IBMS BoneKEy. 2008 January;5(1):16-24
http://www.bonekey-ibms.org/cgi/content/full/ibmske;5/1/16
doi: 10.1138/20080294

PERSPECTIVES

Cathepsin K – A New Molecular Target for Osteoporosis

Sevgi B. Rodan[1] and Le T. Duong[2]

[1]Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA
[2]Department of Molecular Endocrinology, Merck Research Laboratories, West Point, Pennsylvania, USA

Abstract

Cathepsin K (Cat K) is a cysteine protease of the papain family, now considered to be the major enzyme responsible for degradation of the organic bone matrix. It is highly and selectively expressed in osteoclasts and, under acidic conditions, has the unique ability to degrade type I collagen helical regions. Complete deficiency of Cat K activity leads to pycnodysostosis, a severe and rare autosomal recessive bone sclerotic disorder, and Cat K null-mice are osteopetrotic. Thus this protease is an attractive target for inhibition of bone resorption. Cat K inhibitors have been reviewed recently. Therefore, the following *Perspective* will focus on inhibitors that have been extensively characterized in animal models of bone loss, particularly the inhibitors that have advanced to clinical trials for the treatment of osteoporosis. Other disease targets such as osteoarthritis and bone metastasis will also be discussed. *IBMS BoneKEy*. 2008 January;5(1):16-24.

Introduction - Structure, Function, Localization and Genetics of Cathepsin K

Osteoclasts resorb bone by removing both the inorganic (hydroxyapatite) and the organic components (90% is fibrillar type I collagen) of mineralized bone. Following tight attachment to the bone surface, osteoclasts secrete protons into a sealed extracellular compartment. The acidic pH (about 4-5) removes the bone mineral and exposes the underlying matrix (Figure 1). About forty years ago, based on a model of parathyroid hormone-stimulated, resorbing bone explants, Vaes proposed that lysosomal acid hydrolases were the proteases degrading the organic matrix of bone (1) and demonstrated that E-64 and leupeptin, inhibitors of lysosomal cysteine proteases, blocked bone resorption *in vitro* and *in vivo* (2). Although the identity of the enzyme(s) was not known, several cathepsins including B and L were thought to be involved in bone resorption. In 1994, cathepsin K (Cat K) was discovered using differential display of osteoclast and rabbit macrophage cDNA libraries in rabbits (3). Subsequently, the human and murine enzymes were also cloned (4;5).

Cat K is a member of the CA1 family of lysosomal cysteine proteases. In humans, there are eleven functionally diverse members of this family (6), with each cathepsin having an active site comprised of cysteine, asparagine and histidine residues. Cat K and other cysteine proteases are synthesized as inactive pre-pro enzymes from which the propeptide must be removed for activation. *In vitro*, this is an autocatalytic process at pH 4 (7), making this enzyme an ideal candidate for the acidic conditions of the resorption lacunae. Unlike the other cathepsins, Cat K not only degrades type I collagen in the telopeptide regions, but is capable of cleaving the triple helical domains at multiple sites (8). Cat K is abundantly expressed in osteoclasts along the bone and cartilage

IBMS BoneKEy. 2008 January;5(1):16-24
http://www.bonekey-ibms.org/cgi/content/full/ibmske;5/1/16
doi: 10.1138/20080294

