USE OF CATHEPSIN K INHIBITORS IN OSTEOPOROSIS: A CAUTIONARY TALE

Devin Walker

December 11, 2020

An Introduction to Osteoporosis

Osteoporosis is characterized by bone resorption exceeding bone formation [4]. As such, osteoporosis involves a loss of bone mass, strength and microarchitecture, increased risk of fracture, and decreased quality of life. Unfortunately, with an aging population, osteoporosis is on the rise [6]. In this paper, I will 1) examine current pharmacological approaches to treating osteoporosis, 2) introduce Cathepsin K as a novel target, 3) discuss lessons learned from odanacatib (ODN) animal studies, 4) summarize insights from ODN clinical trials, 5) conclude on ODN's promise and shortcomings in osteoporosis treatment, and 6) consider next steps.

Current Pharmacological Approaches to Treating Osteoporosis

Several pharmacological approaches to treating osteoporosis have been developed [4]. First, 'anti-resorptive drugs' mitigate bone resorption and include bisphosphonates, selective estrogen-receptor modulators [7], and monoclonal antibodies against receptor activator of nuclear factor-κB ligand [4]. Second, 'anabolic drugs' increase bone formation and include parathyroid hormone (PTH 1-84) and teriparatide (PTH 1-34). Third, several drugs reduce the risk of fracture. However, these drugs have adverse effects which impede patient compliance. Ultimately, there remains a need for innovation in developing osteoporosis therapies. This need has led to further study of bone remodeling which has revealed a novel target: Cathepsin K.

Cathepsin K as a Novel Target

To understand Cathepsin K's role as a novel target in osteoporosis treatment, it is critical to understand bone resorption. In bone resorption, osteoclasts attach to bone using the "sealing membrane" domains of their bone-facing membranes [4, 8]. This attachment forms a distinct,

acidic compartment called the resorption lacuna. The acidity of the resorption lacuna is essential for demineralization of the bone's hydroxyapatite structure. Next, the osteoclast releases lysosomal enzymes into the resorption lacuna which degrade the bone's organic components. One such enzyme is Cathepsin K, a cysteine protease highly expressed in osteoclasts which specializes in type I collagen degradation [8, 9]. Note that Cathepsin K is not only expressed in osteoclasts, but also in fibroblasts from bone, skin cells, osteoblasts and osteocytic cell lines [10]. Cathepsin K's tissue distribution is important to consider, because drugs targeting Cathepsin K may cause toxicities in these tissues. Also note there are other Cathepsin enzymes - Cathepsins B, L and S - expressed in the skin [4, 10]. Cathepsins B, L and S must be considered, because Cathepsin K inhibitors could theoretically inhibit all Cathepsin enzymes, causing adverse effects.

This context on bone resorption helps elucidate Cathepsin K's role as a novel target in osteoporosis [4]. Since Cathepsin K degrades collagen, inhibiting Cathepsin K may prevent bone loss. Furthermore, because Cathepsin K does not regulate osteoclast maturation, its inhibition should not impact osteoclast viability. This sets Cathepsin K inhibitors apart from other anti-resorptive drugs that upregulate osteoclast apoptosis such as denosumab and bisphosphonates.

Moreover, Cathepsin K represents a novel target, because defects in the gene encoding Cathepsin K, CTSK, lead to the disorder pycnodysostosis characterized by osteosclerosis, a rise in BMD, and decreased levels of bone resorption biomarkers [11, 12]. Similarly, as demonstrated by Saftig et al., global deletion of Cathepsin K in mice leads to an osteopetrotic phenotype with decreased bone resorption and altered osteoclast morphology – what may serve as an animal model of pycnodysostosis [13]. Saftig et al. generated their mouse model by creating a target construct consisting of a Cathepsin K cDNA fragment and the neomycin phosphotransferase gene (*Neo*) which introduces a premature stop codon in the Cathepsin K gene, interfering with its

translation. This target construct, pCK-Kpn(*Neo*) was then utilized to disrupt Cathepsin K in embryonic stem cells. The mutated embryonic stem cells were next injected into the blastocysts of female mice. This led to the creation of heterozygous mice and eventually homozygous mutant offspring. Northern blotting, Reverse Transcription-PCR and Western blotting confirmed the lack of Cathepsin K RNA and protein in the homozygous Cathepsin K-deficient mice. Saftig et al. explain that they created this mouse model to further elucidate Cathepsin K's role in bone resorption.

