the dosing period. These reductions were comparable to those previously reported for alendronate.^{83,84}

Two additional osteoclastic biomarkers were evaluated: ICTP and serum TRAP5b. ODN produced a rapid and dose-dependent increase in serum ICTP, consistent with the role of cathepsin K in the degradation of ICTP. Neither weekly nor daily dosing of ODN changed serum TRAP5b levels during 3 weeks of dosing. This agreed with previous studies that showed that osteoclast number was either unaffected or tended to increase in cathepsin K-null mice.⁴⁸

In contrast to the results relating to biomarkers of bone resorption, no significant effect was observed with serum bone formation markers, BSAP, and osteocalcin, supporting the idea that this inhibitor is capable of dissociating these two processes.

Data from the two Phase I studies showed that ODN was well tolerated.^{33,80–82} There was no difference in adverse effects between placebo and ODN. ODN had a long apparent half-life ($t_{1/2}$) of ~66–93 hours across all doses and regimens studied. Trough concentration data suggested that steady-state conditions were reached in the third week of dosing with either regimen. The sustained pharmacokinetics and pharmacodynamics of ODN supported the adoption of a once-weekly regimen for the treatment of diseases associated with bone loss, given that the once-daily regimen showed no obvious advantage over the once-weekly regimen.

Phase II trials

Two-year results of Phase II trial

This was a double-blind, randomized, placebo-controlled trial of 12 months duration with an extension period of 12 months to evaluate the safety and efficacy of weekly doses of placebo or 3, 10, 25, or 50 mg of ODN on BMD and biomarkers

of skeletal remodeling.⁸⁵ It recruited 399 postmenopausal women aged between 45 and 85 years (mean age 63 years), with a T-score of less than -2 but not less than -3.5 at any site. All patients received vitamin D with calcium supplementation as needed. The primary objective was change in bone mass in the lumbar spine and secondary objectives were changes in BMD in other sites, changes in bone remodeling and adverse treatment effects. A total of 331 (83%) subjects completed 12 months of treatment and 320 participated in the extension study, which was completed by 270 (70%) at 24 months. Twenty-four months of treatment produced progressive dose-related increases in BMD. With the 50 mg dose of ODN, lumbar spine and total hip BMD increased 5.5% and 3.2% respectively, whereas BMD at these sites was unchanged with placebo. Resorption markers fell in a dose-dependent manner. The urinary NTX/Cr ratio decreased by 52%, while the BSAP levels decreased initially but then recovered gradually from month 6 onward to reach -13% with the 50 mg dose at month 24.⁸⁵ Significant difference from control for BSAP was observed only for the 50 mg group. The decrease in BSAP level associated with ODN treatment was less than what is typically seen with other antiresorptive agents, such as bisphosphonates.⁸⁶ The safety and tolerability of ODN generally were similar to those of placebo.

Three-year results of Phase II trial

At the end of 2 years, a 1-year extension study was carried out to further assess ODN efficacy, safety, and the effects of discontinuing therapy. After 2 years, patients ($n = 189$) were re-randomized to ODN 50 mg weekly or placebo for another year in such a way that some participants received placebo for the entire 3 years (PLB/PLB), some were treated with ODN for the entire 3 years (ODN/ODN), and some received ODN 50 mg for 2 years followed by placebo (ODN/PLB) for 1 year.⁸⁷

Women in the ODN/ODN group showed further improvement in BMD and the cumulative gain in BMD after 3 years of treatment with ODN 50 mg was 7.9% at the lumbar spine, 5.8% at the total hip, 5.0% at the femoral neck, and 7.4% at the trochanter. Urine NTX/Cr remained suppressed at year 3 (-50.5%) compared with a 17.5% decline for those who received placebo for the 3-year period (PLB/PLB). TRAP5b levels were not significantly different from the placebo group and bone formation markers returned to near baseline.