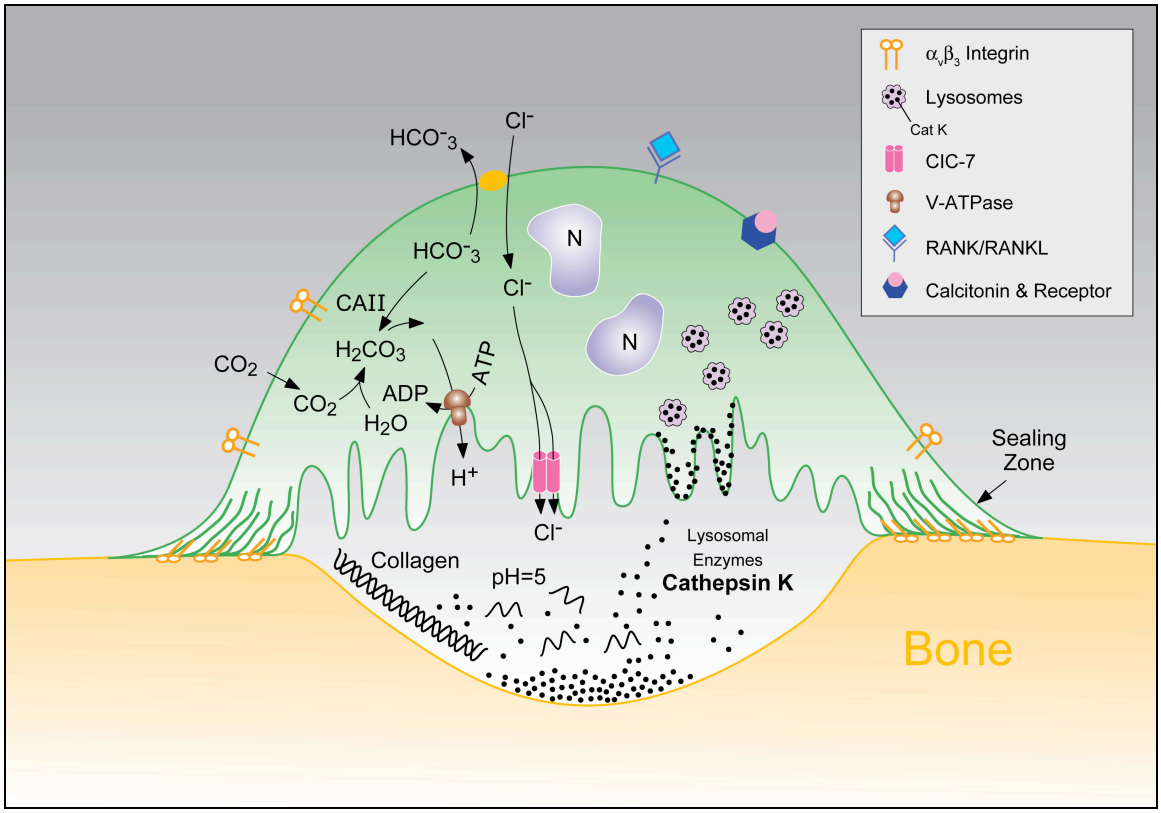

Figure 1: Schematic representation of a resorbing osteoclast. The osteoclast acidifies the resorption lacunae by secreting H^+ and Cl^- ions for demineralization, and lysosomal cathepsin K for degradation of type I collagen.

resorption lacunae, near the ruffled border (9), and in intracellular lysosomes and transcytotic vesicles (10). Cat K expression in other cells is much lower than that observed in osteoclasts either at the level of mRNA or protein (embryonic lung, and neonatal dermal fibroblasts (11;12)), but is higher in synovial fibroblasts and macrophages of rheumatoid arthritic joints (13) and in breast and prostate tumors (14; 15).

The proof of concept for Cat K being the major collagenase responsible for degradation of the bone matrix was provided by the discovery that deficiency of this enzyme causes a rare autosomal recessive bone sclerosing disorder called pycnodysostosis (16) triggered by mutations in the Cat K gene. Affected individuals are typically short in stature and, in spite of dense bones, suffer from increased non-traumatic fractures and were shown to accumulate undigested collagen fibrils in osteoclasts (17). While targeted disruption of the Cat K (*CSTK*) gene in mice results in osteopetrosis without an effect on bone quality (18-20), in one study Cat K knockout mice backcrossed into a C57BL/6J background developed bone fragility (21). Furthermore, transgenic mice that overexpress Cat K have reduced trabecular bone volume as a result of accelerated bone turnover (22).

Based on its localization, selectivity of expression, biochemical activity, and (mouse and human) genetics, Cat K has become a pharmaceutical target for the treatment of osteoporosis and potentially for other diseases associated with high bone turnover such as osteoarthritis, and metastatic bone disease. Cat K inhibitors designed to diminish bone resorption *in vitro* and *in vivo* have been recently reviewed (6; 23;24), therefore, the following *Perspective* is intended to provide an update on inhibitors that have been extensively

characterized in animal models of bone loss, particularly focusing on those that have advanced to clinical trials for the treatment of osteoporosis.