Interestingly, Saftig et al. found the homozygous Cathepsin K-deficient mice survive and are fertile but demonstrate osteosclerosis upon radiological examination. More specifically, the Cathepsin K-deficient mice exhibited trabeculation of the bone-marrow spaces of the long bones and vertebrae that was abnormally dense – a result that is characteristic of osteopetrosis. Additionally, Saftig et al. observed through electron microscopy that Cathepsin K-deficient osteoclasts have a poorly defined resorptive surface with undigested fine collagen fibrils along the matrix fringe and lack collagen-fibril-containing vacuoles in the cytoplasm. Finally, Saftig et al. predicted the activity of Cathepsin K-deficient osteoclasts in vitro using confocal laser reflection microscopy and determined their resorptive activity was severely limited. Saftig et al. conclude that the abnormalities they observed in the Cathepsin K-deficient mice mimic those seen in pycnodysostosis and can be explained by limited bone resorption. Most importantly, this supports Cathepsin K's role as a novel target in osteoporosis treatment.

Furthermore, Lotinun et al. have shown that osteoclast-specific deletion of Cathepsin-K in mice is associated with decreased bone resorption, increased bone formation and high bone mass – characteristics of pcynodysostosis [14]. Lotinun et al. accomplished osteoclast-specific deletion in their mouse model by using the Mx1-Cre system. In this system, the gene segment of

interest (in this case, a CTSK fragment) is flanked by *loxP* sites, allowing cell- or tissue- specific excision via *Cre* recombinase. Lotinun et al. created this mouse model because prior studies (such as Saftig et al.'s study discussed previously) had explored global CTSK deletion and observed not only decreased bone resorption linked to osteopetrosis, but also increased bone formation rate (BFR). Lotinum et al. thus generated this osteoclast-targeted CTSK knockout mouse model to understand the cause of increased BFR.

Lotinum et al.'s major findings include that osteoclast-specific CTSK deletion increases bone volume, BFR, and osteoclast and osteoblast numbers. It is worth noting that by demonstrating osteoblast-targeted CTSK deletion does not increase BFR, Lotinum et al. established that the BFR effect is osteoclast dependent. Additionally, osteoclast-specific CTSK deletion led to increased sphingosine kinase 1 expression, and in turn, increased sphingosine-1-phosphate (S1P). When media from CTSK-deficient osteoclasts containing elevated S1P was introduced to osteoblast cultures, increased alkaline phosphatase (involved in bone mineralization) and mineralized nodules were observed. Moreover, the addition of a SIP receptor antagonist blocked these effects. Together, Lotinum et al.'s findings support that bone formation in vivo can be increased by osteoclast-targeted CTSK deletion through increased osteoclast-derived SIP. Overarchingly, Lotinum et al.'s results support the idea that Cathepsin K inhibition can limit bone resorption and simultaneously maintain or enhance bone formation – further solidifying Cathepsin K's role as a novel target in osteoporosis.

ODN acts as a competitive inhibitor of Cathepsin K, binding to its active site [15]. The drug was developed by Merck with the goal of mitigating the metabolic limitations – such as short half-life and clearance - of the prior Cathepsin K inhibitor L-873724 [16, 17]. By substituting L-873724's P1 residue with a 1-cyclopropane ring and modifying its P2 side chain

by introducing a 4-fluoroleucine derivative, Merck generated a molecule with a longer half-life: ODN [16]. Although several Cathepsin K inhibitors have been investigated, only ODN has been studied in phase III trials [4]. This is largely due to ODN's improved specificity; while other Cathepsin K inhibitors also target Cathepsins B, L and S, causing skin thickening and rashes, ODN uniquely targets Cathepsin K [4, 10]. Thus, this paper will focus on results from ODN animal studies and clinical trials with the goal of analyzing ODN's promise and shortcomings.