In the ODN/PLB group, women who were previously treated with ODN 50 mg for 2 years showed significant bone loss in all sites and this was most rapid during the first 6 months after discontinuation of active treatment. BMD at

the lumbar spine, total hip, and trochanter returned to baseline levels after 12 months off medication, although femoral neck BMD remained slightly increased (+2.3%). After ODN discontinuation at month 24, bone turnover markers increased transiently above baseline, but this increase largely resolved by month 36. The results of this 1-year extension showed that ODN effects were reversible; bone resorption increased transiently and BMD decreased following treatment discontinuation.⁸⁷ The rapid reversibility observed upon discontinuation of ODN was similar to that seen with most other antiresorptive agents such as estrogens, selective estrogen receptor modulators, and denosumab but not oral bisphosphonates or zoledronic acid.^{2,3,88-91}

Clinical adverse experiences (AEs) were reported by 150 (79.4%) of the 189 extension participants. The most common clinical AEs, regardless of treatment group, were back pain (10.1%), arthralgia (7.9%), pain in an extremity (8.5%), and nasopharyngitis (9.5%). The incidence of adverse experiences of any type generally was balanced between the 50 mg ODN and placebo groups. Eight patients (4.2%; four placebo, four ODN) discontinued study therapy owing to a clinical AE; discontinuations were not due to any one particular clinical AE.⁸⁷

Five-year results of Phase II trial

At the end of 3 years, the Phase II trial was extended for a further 2 years.⁹² After 5 years, women who received ODN 50 mg continuously from year 1 ($n = 13$), showed BMD increases from baseline of 11.9% at the lumbar spine, 9.8% at the femoral neck, 10.9% at the hip trochanter, and 8.5% at the total hip. Additionally, women treated continuously with ODN 50 mg maintained a low level of urine NTX/Cr (-67.4% from baseline) through 5 years of treatment; while levels of serum BSAP remained only slightly reduced relative to baseline (-15.3%). In women who were switched from ODN to placebo after the 2-year base study ($n = 14$), BMD gains were reversed.

Pooled data showed no statistical difference in the incidence of AEs and serious AEs between the placebo and ODN groups. None of the women in the placebo group and two women (3%) in the ODN group discontinued the study drug due to an AE. The incidence of skin-related adverse experiences was 17% and 12% in the placebo and ODN groups, respectively.⁹²

Potential off-target effects

While cathepsin K is highly expressed in osteoclasts, very low levels exist in other adult tissues, including the heart,

liver, skeletal muscle, placenta, ovary, testis, small intestine, and colon as well as embryonic lung and neonatal dermal fibroblasts.⁹³⁻⁹⁶ It is also expressed in synovial fibroblasts and macrophages of rheumatoid arthritic joints and breast and prostate tumors.⁹⁷⁻⁹⁹ Therefore, inhibition of cathepsin K could affect the turnover of type I collagen in organs other than bone. Indeed, histological analysis of human pycnodystosis tissue specimens revealed the accumulation of collagen fibrils not only in osteoclasts but also in various types of fibroblasts.¹⁰⁰ Cathepsin K participates in the degradation of extracellular matrix of the lung and may be involved in the pathogenesis of pulmonary fibrosis as suggested by a more pronounced pulmonary fibrosis in cathepsin K-null mice, compared with their wild-type littermates.¹⁰¹ In contrast, when challenged with bleomycin, mice overexpressing cathepsin K had a lower degree of pulmonary fibrosis than wild-type mice.¹⁰² The expression of cathepsin K in dermal fibroblasts may account for some of the adverse effects seen with cathepsin K inhibition in clinical trials.¹⁰³ However, the degradation of collagen by fibroblasts appears to be a predominantly lysosomal event and this may explain the advantage of the non-lysosomotropic ODN over balicatib.⁹⁷ The ODN clinical trials reported neither skin- nor lung-related adverse side effects, although the trials were limited by the small number of subjects treated.^{85,87}

Preliminary findings suggesting that cathepsin K appears to be involved in thyroid hormone synthesis, blood pressure regulation, and certain brain functions are also of concern for the long-term use of anti-osteoporotic cathepsin K inhibitors.⁵² Cathepsin K is expressed in the thyroid epithelium and the protease is likely involved in thyroid-globulin processing as revealed in studies using cathepsin K deficient mice.^{104,105} Cathepsin K messenger RNA is detectable in all brain regions of wild-type mice, so cathepsin K activity could have an important impact on the development and maintenance of the central nervous system. Deletion of cathepsin K in mice led to deregulation of related proteases such as cathepsin B and L, resulting in a wide range of molecular and cellular changes in the central nervous system. An overall induction of the dopaminergic system was found in cathepsin K-deficient mice, which exhibited reduced anxiety levels as well as short- and long-term memory impairments in behavioral assessments.¹⁰⁶

