# Src Inhibitors in Metastatic Bone Disease

Brendan F. Boyce,[1] Lianping Xing,[1] Zhenqiang Yao,[1] Teruhito Yamashita,[1] William C. Shakespeare,[2] Yihan Wang,[2] Chester A. Metcalf III,[2] Raji Sundaramoorthi,[2] David C. Dalgarno,[2] John D. Iuliucci,[2] and Tomi K. Sawyer[2]

**Abstract** Src tyrosine kinase was the first gene product shown to have an essential function in bone using recombinant DNA technology after its expression was knocked out in mice ~15 years ago. Since then, our understanding of the regulation of bone catabolism has advanced significantly with the identification of other key enzymes that regulate osteoclast formation, activation, and survival after their knockout in mice or recognition of mutations in them in humans. This led to the discovery or development of specific inhibitors of some of these key enzymes, including Src, as proof-of-concept lead compounds or potential clinical candidates for the prevention of diseases associated with increased bone resorption, such as osteoporosis and metastatic bone disease. Although bisphosphonates have been prescribed with proven and improving efficacy for the prevention of bone loss for >30 years, adverse effects, such as upper gastrointestinal tract symptoms, and the requirement to take them at least 2 hours before food have limited patient compliance. Thus, with growing knowledge of the pathways regulating osteoclast function and the appreciation that some of these are active also in tumor cells, drug companies have made efforts to identify small-molecular lead compounds for development into new therapeutic agents for the prevention of bone loss with efficacy that matches or supersedes that of bisphosphonates. In this article, we review our current understanding of the signaling pathways that regulate osteoclast formation, activation, and survival with specific reference to the role of Src tyrosine kinase and downstream signaling and highlight in a variety of models of increased bone resorption the effects of Src kinase inhibitors that have been targeted to bone to limit potential adverse effects on other cells.

Understanding of the regulation of osteoclast formation, activation, and survival has increased significantly in the past 15 years because the identification initially of a role for Src tyrosine kinase (1) in osteoclast ruffled border formation (2). Furthermore, such knowledge has advanced even more significantly during recent years after the receptor activator of nuclear factor-κB ligand (RANKL)/RANK signaling pathway was identified as the key regulatory mechanism for osteoclastogenesis (3–6). RANKL/RANK interaction on the cell surface of osteoclasts and their precursors triggers signaling through several enzymatic pathways, which leads to the activation of the transcription factors nuclear factor-κB, c-Fos, and nuclear factor of activated T cells (3–6), resulting in activation of the enzymes that mediate the secretion of protons (7) and chloride ions (8) for dissolution of the mineral and degradation of the matrix of bone (refs. 9, 10; reviewed in ref. 11). These studies led to the development or identification from screens of compound libraries of several small molecules that specifically inhibit these enzymes, including cathepsin K, vacuolar adenosine triphosphatase, the mammalian target of rapamycin, and Src tyrosine kinase.

In addition to studies that describe the effects of synthetic small-molecule agents on bone, several studies have suggested that the active ingredients in a variety of natural product-related herbal remedies or foods have osteoprotective effects. These include tanshinone, the main active diterpene quinone of a herbal medicine used to treat coronary heart disease and also present in Chinese green tea (12), the water extract of *Dioscorea spongiosa* (13), and the soybean isoflavones, genistein, and daidzein (14). Most of these have been studied in patients with osteoporosis and not in those with metastatic bone disease. However, the osteoprotective effects of these compounds and phytoestrogens remain controversial (15), and double-blind controlled clinical trials will be required to prove efficacy of all herbal compounds in humans.

## Src Tyrosine Kinase

A role for Src, a protein tyrosine kinase, in osteoclasts was identified unexpectedly when $src^{-/-}$ mice were generated (1). Despite the ubiquitous expression of Src, the only cells that seem to have a required function in the knockout mice were osteoclasts. $src^{-/-}$ mice formed increased numbers of

**Authors' Affiliations:** [1]University of Rochester Medical Center, Rochester, New York and [2]ARIAD Pharmaceuticals, Cambridge, Massachusetts

Received 4/21/06; revised 7/13/06; accepted 8/2/06.
Presented at the First Cambridge Conference on Advances in Treating Metastatic Bone Cancer, a symposium held in Cambridge, Massachusetts, October 28-29, 2005.