Lessons Learned from Odanacatib Animal Studies

Since ODN was created to bind to human Cathepsin K, it has limited potency for rat and mouse Cathepsin K which share only 87-88% homology with human Cathepsin K [10, 18]. As such, rabbits and primates, which share 94% and 100% homology with human Cathepsin K, respectively, serve as the best animal models for studying ODN in vivo.

First, consider ODN use in ovariectomized (OVX) rabbits [3, 19]. Notably, OVX rabbits show the key features of estrogen deficiency-induced bone loss and thus serve as an effective way of modeling postmenopausal osteoporosis. One particular study compared the effects of ODN to the effects of alendronate (ALN), a bisphosphonate, on bone resorption and formation [3, 20]. This investigation is especially fascinating, because bisphosphonates have served as effective antiresorptive drugs for years. As such, this study's comparative design offers insights on how ODN's efficacy 'shapes up' to that of a well-established osteoporosis therapy. This comparative information is relevant, because new therapies must offer improved efficacy or reduced toxicity (compared to available drugs) to be approved by the FDA. Interestingly, doses of 4 or 9 μM ODN/day prevented the decline in lumbar spine BMD observed in OVX-vehicle control rabbits to an extent comparable with sham rabbits or OVX-ALN rabbits (see figure 1)

[3]. Additionally, ODN did not reduce bone formation at the sites studied. For example, while ALN significantly reduced endocortical bone formation in the central femur compared to OVX-vehicle control rabbits, ODN did not (see figure 2). Pennypacker et al.'s results indicate that ODN prevented bone loss with an efficacy similar to that of ALN but did not impair bone formation. This differentiates ODN from ALN and other anti-resorptive drugs.

ODN was further investigated in primates. In one experiment, 11 intact and 31 OVX monkeys were treated for 21 months with 6 mg/kg/day ODN, 30 mg/kg/day ODN, or placebo [5]. More specifically, the 11 intact monkeys received placebo, while 11 OVX monkeys received placebo, 10 received 6 mg/kg/day ODN and 10 received 30 mg/kg/day ODN. In this study, ODN decreased bone resorption. This was demonstrated by decreases in urinary amino-terminal collagen cross-links (NTX) and serum carboxyl-terminal collagen cross-links (CTX) [5]. NTX and CTX are considered valuable markers of bone resorption, because they are generated through degradation of cross-linked collagen fibers which make up 90-95% of bone's organic matrix [21]. Therefore, NTX and CTX are byproducts of osteoclast activity. NTX and CTX are often used over other emerging bone turnover markers such as Sclerostin, RANKL and osteoprotegrin, because there is still a need for large trials to establish the clinical utilities of such new markers [21]. In this study, ODN reduced urinary NTX by 75% to 90% and serum CTX by 40% to 55% compared to placebo [5]. In addition, ODN did not decrease the primates' tartrate-resistant acid phosphatase type 5b (TRAP-5b) levels. As TRAP-5b is widely regarded as an indicator of osteoclast number [21], ODN's lack of effect on TRAP-5b levels suggests the drug does not interfere with osteoclast numbers. In other words, this result indicate that ODN impairs osteoclast activity without inhibiting osteoclast maturation or differentiation [5]. This was further substantiated with histomorphometric analysis which demonstrated that osteoclast levels

remained consistent or increased during ODN treatment. Similarly, long-term ODN treatment has been shown to increase Cathepsin K expression [22]. As such, after ODN discontinuation, bone resorption can transiently increase over baseline. Together, these findings support the reversible nature of ODN treatment. Moreover, ODN dose-dependently increased BMD at the lumbar spine compared to OVX-vehicle control monkeys (see figure 3) [5]. More specifically, 6 mg/kg/day ODN increased the OVX-monkeys' lumbar spine BMD by 7.2%, while 30 mg/kg/day ODN increased the OVX-monkeys' lumbar spine BMD by 15% over 20 months [5]. Importantly, both BMD increases of 7.2% and 15% represent clinically significant BMD increases and were observed through dual x-ray absorptiometry [5, 23].