Cathepsin K is the only known mammalian cysteine cathepsin that exhibits a potent kininase activity *in vitro*.¹⁰⁷ Kinins are short-term mediators involved in various physiological and pathophysiological events including vasodilation and constriction of smooth muscle, cardiac homeostasis,

regulation of blood pressure, and proinflammatory properties in the lung. In rats, cathepsin K modulates the bradykinin-dependent contraction of isolated bronchial smooth muscles and impairs bradykinin-induced transient falls in systemic blood pressure. Since bradykinin induces bronchial constriction and hyper-responsiveness in asthmatic patients, cathepsin K may have a positive effect during an asthma crisis by inactivating pro-inflammatory kinins. Thus, inhibition of cathepsin K could potentially impair the metabolism of regulatory peptides such as kinins.

Finally, cathepsin K inhibitors are metabolized by enzymes such as the cytochrome CYP3 A4, making them susceptible to pharmacokinetic interactions with other drugs.^{60,108}

Potential uses for cathepsin K inhibitors

There is potential for cathepsin K inhibitors to be used in the treatment of metastatic bone disease as well as in osteoarthritis and rheumatoid arthritis.

Metastatic bone disease is characterized by very high levels of bone turnover in proximity to tumors.¹⁰⁹ Bone resorption inhibitors such as bisphosphonates, and more recently denosumab, have demonstrated their value as adjunctive therapy in preventing bone loss and reducing disease recurrence in patients with breast cancer.^{110–114} Cathepsin K inhibitors could have a role in the treatment of tumor metastases as well, since cathepsin K is expressed in breast and prostate tumors, although their role is not yet known.^{98,99,115}

Cathepsin K expression is increased in synovial tissue isolated from joints of human osteoarthritis patients and is specifically found in synovial fibroblasts and articular chondrocytes.¹¹⁶ Cathepsin K not only efficiently degrades type II collagen but also degrades aggrecan at acidic pH, suggesting a possible role in articular cartilage degradation.¹¹⁷ In rheumatoid arthritis models, overexpression of cathepsin K led to spontaneous synovitis and cartilage erosion, while cathepsin K-null mice in the collagen-induced arthritis model revealed decreased bone and articular cartilage resorption, suggesting that cathepsin K inhibition could be beneficial in this context.^{52,118}

Discussion

Cathepsin K was identified as a therapeutic target for the treatment of osteoporosis because of its key role in the resorption of the organic matrix of bone. Targeting one particular function of the osteoclast rather than its production or survival has conferred some theoretical advantages over the antiresorptive agents currently in use.

Evidence to date has shown that selective cathepsin K inhibitors substantially reduced bone resorption by preventing cathepsin K degradation of type I collagen in several animal models and in clinical trials. At 36 months, increases in BMD similar to those of zoledronate and denosumab were observed, although these changes are not from head-to-head comparisons.^{19,87,119} However, cathepsin K inhibition has shown a quality that is not present among other classes of antiresorptive agents in that it has resulted in greater suppression of bone resorption than bone formation, suggesting a dissociation between bone resorption and bone formation. Even after 5 years of treatment with ODN in humans, while lumbar spine and hip BMD increases correlated with sustained suppression of bone resorption, there was little suppression of bone formation markers, in comparison with the known reduction of these markers by bisphosphonates.^{120,121} In OVX monkeys, ODN not only suppressed bone resorption but also showed a compartment-specific action on bone formation with increased periosteal bone formation and cortical thickness in the femur. These results of the effects of cathepsin K inhibitors are consistent with the dissociation of bone resorption and formation, suggesting an additional influence on bone modeling. Whether a similar compartment-specific action of ODN on bone resorption and formation can be demonstrated in humans remains to be determined.