Cat K Inhibitors for Suppression of Bone Loss - *In Vitro* and *In Vivo* Pharmacology

Briefly, cysteine protease inhibitors are low molecular weight compounds carrying an electrophilic moiety, referred to as a warhead, which targets a nucleophilic cysteine in the active site of this enzyme. Ideally, effective and safe Cat K inhibitors should bind reversibly, have good bioavailability and potency and be selective for Cat K with limited inhibition of other cysteine protease family members. For characterization of potency and selectivity, the Cat K inhibitors are evaluated in enzyme assays using purified recombinant human Cat K and other related cathepsins L, B and S. In addition, detailed interactions of Cat K and its inhibitors can be viewed as high resolution X-ray crystallographic structures (25). For assessing functional efficacy *in vitro*, some inhibitors were tested in isolated rabbit osteoclast-mediated bone resorption assays, since rabbit Cat K is 94% homologous to human Cat K. A covalent and reversible inhibitor with a nitrile warhead, L-006235, was thus identified with the following properties: (i) forms a reversible thioimidate bond with the active site cysteine of cathepsin K; (ii) inhibits human Cat K with a Ki of 0.25 nM; and (iii) bone resorption by rabbit osteoclasts (IC_{50} = 5 nM); and (iv) is >4000- fold selective against cathepsins L, B and S (26).

The traditional rat and mouse models of bone loss are not appropriate for testing *in vivo* efficacy because of the differences between the rat/mouse and human Cat K enzymes (87-88% homology). Therefore, the *in vivo* efficacy of this compound was tested in ovariectomized (OVX) rabbits. When given orally, once daily for 27 weeks to adult newly-OVX rabbits, L-006235 partially (2 mg/kg/d) or fully (10 mg/kg/d) blocked bone loss due to estrogen deficiency with efficacy similar to alendronate, which was used as a control. However, unlike alendronate, L-006235 had no effect on cancellous and endocortical mineralizing surface (MS/BS) or on Haversian bone formation (27). These results were surprising given that in adult bone turnover, formation is tightly coupled to bone resorption. Furthermore, SB-553484, which inhibits mouse Cat K with an IC_{50} of 26 nM, prevented bone loss in OVX mice while increasing cortical bone volume and cortical thickness (28). There is some precedence for these data in Cat K knockout mice, where histomorphometric analysis of cancellous bone revealed that bone formation rates were increased (18-22;29).

In non-human primates, as in other animal models of bone loss, serum and urinary biomarkers of bone resorption and formation are increased as a result of OVX. In addition, rhesus and cynomolgus monkeys have identical amino acid sequences to human Cat K (30;31). Proof of concept for L-006235 as an anti-resorptive agent was therefore further tested in OVX rhesus monkeys treated for 11 days, and was found to dose-dependently suppress the increases in collagen degradation products (uNTx) (26). Other Cat K inhibitors, balicatib (AAE581 from Novartis), relacatib (SB-462795 from GlaxoSmithKline) and odanacatib (MK-0822 from Merck), when tested for longer periods (9 to 18 months) in OVX cynomolgus (balicatib and relacatib) and in rhesus (odanacatib) monkeys, all significantly reduced bone resorption markers. However, balicatib and relacatib also increased bone formation markers relative to vehicle-treated animals. In one of these studies, alendronate was used as a control and was demonstrated to suppress both bone resorption and formation markers as expected.

The effects of the Cat K inhibitors on OVX monkey bones can be summarized as follows: (i) balicatib prevented the loss of vertebral and femoral BMD, increased bone strength, and suppressed cancellous bone formation rate (MAR), while increasing periosteal bone formation and cortical thickness (32); (ii) relacatib also reduced bone formation at cancellous sites while increasing femur periosteal bone (33) possibly due to transiently increased PTH

plasma levels (34); (iii) odanacatib increased BMD of the spine and hip, and reduced bone turnover in trabecular and endocortical surfaces in rib and ilium biopsies (35). In conclusion, Cat K inhibitors, in mouse, rabbit and in some monkey bone loss models, suppressed bone resorption without affecting bone formation.