Insights from Major Odanacatib Clinical Trials

After animal studies, ODN was introduced to humans in a trial with 44 healthy volunteers [12]. Among the 44 participants were eight postmenopausal women, representing ODN's target population. The study investigated ODN doses ranging from 2-600 mg/week. In terms of safety, the drug was well tolerated, with transient, mild to moderate adverse effects such as headache. In terms of pharmacokinetics, ODN reached its peak concentration 4-6 hours after administration and showed monophasic decline with a half-life of ~40-80 hours. Regarding pharmacodynamics, ODN treatment led to a decrease in CTX and NTX. For example, in the postmenopausal women, 50 mg/week ODN reduced serum CTX by 66% and urinary NTX/creatinine by 51% compared to placebo at 24 hours. Moreover, at 168 hours, serum CTX had declined by 70% and urinary NTX/creatinine had dropped by 78% compared to placebo. Importantly, these decreases in serum CTX and urinary NTX/creatinine demonstrate ODN's reduction of bone resorption. Overall, Stoch et al.'s results support ODN's tolerability, pharmacokinetic and pharmacodynamic

properties, and suggest the drug should be studied in larger trials more representative of osteoporosis patients. Furthermore, as this study was conducted over a relatively short time span, ODN must be studied in longer trials to note long-term effects [10].

In a phase II trial, ODN was studied in 399 postmenopausal women [2]. Subjects received either placebo or 3, 10, 25 or 50 mg/week ODN. Endpoints measured include percent change in BMD at all measured sites and percent change in markers of bone resorption and formation. Results of this trial were promising; ODN doses of ≥10 mg dose-dependently increased BMD at the lumbar spine and all femoral sites at 12 months (with further increases observed by 24 months). For example, 50 mg/week ODN led to BMD increases of 5.7% at the lumbar spine, 4.1% at the hip and 4.7% at the femoral neck compared to placebo at 24 months. Moreover, ≥10 mg/week ODN decreased bone resorption markers. For example, on average, urinary NTX decreased by 60.2% at 12 months and by 51.8% at 24 months. However, ≥10 mg/week ODN decreased bone formation markers such as bone-specific alkaline phosphatase in the first 6 months of treatment. While gradual increases were observed after 6 months for most doses, allowing the markers to approach baseline levels, 50 mg/week ODN did not facilitate such gradual increases (see figure 4). As such, 50 mg/week ODN reduced bone formation markers under baseline. While Bone et al. do not offer an explanation for this observation, the researchers do note that the decreases observed were milder and shorter in duration than those observed with other antiresorptive drugs like ALN [2]. Nevertheless, this represents a potential limitation of high dose ODN use in osteoporosis patients. More positively, although Cathepsins are expressed in the skin, adverse skin reactions were equivalent across treatment groups. Additionally, other phase II trials including different patient populations have demonstrated that ODN exerts similar effects in Asian and Caucasian people and in older men and older women [24, 25].