**Requests for reprints:** Brendan F. Boyce, Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, Box 626, Rochester, NY 14642. Phone: 585-275-5837; Fax: 585-273-3637; E-mail: brendan_boyce@urmc.rochester.edu.



doi:10.1158/1078-0432.CCR-06-0991

osteoclasts, but the cells failed to resorb bone because they did not form ruffled borders (2), which severely limited their ability to resorb bone matrix. Thus, the mice were osteopetrotic and their teeth did not erupt because of defective osteoclastic resorption. Src is activated in osteoclasts after integrin binding when the cells attach to bone matrix to initiate bone resorption (16), and it mediates the complex intracellular cytoskeletal reorganization that is required for polarization of the cytoplasm, including ruffled border formation. It is activated also in response to RANKL/RANK interaction in osteoclasts after the recruitment of tumor necrosis factor receptor–associated factor 6 (TRAF6) to the intracellular domain of RANK to mediate downstream signaling (17). Src binds to TRAF6 and recruits several signaling proteins, including Cbl (18), Pyk-2 (19), and cortactin (20), which mediate polarization of the cell, actin ring, and ruffled border formation in an as yet incompletely understood process.

In addition to these osteoclast-activating actions, Src also seems to mediate osteoclast survival in response to RANKL *in vitro*. In studies in which RANKL and macrophage colony-stimulating factor were withdrawn from $src^{-/-}$ or wild-type (WT) osteoclasts, the WT cells survived in the presence of RANKL alone, whereas $src^{-/-}$ osteoclasts died by apoptosis (17, 21). Src seems to mediate this protective effect by recruiting phosphatidylinositol 3-kinase to the intracellular domain of TRAF6 and activating the Akt/mammalian target of rapamycin pathway. This promotes osteoclast survival by phosphorylating the apoptosis inducer caspase-3 and thus inactivating it (22), although recent studies have questioned this role for Akt (23). Therefore, Src expression in osteoclasts could influence bone mass by regulating not only osteoclast activation but also osteoclast life span. In contrast, the $src^{-/-}$ cells survived, albeit in slightly reduced numbers, when they were treated with macrophage colony-stimulating factor alone, indicating that Src does not mediate macrophage colony-stimulating factor survival signaling. Interestingly, osteoclast apoptosis is not increased in $src^{-/-}$ mice *in vivo*, and indeed, osteoclast numbers are actually higher in $src^{-/-}$ mice than in WT controls (21). Thus, either these *in vitro* findings reflect a phenomenon that does not apply *in vivo* or macrophage colony-stimulating factor or perhaps other Src family kinase members substitute for Src *in vivo* in the $src^{-/-}$ mice to enhance their survival. Further studies will be required to determine which of these theories is correct.

There are three major structural domains in Src that could mediate its functions in osteoclasts: the kinase domain, which phosphorylates tyrosine residues in molecules that bind to the Src homology 2 domain, and the Src homology 3 domain, which binds proline-rich portions of interacting molecules. Studies designed to determine which domain(s) of the molecule mediates its resorptive function in osteoclasts suggested surprisingly that, although the kinase domain is required, Src kinase activity is not essential for bone resorption *in vivo* (24). In these studies, the TRAP promoter was used to drive expression of WT and various mutated *src* genes in crossed *src* knockout/transgenic mice. As expected, expression of WT Src in the $src^{-/-}$ transgenic mice rescued the defect in ruffled border formation and resorption in the mice, which had normal bone volumes and tooth eruption. However, a surprising observation was that, in 25% of the $src^{-/-}$ mice expressing the kinase-inactive mutant K295M in which the ATP-binding site of Src is mutated to prevent phosphorylation of tyrosine residues on interacting molecules, bone volumes were normal, and the mice also had normal tooth eruption and ruffled border formation. Equally surprising was the observation that the osteopetrosis became more pronounced in 25% of the mice, which had increased osteoclast apoptosis, whereas no phenotypic change was observed in the remaining 50% of the mice (24).