Recently, there has been great interest in the development of drugs for osteoporosis with these properties, *i.e*., with uncoupling of bone formation and resorption, possibly due to secretion of anabolic factors either from non-resorbing osteoclasts (36-38) or released from bone matrix by resorbing cells (39). Although patients with pycnodysostosis have reduced bone turnover with no suggestion of uncoupling of resorption and formation (40), these studies were done in only two patients, a child and a young adult. More studies are clearly needed, but given the rarity of patients with this condition, may be slow to emerge.

Clinical Trials and Safety of Cat K Inhibitors

Relacatib, balicatib and odanacatib have all been evaluated in humans for safety and efficacy. Relacatib is an equipotent inhibitor of Cat K, L and V, while exhibiting some selectivity against Cat S and B (41). There are currently no published data on relacatib in clinical trials as it was reportedly discontinued following Phase I studies. In a one-year dose ranging study (5, 10, 25 and 50 mg/day) 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, bone specific alkaline phosphatase and N-terminal propeptide of type I collagen (P1NP)) were not reduced by the 10, 25 and 50 mg doses, and were increased in the 5 mg dose group. Although there were dose-dependent BMD increases in lumbar spine and hip of 4.5 and 2.2%, respectively, the development of balicatib has been discontinued, reportedly due to skin adverse events including scleroderma-like skin thickening and rashes (42). Several Cat K inhibitors, including balicatib and L-006235, are basic, nitrogen-containing compounds that demonstrate lysosomotropic properties, *i.e.*, increased accumulation in acidic lysosomes. In whole cell assays, both compounds accumulated in lysosomes and displayed increased potency (~100-fold) against off-target cathepsins L, B and S (43). In a recent *in vivo* study, balicatib and L-006235 were found to have activities against Cat L, B and S in rats and mice (44). It is therefore possible that the skin toxicities in human trials were caused by increased accumulation of balicatib in lysosomes of human skin fibroblasts, in which cathepsins B and L are highly expressed and localized along with Cat K.

These findings led to the synthesis and evaluation of non-basic inhibitors, including odanacatib, which do not accumulate in lysosomes, therefore maintaining selectivity in whole cell assays (45). Odanacatib inhibits human cathepsin K with an IC_{50} of 0.2 nM, and rabbit osteoclast-induced bone resorption *in vitro* with an IC_{50} of 23 nM. In addition, odanacatib exhibited long half-lives in several preclinical species, including 6 h in the rat, 57 h in the dog and 18 h in the monkey. In Phase I clinical trials, odanacatib was shown to have an apparent half-life of 45-50 hours (46) in humans, making it possible to evaluate the safety and tolerability of weekly doses in clinical trials (47). In a 12-month, randomized, double blind, placebo-controlled study in postmenopausal women, odanacatib at the highest dose tested (50 mg/week) increased lumbar spine BMD by 3.4%, femoral neck BMD by 2.5%, and reduced uNTx/Cr by 58% and produced less reduction of serum bone formation markers compared to that seen historically with bisphosphonates. Odanacatib was generally safe and well-tolerated. Patients in this study continue to be evaluated for 24 months and the results will be presented in 2008 (47).

Other Indications

Metastatic bone disease (MBD) is characterized by very high levels of bone turnover in proximity to tumors (48;49). Bone resorption inhibitors such as bisphosphonates represent the current

standard of care for the treatment of bone metastases primarily due to breast, prostate or multiple myeloma; yet, this mode of treatment is palliative. It has been proposed that other strong anti-resorptives such as Cat K inhibitors could be useful in the treatment and prevention of bone metastases. Cat K is expressed in breast and prostate cancers (14;15). Evidence for a treatment effect of cathepsin K inhibitors has been presented in the form of preclinical MBD models in which human breast cancer cells are implanted in nude mice or rats. Cat K inhibitors, either dosed immediately after tumor implantation or following detectable osteolytic lesions at the tumor injected site, were demonstrated to prevent or reduce breast cancer-induced osteolysis and skeletal tumor burden (50;51). When prostate cancer cells were injected into the tibia of SCID mice, treatment with a Cat K inhibitor either in prevention or in treatment mode, was also reported to effectively block the progression of skeletal lesions and cancer growth in bone (52).