Finally, ODN has been analyzed in a phase III trial called Long-Term Odanacatib Fracture Trial (LOFT) which included 16,000 postmenopausal women [1]. Participants either received 50 mg ODN/week or placebo. LOFT results indicated that 50 mg ODN/week over 3 years reduces risk of new and worsening morphometric vertebral fractures by 54%, clinical hip fractures by 47%, clinical nonvertebral fractures by 23% and clinical vertebral fractures by 72% compared to placebo. Based on concerns raised in phase II trials, four categories of adverse effects were monitored in LOFT: skin problems, respiratory infections, skeletal concerns and adverse cardiovascular events. Alarmingly, ODN increased the risk of (mostly ischemic) strokes compared to placebo (see figure 5). This may be partially explained by the cardioprotective role Cathepsin K has been hypothesized to play [1]; many researchers have suggested that Cathepsin K's breakdown of collagen and elastin may promote instability of atherosclerotic plaques by compromising the structural integrity of blood vessel walls. Thus, Cathepsin K inhibition may increase plaque formation and contribute to atherosclerosis – a leading cause of stroke. Unfortunately, ODN's increase of stroke risk prompted its withdrawal from FDA consideration.

Odanacatib's Promise and Shortcomings in Osteoporosis Treatment; What's Next?

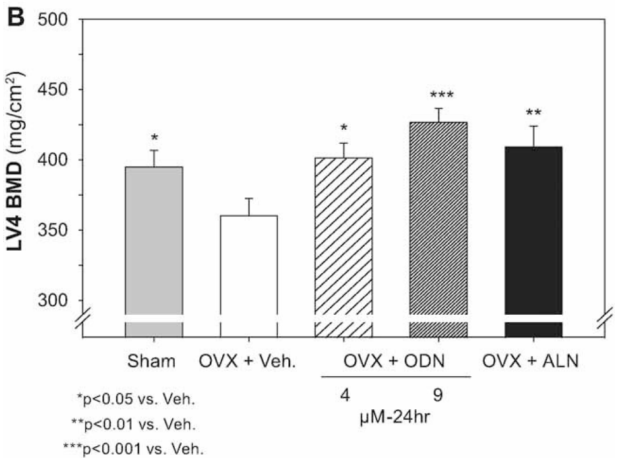
In summary, one of the most promising aspects of ODN is its retention of coupling of bone formation and resorption [10]. As Cathepsin K inhibitors prevent bone resorption while not impacting osteoclasts' ability to release 'coupling' factors which recruit, differentiate or increase osteoblast activity, impairment of bone formation is milder and more transient than that seen with other antiresorptive drugs like ALN [2, 10]. This represents a significant step forward in osteoporosis treatment. Another advantage of ODN is its half-life of ~40-80 hours - a significant improvement over the prolonged biologic half-lives of bisphosphonates [19]. Additionally, as

discussed previously, ODN's reversibility is supported by its lack of impact on osteoclast number as well as the increase in Cathepsin K expression observed in long-term ODN treatment. The reversibility of ODN may offer advantages over other antiresorptive therapies.

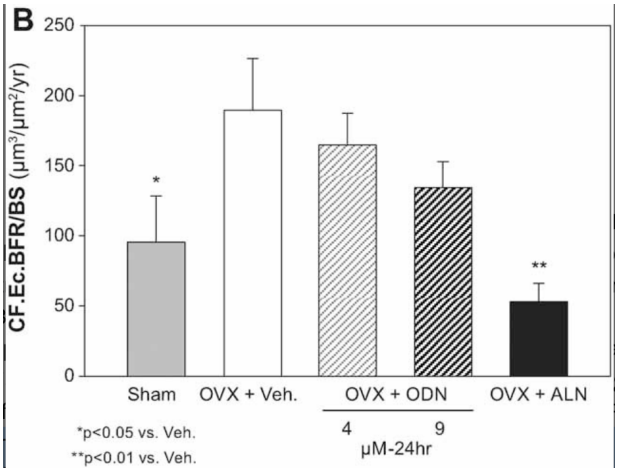
Despite ODN's promising retention of bone formation and resorption coupling, half-life, and reversibility, the drug failed to reach osteoporosis patients. One potential reason why ODN failed is its mechanism of competitive inhibition [15]. This represents a limitation of ODN, because Cathepsin K is not perfectly specific for collagen, but instead catalyzes many reactions with diverse substrates. Thus, occupation of Cathepsin K's active site inhibits not only breakdown of collagen, but also breakdown of elastin, gelatin and other Cathepsin K substrates [15]. Inhibition of diverse cellular reactions may lead to adverse side effects such as the cerebrovascular effects seen in LOFT. Therefore, if moving forward with Cathepsin K as a target in osteoporosis drug development, one aim should be selective inhibition of Cathepsin K's collagenase activity. Law et al.'s work demonstrates that blocking the formation of collagen-degrading Cathepsin K oligomers may be an effective approach. Secondly, as Cathepsin K is expressed not only in osteoclasts, but also in fibroblasts, osteoblasts, and osteocytes, a valuable next step may be developing a Cathepsin K inhibitor specific to osteoclasts [10].