We found it difficult to explain these divergent findings definitively, but based on our previous experience that expression levels in osteoclasts of SV40 large T antigen driven by this same TRAP promoter in transgenic mice correlated with the number of apoptotic osteoclasts and the severity of osteopetrosis (25), we speculated that K295M expression levels varied in the $src^{-/-}$ transgenic mice. We further hypothesized that at high levels of expression Src K295M was acting in a dominant-negative fashion to inhibit recruitment of interacting molecules, whereas optimal expression levels rescued the defect, and that there was low expression in most mice, which thus had no change in the intensity of their osteopetrosis. Unfortunately, we were unable to assess the levels of K295M protein expression in these mice with the antibodies available at the time. Nevertheless, our interpretation was supported by our observation that the percentage of apoptotic osteoclasts correlated with the severity of the osteopetrosis in the Src K295M transgenic mice. Furthermore, expression of the Src homology 2 and Src homology 3 domains of the molecule without the kinase domain also induced more pronounced osteopetrosis in $src^{-/-}$ transgenic mice and in WT mice due to increased osteoclast apoptosis (21). Osteoclasts from these mice had reduced phosphorylated Akt and phosphatidylinositol 3-kinase, consistent with a failure of Src activation of the survival pathway.

Our conclusion that Src kinase activity is not essential for osteoclastic resorption *in vivo* has been challenged recently (26). These authors overexpressed the Src K295M mutant and other Src mutant molecules in osteoclasts *in vitro* using adenoviral infection and concluded that kinase activity was essential for resorption based on profoundly reduced resorptive activity by osteoclasts expressing Src K295M. This reduced resorptive activity was not rescued by concomitant expression of the constitutively active Src Y527F src mutant, which rescued the defective resorption of the kinase-deficient mutant Src Y416M. Although these are interesting and provocative findings, we believe that, based on their reported findings, there is an alternative explanation to the authors' conclusions. Specifically, the level of expression of the Src K295M mutant assessed by Western blot analysis was slightly higher in the $src^{-/-}$ osteoclasts than that of native Src in the control $src^{-/-}$ mice. Thus, we propose that, in these experiments, the authors had achieved a level of expression of Src K295M in osteoclasts *in vitro* that was equivalent to the levels we had achieved *in vivo* in osteoclasts in our Src K295M mice, which had a slight increase in the degree of their osteopetrosis. The osteoclasts infected with the K295M retroviral construct did not have increased apoptosis; thus, the level of expression of this construct is likely to have been lower than that which induces detectably increased osteoclast apoptosis *in vivo* ($0.5 \pm 0.1\%$ osteoclasts apoptotic in rescued K295M transgenic mice versus $4.3 \pm 1.2\%$ in osteopetrotic K295M transgenic mice).[3] Further

[3] Unpublished data.

studies will be required to determine the role of the kinase domain of Src in osteoclasts for their resorptive activity.

In addition to its role in osteoclasts, Src tyrosine kinase also has important functions in malignant cells, including regulation of cell division, growth factor signaling, and movement (27). Its activity is up-regulated in certain cancers. Thus, in patients with metastatic bone disease, Src inhibitors could potentially have negative effects not only on osteoclasts but also on tumor cells and their interactions with osteoclasts. Because of these roles for Src in osteoclasts and tumor cells, several pharmaceutical companies have advanced Src inhibitors as potential therapeutic agents for use in osteoporosis, osteolytic bone metastasis, and related diseases with increased resorption. Example of such Src inhibitors include the ATP-based lead compounds NVP-AAK980 (28) and AP23451 (see below); the pyrazolopyrimidines PP1 and PP2 (29), the pyrrolopyrimidines CGP-76775 and CGP-76030 (30), the pyridopyrimidinones PD166326 and PD18097 (31), the quinazoline AZM475271 (32), the quinoline SKI-606 (33), and the indolinone SU6656 (34). Hereinafter, we will briefly describe the *in vitro* and *in vivo* effects of Src kinase inhibitors AP23451 and AZD0530.