The exact molecular mechanism(s) linking cathepsin K inhibition and bone formation remain(s) largely unknown; however, they have been predicted to involve complex networks of cell–cell communications.¹²² Histomorphometric and serum TRAP5b data showed that cathepsin K inhibition, unlike the actions of bisphosphonates and denosumab, did not reduce the number of osteoclasts or affect their survival, thereby providing a possible mechanism by which bone formation can be uncoupled from bone resorption. Khosla hypothesized that in the setting of ODN treatment, while the reduction in bone resorption would lead to a reduction in the release of growth factors from the bone matrix, direct communication between non-resorbing osteoclasts and osteoblasts through the ephrin 2-Eph B4 system may not be affected.^{12–14,123} The same may be true of coupling factors, if they are also secreted by non-resorbing osteoclasts. Thus, the net effect of cathepsin K inhibition on bone formation could depend on offsetting the effects of the loss of growth factor release from bone matrix with the ongoing, perhaps enhanced, effects of coupling factors from the increased numbers of relatively healthy osteoclasts. Furthermore, different remodeling or modeling rates of specific bone surfaces could be linked to the compartment-specific action of ODN on

bone formation. In trabecular bone, with its high remodeling rate, and where inhibition of bone resorption was associated with reduction in bone formation, the release of growth factors from the bone matrix may be particularly important.^{75,76} On periosteal surfaces, however, where the remodeling rate is much lower and the activity is predominantly modeling, the direct stimulatory effects of osteoclasts on osteoblasts could be responsible for the increased periosteal bone formation and cortical thickness.^{5,124}

Bone biopsies of subjects with pycnodysostosis show severe osteosclerosis, large amounts of undigested mineralized matrix, and dysfunctional bone remodeling, which may contribute to the increase in fragility fractures.¹²⁵ However, the relevance of these findings in pycnodysostosis patients, who have a lifelong deficiency of the enzyme, to any potential impact on bone strength in patients with osteoporosis treated with cathepsin K inhibitors is not known.

The onset and resolution for the antiresorptive effect of cathepsin K inhibitors were rapid.^{73,87} While a rapid resolution of effect might be preferable if there are concerns about side effects with prolonged exposure, it could also increase the risk of fracture shortly after discontinuation of treatment. This is particularly important because intended and unintended interruptions in therapy, including noncompliance, are very common in clinical practice.¹²⁶

If cathepsin K inhibitors are safe and at least as effective in fracture reduction as other inhibitors, they could offer theoretical advantages over bisphosphonates. For instance, ODN and other selective cathepsin K inhibitors might be more effectively combined with anabolic therapy like PTH than resorption inhibitors such as bisphosphonates and denosumab, which lead to inhibition of bone formation.^{79,127}

Conclusion

Available data suggest that cathepsin K inhibition could be a promising intervention with which to treat osteoporosis. Phase I and II studies conducted in postmenopausal women showed ODN to be safe and well tolerated. It is orally available and effective in a once-weekly dose. After 5 years, women who received ODN 50 mg weekly showed significant BMD increases at the lumbar spine and total hip. Antiresorptive activity, shown by the low level of urine bone resorption marker, NTX/Cr, was maintained through 5 years of treatment, while levels of serum bone-specific alkaline phosphatase remained only slightly reduced relative to baseline. In women who were switched from ODN to placebo after 2 years, bone turnover markers reverted to baseline levels after 12 months off medication. Adverse experiences in

the ODN-treated group were not significantly different from the placebo group. However, there is yet no fracture data for ODN. Three Phase III studies with ODN are ongoing, two in postmenopausal women (ClinicalTrials.gov registration numbers NCT00529373 and NCT00729183) and one in men (NCT0112600) to assess its safety, efficacy and effects on fracture risk.^{128–130} The results of the “BMD efficacy and safety of odanacatib in postmenopausal women (0822-031)” trial, NCT00529373, are expected in the later half of 2012. This is a clinical, randomized, double-blind trial with 16,716 patients. The target population is postmenopausal osteoporotic women aged ≥ 65 years not previously treated for osteoporosis. ODN at a dose of 50 mg weekly will be used and placebo will include calcium and vitamin D. The primary objective of the study is the reduction in osteoporotic fractures (vertebral, non-vertebral, and hip). These ongoing fracture-outcome studies are necessary to determine whether the increased bone mass demonstrated in the Phase II studies is translatable into better fracture-risk reduction.^{82,122}

Disclosure

The author reports no conflicts of interest in this work.

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