Cat K inhibitors may be beneficial for the treatment of osteoarthritis. Cat K expression is increased in tissues isolated from synovia from joints of human OA patients and is specifically found in synovial fibroblasts and articular chondrocytes (recently reviewed in (53)). Cat K not only efficiently degrades triple helical type II collagen, one of the two major extracellular components of cartilage, but it also degrades aggrecan at acidic pH (6). This enzyme is thus suggested to play a direct role, along with metalloproteinases, in articular cartilage degradation. Cat K inhibitors, through antiresorptive activity, may also reduce subchondral bone turnover, thus producing an indirect benefit, contributing to modifying disease progression in osteoarthritic joints (54).

Summary

Cat K inhibitors have been shown to inhibit bone resorption in several animal models and in clinical trials. In some animal models of bone loss, Cat K inhibitors are effective without inhibiting bone formation. In clinical trials after 12 months of treatment, balicatib and odanacatib increased both lumbar spine and hip BMD, and reportedly showed limited suppression of bone formation markers, as compared to the known reduction of these markers by bisphosphonates. These results suggest an apparent decoupling of bone formation and resorption. A phase III fracture prevention trial with odanacatib is underway. Further human studies of these new agents should determine bone safety, and possible neutral or stimulatory effects on bone formation.

Conflict of Interest: Dr. Rodan reports that she is a consultant to Merck & Co. and owns stock in the company. Dr. Duong reports that she is an employee of Merck & Co. and owns stock in the company.

References

1. Vaes G. On the mechanisms of bone resorption. The action of parathyroid hormone on the excretion and synthesis of lysosomal enzymes on the extracellular release of acid by bone cells. *J Cell Biol*. 1968 Dec;39(3):676-97.

2. Delaissé JM, Eeckhout Y, Vaes G. In vivo and in vitro evidence for the involvement of cysteine proteinases in bone resorption. *Biochem Biophys Res Commun.* 1984 Dec 14;125(2):441-7.

3. Tezuka K, Tezuka Y, Maejima A, Sato T, Nemoto K, Kamioka H, Hakeda Y, Kumegawa M. Molecular cloning of a possible cysteine proteinase predominantly expressed in osteoclasts. *J Biol Chem*. 1994 Jan 14;269(2):1106-9.

4. Inaoka T, Bilbe G, Ishibashi O, Tezuka K, Kumegawa M, Kokubo T. Molecular cloning of human cDNA for cathepsin K: novel cysteine proteinase predominantly expressed in bone. *Biochem Biophys Res Commun*. 1995 Jan 5;206(1):89-96.

5. Rantakokko J, Aro HT, Savontaus M, Vuorio E. Mouse cathepsin K: cDNA cloning and predominant expression of the gene in osteoclasts, and in some hypertrophying chondrocytes during mouse development. *FEBS Lett*. 1996 Sep 16;393(2-3):307-13.

6. Yasuda Y, Kaleta J, Brömme D. The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. *Adv Drug Deliv Rev*. 2005 May 25;57(7):973-93.

7. McQueney MS, Amegadzie BY, D'Alessio K, Hanning CR, McLaughlin MM, McNulty D, Carr SA, Ijames C, Kurdyla J, Jones CS. Autocatalytic activation of human cathepsin K. *J Biol Chem*. 1997 May 23;272(21):13955-60.

8. Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, McQueney MS, Foged NT, Delmas PD, Delaissé JM. The collagenolytic activity of cathepsin K is unique among mammalian proteinases. *J Biol Chem*. 1998 Nov 27;273(48):32347-52.

9. Yamaza T, Goto T, Kamiya T, Kobayashi Y, Sakai H, Tanaka T. Study of immunoelectron microscopic localization of cathepsin K in osteoclasts and other bone cells in the mouse femur. *Bone*. 1998 Dec;23(6):499-509.