## Src Tyrosine Kinase Inhibitors

AP23451 is a purine-based Src tyrosine kinase inhibitor that incorporates a bisphosphonate group that confers bone-targeting and tissue selectivity properties, therefore potentially minimizing adverse effects *in vivo*. It exemplifies a key lead compound among a series of bone-targeted Src kinase inhibitors of varying ATP-related templates (35). AP23451 inhibited osteoclast formation and induced osteoclast apoptosis *in vitro* in the 0.1 to 1 μmol/L range. Both a non-bone-targeted analogue of AP23451 and the bone-targeted aniline related to AP23451 were synthesized and tested to understand the biological activities of AP23451 (Fig. 1). In contrast to AP23451, neither of these two compounds was found to be biologically active *in vitro* and *in vivo* (data not shown). These studies showed that the bisphosphonate group of AP23451 does not possess significant antiresorptive activity, and additional studies determined that the effects of AP23451 to induce osteoclast apoptosis were not prevented by addition of geranylgeraniol, which otherwise prevents alendronate-induced apoptosis. AP23451 dose dependently prevented parathyroid hormone–induced bone resorption (Fig. 2) hypercalcemia and ovariectomy-induced bone loss (36). When given to mice inoculated with MDA-231 breast cancer cells into the left cardiac ventricle, AP23451 prevented metastasis-induced osteolysis similar to zoledronic acid in the same experiment (37), but it also significantly reduced the volume of tumor cells inside the bone marrow cavities of the mice. This inhibitory effect on tumor cell volume was not observed in mice treated with zoledronic acid, suggesting that the Src inhibitor may have had an inhibitory effect on the tumor cells distinct from the effect on osteoclasts. Inhibition of resorption reduces the efflux of bone-derived growth factors that participate in a vicious cycle to stimulate tumor cell growth or release of osteoclast-stimulating factors, such as parathyroid hormone–related protein (38). As mentioned previously, Src is expressed by many tumor cells and is thus a target for inhibitors in tumor cells and osteoclasts.

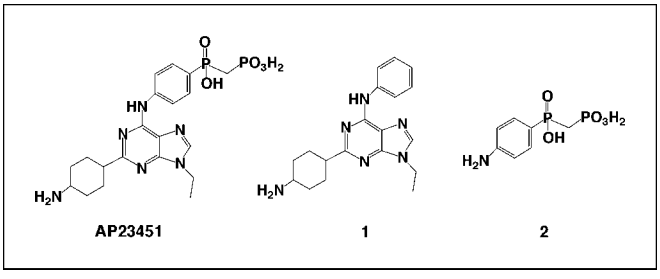


**Fig. 1.** Chemical structures of AP23451, a non-bone-targeted analogue (*1*) and a bone-targeted aniline (*2*) related to AP23451. See text for discussion of the comparative biological properties of these compounds.

AZD0530 is an orally active, highly selective, dual-specific, small-molecule inhibitor of Src kinase and Bcr-Abl that has been developed by AstraZeneca (39). The safety and efficacy of AZD0530 have been tested on bone resorption in two randomized, double-blind, and placebo-controlled phase 1 clinical trials: one single ascending dose (2.5-1,000 mg) and one multiple ascending dose (60-250 mg) study in healthy, male volunteers (ages 18-55 years). The single ascending dose study comprised 27 volunteers (cohorts of nine) who received a single AZD0530 dose ($n = 6$) or placebo ($n = 3$). In the multiple ascending dose study, five cohorts of volunteers received a single dose ($n = 9$) or placebo ($n = 3$) and then, 7 to 10 days later, multiple doses (10-14 days). Mean serum and urine levels, respectively, of the bone resorption markers, serum cross-linked C-telopeptides and urinary cross-linked N-telopeptides, decreased significantly from baseline with the 1,000-mg dose in the single ascending dose study and with the highest doses in the multiple ascending dose study (40), highly suggestive of inhibition of osteoclast-mediated bone resorption via suppression of Src tyrosine kinase. Together, these murine and human findings suggest that Src inhibitors could have a dual action in metastatic bone disease to prevent the growth of metastatic lesions and their progression.