Ultimately, although ODN was withdrawn from FDA consideration, there are insights to be gleaned from its failure; the drug's severe cerebrovascular side effects should motivate development of osteoclast-specific and/or substrate-specific Cathepsin K inhibitors selective for collagenase activity. Moreover, ODN's story of triumph and tragedy serves as a cautionary tale; while the osteoporosis sphere urgently needs innovation, this innovation must be driven by prior drugs' successes and failures and informed by clinical trial data.

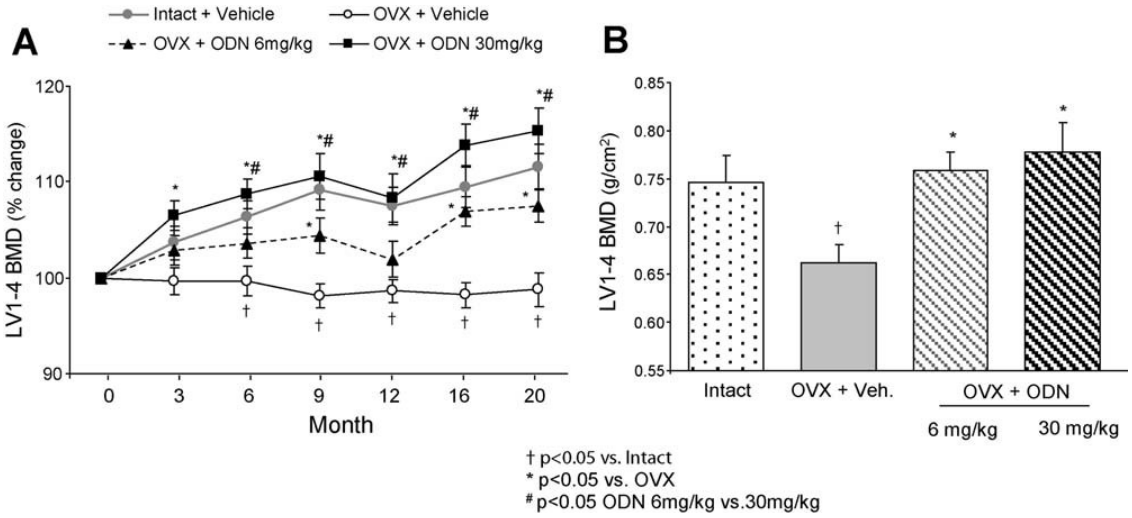
Figures:

1. 