10. Vääräniemi J, Halleen JM, Kaarlonen K, Ylipahkala H, Alatalo SL, Andersson G, Kaija H, Vihko P, Väänänen HK. Intracellular machinery for matrix degradation in bone-resorbing osteoclasts. *J Bone Miner Res*. 2004 Sep;19(9):1432-40.

11. Bühling F, Waldburg N, Gerber A, Häckel C, Krüger S, Reinhold D, Brömme D, Weber E, Ansorge S, Welte T. Cathepsin K expression in human lung. *Adv Exp Med Biol*. 2000;477:281-6.

12. Rünger TM, Quintanilla-Dieck, MJ, Bhawan J. Role of cathepsin K in the turnover of the dermal extracellular matrix during scar formation. *J Invest Dermatol*. 2007 Feb;127(2):293-7.

13. Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, Levy R, Keysser M, Keyszer G, Brömme D. Cathepsin k is a critical protease in synovial fibroblast-mediated collagen degradation. *Am J Pathol*. 2001 Dec;159(6):2167-77.

14. Littlewood-Evans, AJ, Bilbe G, Bowler WB, Farley D, Wlodarski B, Kokubo T, Inaoka T, Sloane J, Evans DB, Gallagher JA. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Res*. 1997 Dec 1;57(23):5386-90.

15. Brubaker KD, Vessella RL, True LD, Thomas R, Corey E. Cathepsin K mRNA and protein expression in prostate cancer progression. *J Bone Miner Res*. 2003 Feb;18(2):222-30.

16. Gelb BD, Shi GP, Chapman HA, Desnick RJ. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science*. 1996 Aug 30;273(5279):1236-8.

17. Everts V, Hou WS, Rialland X, Tigchelaar W, Saftig P, Brömme D, Gelb BD, Beertsen W. Cathepsin K deficiency in pycnodysostosis results in accumulation of non-digested phagocytosed collagen in fibroblasts. *Calcif Tissue Int*. 2003 Oct;73(4):380-6.

18. Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W, Moritz JD, Schu P, von Figura K. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. *Proc Natl Acad Sci U S A*. 1998 Nov 10;95(23):13453-8.

19. Gowen M, Lazner F, Dodds R, Kapadia R, Feild J, Tavaria M, Bertoncello I, Drake F, Zavarselk S, Tellis I, Hertzog P, Debouck C, Kola I. Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not mineralization. *J Bone Miner Res*. 1999 Oct;14(10):1654-63.

20. Pennypacker B, Kimmel DB. Bone formation phenotype in cathepsin K null mice. *J Bone Miner Res*. 2004 Oct;19(Suppl 1):S22

21. Li CY, Jepsen KJ, Majeska RJ, Zhang J, Ni R, Gelb BD, Schaffler MB. Mice lacking cathepsin K maintain bone remodeling but develop bone fragility despite high bone mass. *J Bone Miner Res*. 2006 Jun;21(6):865-75.

22. Kiviranta R, Morko J, Uusitalo H, Aro HT, Vuorio E, Rantakokko J. Accelerated turnover of metaphyseal trabecular bone in mice overexpressing cathepsin K. *J Bone Miner Res*. 2001 Aug;16(8):1444-52.

23. Turk B. Targeting proteases: successes, failures, and future prospects. *Nat Rev Drug Discov.* 2006 Sep;5(9):785-99.

24. Vasiljeva O, Reinheckel T, Peters C, Turk D, Turk V, Turk B. Emerging roles of cysteine cathepsins in disease and their potential as drug targets. *Curr Pharm Des*. 2007;13(4):387-403.