## Summary

Although Src tyrosine kinase has been implicated in osteoclast function for >15 years and for longer in tumor cell growth and metastasis, no specific inhibitors of its function have been developed thus far as approved therapeutic agents to treat common human diseases in which bone resorption is increased, such as osteoporosis and metastatic cancer. Several potential lead compounds have been developed by several pharmaceutical companies, and preclinical studies with these small-molecule Src inhibitors have shown efficacy in reducing ovariectomy-induced bone resorption and progression of osteolytic bone metastases. Thus, there is growing optimism that Src inhibitors may be available in the near future as new therapeutics for use in several clinical settings.

## Open Discussion

**Dr. Roodman:** It's important that you have pointed out the autocrine loops in osteoclasts. We spent the last 10 years working on that. Osteoclasts make a lot of things that are up-regulated in inflammatory arthritides, such as IL-6 and eosinophil chemotactic factor, which is a late differentiation

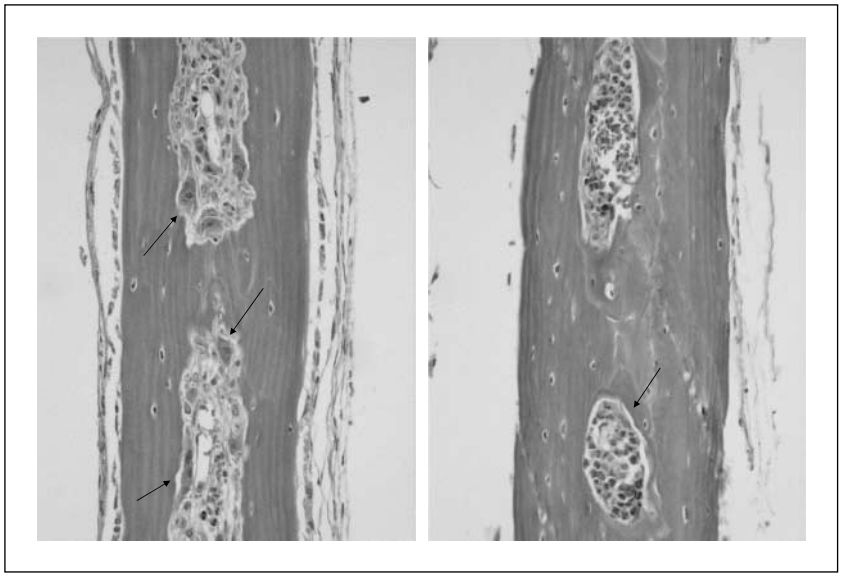

**Fig. 2.** Inhibitory effect of the Src inhibitor AP23451 on parathyroid hormone – induced bone resorption in mouse. Parathyroid hormone (20 μg/kg, given s.c. four times daily for 3 days) stimulated increased osteoclast formation (*arrows*) and the size of the bone marrow spaces *in vivo* in a mouse calvaria (*left*). AP23451 (10 mg/kg) given s.c. twice daily for 2 days before the start of the parathyroid hormone injections and continued for 3 days prevented this effect, and only occasional osteoclasts (*arrow*) were observed (*right*).

fusion molecule. We need to look at these areas carefully. However, tumor necrosis factor may be a little more murky because stromal cells make enough tumor necrosis factor in response to tumors, especially myeloma, to start the NF-κB signaling pathway.

**Dr. Boyce:** There's a paper published showing that an NF-κB inhibitor is able to prevent bone loss in an animal model of rheumatoid arthritis (Jimi, E, Aoki K, Saito H, et al. Selective inhibition of NF-κB blocks osteoclastogenesis and prevents inflammatory bone destruction *in vivo*. *Nat Med* 2004;23:617-624). It is important to have drugs that can specifically target the osteoclast and tumor cells.

**Dr. Berenson:** We're publishing data on an oncogene that's a potent inhibitor of osteoclast development and also knocks out the tumor.