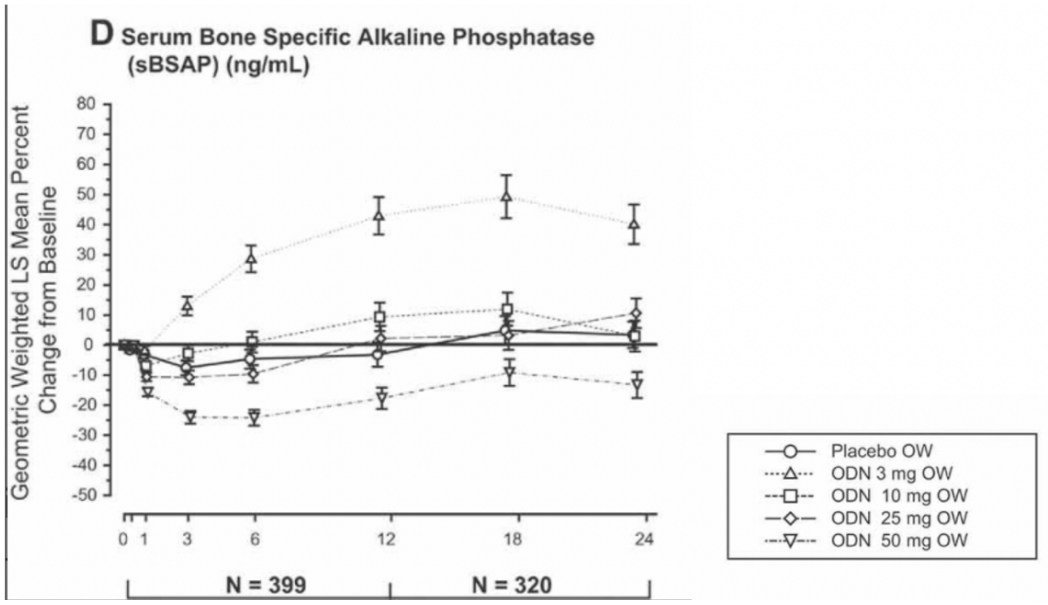
ODN dose-dependently prevented lumbar spine BMD loss observed in OVX-vehicle control rabbits. The lower dose of ODN had similar efficacy to ALN [3].

2. 

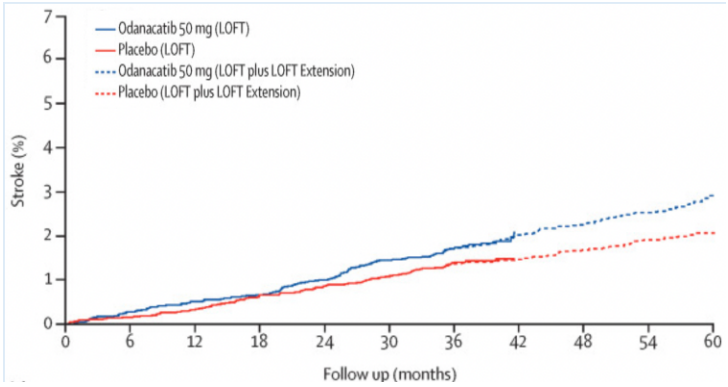
While ALN significantly reduced endocortical bone formation in the central femur compared to OVX-vehicle control rabbits, ODN did not [3].



3. ODN dose-dependently increased lumbar spine BMD compared to OVX-vehicle control monkeys [5].



4. 50 mg/week ODN reduced the bone formation marker bone-specific alkaline phosphatase below baseline in postmenopausal women [2].

5. 

ODN treatment increased the risk of stroke compared to placebo in postmenopausal women [1].