25. Lecaille F, Brömme D, Lalmanach G. Biochemical properties and regulation of cathepsin K activity. *Biochimie*. 2007 Sep 2; [Epub ahead of print]

26. Palmer JT, Bryant C, Wang DX, Davis DE, Setti EL, Rydzewski RM, Venkatraman S, Tian ZQ, Burrill LC, Mendonca RV, Springman E, McCarter J, Chung T, Cheung H, Janc JW, McGrath M, Somoza JR, Enriquez P, Yu ZW, Strickley RM, Liu L, Venuti MC, Percival MD, Falgueyret JP, Prasit P, Oballa R, Riendeau D, Young RN, Wesolowski G, Rodan SB, Johnson C, Kimmel DB, Rodan G. Design and synthesis of tri-ring P3 benzamide-containing aminonitriles as potent, selective, orally effective inhibitors of cathepsin K. *J Med Chem*. 2005 Dec 1;48(24):7520-34.

27. Pennypacker B, Rodan S, Masarachia P, Rodan G, Kimmel DB. Bone effects of a cathepsin K inhibitor in the adult estrogen deficient rabbit. *J Bone Miner Res*. 2006 Sep;21(Suppl 1):S303.

28. Xiang A, Kanematsu M, Kumar S, Yamashita D, Kaise T, Kikkawa H, Asano S, Kinoshita M. Changes in micro-CT 3D bone parameters reflect effects of a potent cathepsin K inhibitor (SB-553484) on bone resorption and cortical bone formation in ovariectomized mice. *Bone*. 2007 May;40(5):1231-7.

29. Chen W, Yang S, Abe Y, Li M, Wang Y, Shao J, Li E, Li YP. Novel pycnodysostosis mouse model uncovers cathepsin K function as a potential regulator of osteoclast apoptosis and senescence. *Hum Mol Genet*. 2007 Feb 15;16(4):410-23.

30. McQueney MS, Field J, Hanning CR, Brun K, Ramachandran K, Connor J, Drake F, Jones CS, Amegadzie BY. Cynomolgus monkey (Macaca fascicularis) cathpesin K: cloning, expression, purification, and activation. *Protein Expr Purif*. 1998 Dec;14(3):387-94.

31. Guay J, Riendeau D, Mancini JA. Cloning and expression of rhesus monkey cathepsin K. *Bone*. 1999 Aug;25(2):205-9.

32. Jerome C, Missbach M, Gamse R. AAE581, a novel cathepsin K inhibitor, protects against ovariectomy-induced bone loss in non-human primates, in part by stimulation of periosteal bone formation. *J Bone Miner Res*. 2005 Sep;20(Suppl 1):S46.

33. Stroup G, Jerome C, Yamashita DS, Kumar S. Histomorphometric and biochemical evidence for a cortical bone-forming effect of a cathepsin K inhibitor in ovariectomized cynomolgus monkeys. *J Bone Miner Res*. 2005 Sep;20(Suppl 1):S80.

34. Stroup GB, Dare L, Vasko-Moser J, Hoffman S, Kumar S. Repeat daily dosing with a highly potent inhibitor of cathepsin K results in significant, transient elevation of plasma PTH in cynomolgus monkeys. *J Bone Miner Res*. 2006 Sep;21(Suppl 1):S160.

35. Masarachia P, Pun S, Kimmel D. Bone effects of cathepsin K inhibitor in ovariectomized rhesus monkeys. *J Bone Miner Res.* 2007 Sep;22(Suppl 1):S126.

36. Martin TJ, Sims NA. Osteoclast-derived activity in the coupling of bone formation to resorption. *Trends Mol Med*. 2005 Feb;11(2):76-81.

37. Karsdal MA, Martin TJ, Bollerslev J, Christiansen C, Henriksen K. Are non-resorbing osteoclasts sources of bone anabolic activity? *J Bone Miner Res*. 2007 Apr;22(4):487-94.

38. Henriksen K, Tanko LB, Qvist P, Delmas PD, Christiansen C, Karsdal MA. Assessment of osteoclast number and function: application in the development of new and improved treatment modalities for bone diseases. *Osteoporos Int*. 2007 May;18(5):681-5.

39. Fuller K, Lawrence KM, Ross JL, Grabowska UB, Shiroo M, Samuelsson B, Chambers TJ. Cathepsin K inhibitors prevent matrix-derived growth factor degradation by human osteoclasts. *Bone*. 2008 Jan;42(1):200-11.