**Dr. Boyce:** Do you have evidence that TRAF-6 signaling is important in tumor cell growth?

**Dr. Berenson:** We've used IL-1 in this model to induce and then we can block that with the TRAF-6.

**Dr. Boyce:** Do you think you are hitting the osteoclast only with your TRAF-6 antibody or antagonist?

**Dr. Berenson:** We attenuate growth of the tumor cells in both cell lines and fresh tumor cells with the construct. It's a common pathway for both and then leads down to NF-κB, so that's why we thought it was a good target.

**Dr. Suva:** You mentioned that the phosphonate group had no effect *in vitro*. Do you have any data on that molecule bringing something, for example, an inactive Src inhibitor or some other molecule? Are you seeing effects of the phosphonate group on resorption and the Src inhibitor, perhaps on the tumor cells? Are you confident that the compound is affecting both of those processes and that it's not a reflection of the activity of the compound on one cell and the phosphonate group, perhaps, on the osteoclast?

**Dr. Boyce:** The phosphonate group itself has no efficacy *in vitro* at inhibiting osteoclasts.

**Dr. Suva:** I wouldn't expect it to have much activity *in vitro*, but what about *in vivo*?

**Dr. Boyce:** We haven't tested that. Based on the lack of effects *in vitro*, we're assuming the phosphonate group isn't having any effect on either the tumor cells or osteoclasts but we believe that you need it to get it to the bone.

**Dr. Guise:** Does Src having any role in osteoblast function, and do the Src inhibitors affect that at all?

**Dr. Boyce:** A few years ago, we published data showing that Src is a negative regulator of osteoblast activity with Anna Teti (Marzia M, Sims NA, Voit S. Decreased c-Src expression enhances osteoblast differentiation and bone formation. *J Cell Biol* 2000;151:311-320). Osteoblasts from Src knockout mice have increased alkaline phosphatase activity and increased nodule formation, and the mice have increased bone formation rate *in vivo*. When we looked at the effects of this particular Src inhibitor on osteoblasts, to our chagrin it killed osteoclasts *in vitro*. We then tested other inhibitors similar to this, which also had an antiosteoclastic activity *in vitro*. In fact, we screened many compounds to look for one that inhibited osteoblasts and stimulated osteoblasts *in vitro* and identified one that stimulated alkaline phosphatase. It's interesting the way that small changes in the molecular structure of these compounds can have a significant affect on their efficacy on cells. So structure-function relationships are vital in this regard. There is reason to be hopeful that a Src tyrosine kinase inhibitor could inhibit bone resorption and potentially stimulate bone formation in osteoporotic patients.

**Dr. Vessella:** Do you have data comparing the effect of the Src compounds compared with zoledronic acid? Which one has a more pronounced effect?

**Dr. Boyce:** They had similar effects in inhibiting bone resorption *in vivo*. There was a significant reduction in tumor volume only with the Src inhibitor. However, when we measured tumor volume in all of the bone sites, the reduction did not reach significance in the zoledronic acid-treated mice. In other studies we've done using PC3 cells with zoledronic acid, given once a week for 4 weeks, 3 days after the tumor cells were injected into the tibia, the mice developed osteopetrosis, but there is clearly significant resorption of the bone. Once you have established metastases, zoledronic acid and other bisphosphonates are not able to fully stop aggressive resorption in this regimen.

**Dr. Roodman:** Do you have any feel for what is the contribution of blocking bone destruction by the osteoclast?

**Dr. Boyce:** In the first paper we published showing that bisphosphonates inhibit osteoclastic resorption and osteolytic lesions, there was some necrosis of tumors growing within those bones and there certainly was increased intraosseous pressure that could have contributed to that (Sasaki A, Boyce BF, Story B, et al. Bisphosphonate risedronate reduces metastatic human breast cancer burden in bone in nude mice. *Cancer Res* 1995;55:3551-3557). If the tumor cells can get out of the bone, then some of the tumors survive. We found that the bone could become necrotic as well. I haven't seen that with zoledronic acid or with the Src tyrosine kinase inhibitor. Generally, if bone resorption is stopped while the tumor cells are in the bone, the bone erosion is inhibited, but tumor cells can still fill the marrow cavity.