Works Cited

1. McClung, M.R., et al., *Odanacatib for the treatment of postmenopausal osteoporosis: results of the LOFT multicentre, randomised, double-blind, placebo-controlled trial and LOFT Extension study.* Lancet Diabetes Endocrinol, 2019. **7**(12): p. 899-911.
2. Bone, H.G., et al., *Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density.* J Bone Miner Res, 2010. **25**(5): p. 937-47.
3. Pennypacker, B.L., et al., *Cathepsin K inhibitors prevent bone loss in estrogen-deficient rabbits.* J Bone Miner Res, 2011. **26**(2): p. 252-62.
4. Rachner, T.D., S. Khosla, and L.C. Hofbauer, *Osteoporosis: now and the future.* Lancet, 2011. **377**(9773): p. 1276-87.
5. Masarachia, P.J., et al., *Odanacatib reduces bone turnover and increases bone mass in the lumbar spine of skeletally mature ovariectomized rhesus monkeys.* J Bone Miner Res, 2012. **27**(3): p. 509-23.
6. Burge, R., et al., *Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025.* J Bone Miner Res, 2007. **22**(3): p. 465-75.
7. D'Amelio, P. and G.C. Isaia, *The use of raloxifene in osteoporosis treatment.* Expert Opin Pharmacother, 2013. **14**(7): p. 949-56.
8. Cappariello, A., et al., *The Great Beauty of the osteoclast.* Arch Biochem Biophys, 2014. **558**: p. 70-8.
9. Lotinun, S., et al., *Cathepsin K-deficient osteocytes prevent lactation-induced bone loss and parathyroid hormone suppression.* J Clin Invest, 2019. **129**(8): p. 3058-3071.
10. Duong, L.T., *Therapeutic inhibition of cathepsin K-reducing bone resorption while maintaining bone formation.* Bonekey Rep, 2012. **1**: p. 67.
11. Toral-Lopez, J., et al., *Familial pycnodysostosis: identification of a novel mutation in the CTSK gene (cathepsin K).* J Investig Med, 2011. **59**(2): p. 277-80.
12. Stoch, S.A., et al., *Odanacatib, a selective cathepsin K inhibitor to treat osteoporosis: safety, tolerability, pharmacokinetics and pharmacodynamics--results from single oral dose studies in healthy volunteers.* Br J Clin Pharmacol, 2013. **75**(5): p. 1240-54.
13. Saftig, P., et al., *Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice.* Proc Natl Acad Sci U S A, 1998. **95**(23): p. 13453-8.

14. Lotinun, S., et al., *Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation.* J Clin Invest, 2013. **123**(2): p. 666-81.
15. Law, S., et al., *Identification of substrate-specific inhibitors of cathepsin K through high-throughput screening.* Biochem J, 2019. **476**(3): p. 499-512.
16. Gauthier, J.Y., et al., *The discovery of odanacatib (MK-0822), a selective inhibitor of cathepsin K.* Bioorg Med Chem Lett, 2008. **18**(3): p. 923-8.
17. Lu, J., et al., *Advances in the discovery of cathepsin K inhibitors on bone resorption.* J Enzyme Inhib Med Chem, 2018. **33**(1): p. 890-904.
18. Rodan, S.B. and L.T. Duong, *Cathepsin K – A New Molecular Target for Osteoporosis.* IBMS BoneKEy, 2008. **5**(1): p. 16-24.
19. Drake, M.T., et al., *Cathepsin K Inhibitors for Osteoporosis: Biology, Potential Clinical Utility, and Lessons Learned.* Endocr Rev, 2017. **38**(4): p. 325-350.
20. Porras, A.G., S.D. Holland, and B.J. Gertz, *Pharmacokinetics of alendronate.* Clin Pharmacokinet, 1999. **36**(5): p. 315-28.
21. Song, L., *Calcium and Bone Metabolism Indices.* Adv Clin Chem, 2017. **82**: p. 1-46.
22. Stone, J.A., et al., *Clinical and translational pharmacology of the cathepsin K inhibitor odanacatib studied for osteoporosis.* Br J Clin Pharmacol, 2019. **85**(6): p. 1072-1083.
23. Nguyen, T.V., N. Pocock, and J.A. Eisman, *Interpretation of bone mineral density measurement and its change.* J Clin Densitom, 2000. **3**(2): p. 107-19.
24. Nakamura, T., et al., *Effect of the cathepsin K inhibitor odanacatib administered once weekly on bone mineral density in Japanese patients with osteoporosis--a double-blind, randomized, dose-finding study.* Osteoporos Int, 2014. **25**(1): p. 367-76.
25. Anderson, M.S., et al., *Odanacatib, a selective cathepsin K inhibitor, demonstrates comparable pharmacodynamics and pharmacokinetics in older men and postmenopausal women.* J Clin Endocrinol Metab, 2014. **99**(2): p. 552-60.