40. Fratzl-Zelman N, Valenta A, Roschger P, Nader A, Gelb BD, Fratzl P, Klaushofer K. Decreased bone turnover and deterioration of bone structure in two cases of pycnodysostosis. *J Clin Endocrinol Metab*. 2004 Apr;89(4):1538-47.

41. Kumar S, Dare L, Vasko-Moser JA, James IE, Blake SM, Rickard DJ, Hwang SM, Tomaszek T, Yamashita DS, Marquis RW, Oh H, Jeong JU, Veber DF, Gowen M, Lark MW, Stroup G. A highly potent inhibitor of cathepsin K (relacatib) reduces biomarkers of bone resorption both in vitro and in an acute model of elevated bone turnover in vivo in monkeys. *Bone*. 2007 Jan; 40(1):122-31.

42. Adami S, Supronik J, Hala T, Brown JP, Garnero P, Haemmerle S, Ortmann CE, Bouisset F, Trechsel U. 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 Sep;21(Suppl 1):S24.

43. Falgueyret JP, Desmarais S, Oballa R, Black WC, Cromlish W, Khougaz K, Lamontagne S, Massé F, Riendeau D, Toulmond S, Percival MD. Lysosomotropism of basic cathepsin K inhibitors contributes to increased cellular potencies against off-target cathepsins and reduced functional selectivity. *J Med Chem*. 2005 Dec 1;48(24):7535-43.

44. Desmarais S, Black WC, Oballa R, Lamontagne S, Riendeau D, Tawa P, Duong le T, Pickarski M, Percival MD. Effect of cathepsin K inhibitor basicity on in vivo off-target activities. *Mol Pharmacol*. 2008 Jan;73(1):147-56.

45. Black WC, Desmarais S, Duong LT, Falgueyret J, Gauthier J, Lamontagne S, Li C, Masse F, Truong V, Wesolowski G, Percival M. MK-0822 is a potent and selective cathepsin K inhibitor that maintains its high selectivity in whole cell assays. *J Bone Miner Res*. 2007 Sep; 22(Suppl 1):S446.

46. Stoch SA, Miller DL, Van Dyck K, Jin B, Panebianco D, Liu Q, Stone J, Gottesdiener KM, Wagner JA. Effect of cathepsin K inhibition on bone resorption markers in healthy postmenopausal women. *J Bone Miner* Res. 2006 Sep;21(Suppl 1):S60.

47. Bone HG, McClung M, Verbruggen N, Rybak-Feiglin A, DaSilva C, Santora AC, Ince A. A randomized double-blind, placebo-controlled study of a cathepsin K inhibitor in the treatment of postmenopausal women with low BMD: one year results. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S37.

48. Rodan GA. The development and function of the skeleton and bone

metastases. *Cancer*. 2003 Feb 1;97(3 Suppl):726-32.

49. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med*. 2004 Apr 15;350(16):1655-64.

50. Le Gall C, Bellahcène A, Bonnelye E, Gasser JA, Castronovo V, Green J, Zimmermann J, Clézardin P. A cathepsin K inhibitor reduces breast cancer induced osteolysis and skeletal tumor burden. *Cancer Res*. 2007 Oct 15;67(20):9894-902.

51. Wesolowski G, Pickarski M, Neusch G, Leung P, Oballa R, Percival M, Black W, Duong LT. Inhibition of osteolytic bone metastases in breast cancer with a cathepsin K inhibitor. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S112.

52. Lu Y, Keller E, Dai J, Escara-Wilke J, Corey E, Yao Z, Zimmermann J, Zhang J. Targeting cathepsin K in prostate cancer skeletal metastasis in vivo. *J Bone Min Res*. 2005 Sep;20(Suppl 1):S215.

53. Salminen-Mankonen HJ, Morko J, Vuorio E. Role of cathepsin K in normal joints and in the development of arthritis. *Curr Drug Targets*. 2007 Feb;8(2):315-23.

54. Felson DT, Neogi T. Osteoarthritis: is it a disease of cartilage or of bone? *Arthritis Rheum*. 2004 Feb;50(2):341-4.