**Dr. Weilbaecher:** We have done experiments where we took the B16 melanoma line, a very aggressive line, and injected it into the Src knockout mice. There were tumor metastases but no tumor-associated bone destruction.

**Dr. Roodman:** If you get resorption, growth factors are released. The question is whether this release of growth factors is purely mechanical or if it's impacting on tumor growth. Osteopetrotic animal experiments won't help because you don't get resorption.

**Dr. Boyce:** I've not done those experiments in mice. I've seen enough metastases in humans in whom cortical bone gets remodeled. This cortical remodeling doesn't happen in mice, so having spaces for osteoclasts to resorb in cortical bone and not have the extensive necrosis that you can see in mice made osteopetrotic by osteoclast inhibitors may not be an issue. Metastases tend to grow at sites where there is red marrow, and they can then spread down the marrow cavity of long bones, for example, or burst out through the sides of vertebral bodies. The challenge in mice is because of failure of cortical bone to remodel. It may be easier to answer these questions in humans, but that's difficult.

## References


1. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64:693–702.
2. Boyce BF, Yoneda T, Lowe C, Soriano P, Mundy GR. Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice. J Clin Invest 1992;90:1622–7.
3. Iotsova V, Caamano J, Loy J, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-κB1 and NF-κB2. Nat Med 1997;3:1285–9.
4. Franzoso G, Carlson L, Xing L, et al. Requirement for NF-κB in osteoclast and B-cell development. Genes Dev 1997;11:3482–96.
5. Dougall WC, Glaccum M, Charrier K, et al. RANK is essential for osteoclast and lymph node development. Genes Dev 1999;13:2412–24.
6. Kong YY, Boyle WJ, Penninger JM. Osteoprotegerin ligand: a common link between osteoclastogenesis, lymph node formation, and lymphocyte development. Immunol Cell Biol 1999;77:188–93.
7. Li YP, Chen W, Liang Y, Li E, Stashenko P. Atp6i-deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nat Genet 1999;23:447–51.
8. Kornak U, Kasper D, Bosl MR, et al. Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 2001;104:205–15.
9. Gowen M, Lazner F, Dodds R, et al. Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. J Bone Miner Res 1999;14:1654–63.
10. Hofbauer LC, Heufelder AE. Osteopetrosis in cathepsin K-deficient mice. Eur J Endocrinol 1999;140:376–7.
11. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337–42.
12. Cui L, Wu T, Liu YY, et al. Tanshinone prevents cancellous bone loss induced by ovariectomy in rats. Acta Pharmacol Sin 2004;25:678–84.
13. Yin J, Tezuka Y, Kouda K, et al. Antiosteoporotic activity of the water extract of *Dioscorea spongiosa*. Biol Pharm Bull 2004;27:583–6.
14. Picherit C, Coxam V, Bennetau-Pelissero C, et al. Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. J Nutr 2000;130:1675–81.
15. Weaver CM, Cheong JM. Soy isoflavones and bone health: the relationship is still unclear. J Nutr 2005;135:1243–7.
16. Insogna KL, Sahni M, Grey AB, et al. Colony-stimulating factor-1 induces cytoskeletal reorganization and c-src-dependent tyrosine phosphorylation of selected cellular proteins in rodent osteoclasts. J Clin Invest 1997;100:2476–85.
17. Wong BR, Besser D, Kim N, et al. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. Mol Cell 1999;4:1041–9.
18. Tanaka S, Amling M, Neff L, et al. c-Cbl is downstream of c-Src in a signaling pathway necessary for bone resorption. Nature 1996;383:528–31.
19. Sanjay A, Houghton A, Neff N, et al. Cbl associates with Pyk2 and Src to regulate Src kinase activity, α(v)β(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. J Cell Biol 2001;152:181–95.
20. Trubert CL, Bernard F, Hivroz C, et al. PP60c-src expression in osteoclasts from osteopetrotic children and in giant tumor cells. Eur J Histochem 1997;41:169–76.
21. Xing L, Venegas AM, Chen A, et al. Genetic evidence for a role for Src family kinases in TNF family receptor signaling and cell survival. Genes Dev 2001;15:241–53.
22. Glantschnig H, Fisher JE, Wesolowski G, Rodan GA, Reszka AA. M-CSF, TNFα, and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell Death Differ 2003;10:1165–77.
23. Sugatani T, Hruska KA. Akt1/Akt2 and mammalian target of rapamycin/Bim play critical roles in osteoclast differentiation and survival, respectively, whereas Akt is dispensable for cell survival in isolated osteoclast precursors. J Biol Chem 2005;280:3583–9.
24. Schwartzberg PL, Xing L, Hoffmann O, et al. Rescue of osteoclast function by transgenic expression of kinase-deficient Src in src−/− mutant mice. Genes Dev 1997;11:2835–44.
25. Boyce BF, Wright K, Reddy SV, et al. Targeting simian virus 40 T antigen to the osteoclast in transgenic mice causes osteoclast tumors and transformation and apoptosis of osteoclasts. Endocrine 1995;136:5751–9.
26. Miyazaki T, Sanjay A, Neff L, et al. Src kinase activity is essential for osteoclast function. J Biol Chem 2004;279:17660–6.
27. Schwartzberg PL. The many faces of Src: multiple functions of a prototypical tyrosine kinase. Oncogene 1998;17:1463–8.
28. Missbach M, Jeschke M, Feyen J, et al. A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption *in vitro* and in rodent models *in vivo*. Bone 1999;24:437–49.
29. Hanke JH, Gardner JP, Dow RL, et al. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor: study of Lck- and FynT-dependent T cell activation. J Biol Chem 1996;271:695–701.
30. Huron DR, Gorre ME, Kraker AJ, et al. A novel pyridopyrimidine inhibitor of abl kinase is a picomolar inhibitor of Bcr-abl-driven K562 cells and is effective against STI571-resistant Bcr-abl mutants. Clin Cancer Res 2003;9:1267–73.
31. Kraker AJ, Hartl BG, Amar AM, et al. Biochemical and cellular effects of c-Src kinase-selective pyrido[2,3-d]pyrimidine tyrosine kinase inhibitors. Biochem Pharmacol 2000;60:885–98.
32. Ple PA, Green TP, Hennequin LF, et al. Discovery of a new class of anilinoquinazoline inhibitors with high affinity and specificity for the tyrosine kinase domain of c-Src. J Med Chem 2004;47:871–87.
33. Boschelli DH. 4-anilino-3-quinolinecarbonitriles: an emerging class of kinase inhibitors. Curr Top Med Chem 2002;2:1051–63.
34. Blake RA, Broome MA, Liu X, et al. SU6656, a selective src family kinase inhibitor, used to probe growth factor signaling. Mol Cell Biol 2000;20:9018–27.
35. Wang Y, Metcalf CA III, Shakespeare WC, et al. Bone-targeted 2,6,9-trisubstituted purines: novel inhibitors of Src tyrosine kinase for the treatment of bone diseases. Bioorg Med Chem Lett 2003;13:3067–70.
36. Boyce BF, Shakespeare W, Xing L, et al. Development of a novel bone-targeted Src tyrosine kinase inhibitor AP23451 having potent *in vitro* and *in vivo* anti-resorptive properties. J Bone Miner Res 2002;17:S159.
37. Boyce BF, Xing L, W Shakespeare, et al. Regulation of bone remodeling and emerging breakthrough drugs for osteoporosis and osteolytic bone metastases. Kidney Int Suppl 2003;S2–5.
38. Yin JJ, Selander K, Chirgwin JM, et al. TGF-β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J Clin Invest 1999;103:197–206.
39. Eastell R, Hannon RA, Gallagher N, et al. The effect of AZD0530, a highly selective, orally available Src/Abl kinase inhibitor, on biomarkers of bone resorption in healthy males. J Clin Oncol 2005;23:16S.
40. Hannon RA, Clack G, Gallagher N, et al. The effect of AZD0530, a highly selective Src inhibitor, on bone turnover in healthy males. Bone 2005;36:S135.