

## **PERSPECTIVES**

### **New Agents for the Treatment of Osteoporosis**

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#### **Abstract**

Discoveries in bone biology are directing early efforts to find new drugs for the treatment of osteoporosis. For anti-resorptive therapy, much current attention is aimed at inhibiting osteoclast activity, in some cases even doing so without inhibiting bone formation as a coupled response. Human and mouse genetics have provided fascinating possibilities for new anabolic agents by identifying the activated Wnt signaling pathway as a key process in the regulation of osteoblast differentiation and bone formation. *BoneKEy-Osteovision*. 2007 November;4(11):287-298.

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The successful fracture prevention trial of alendronate showed, a little over ten years ago, the drug's efficacy in fracture prevention (1). This was a landmark event in bone research, resulting in a major investment in bone biology by big pharmaceutical companies. It has been followed by successful trials of other bisphosphonates and of selective estrogen receptor modulators (SERMs). In the process, osteoporosis has evolved from an orphan disease to one where treatments have at last changed the landscape, with prospects of further therapeutic advances to come as the huge investment in basic and clinical research in academia and industry has identified a variety of pathways and targets for intervention.

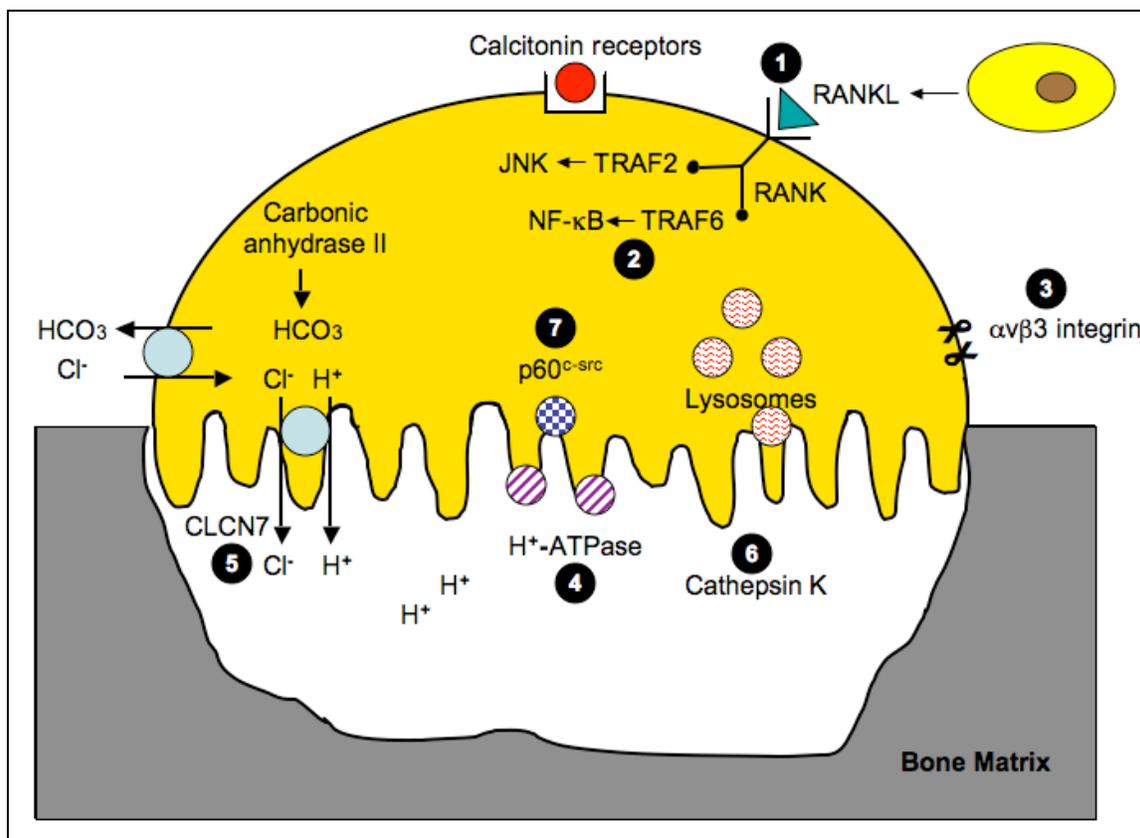
These advances have come from studies of osteoclast and osteoblast differentiation and function, including transcriptional controls, and most importantly from mouse and human genetics. Highlights in this period have been the discoveries of the detailed regulation of osteoclast formation and activity by the osteoblast lineage, through members of the TNF ligand and TNF receptor families (2), the signaling pathways used in these mechanisms (3), the actions of osteoclast enzymes in the resorption process (4), and the central role of certain

genes in bone formation, revealed by genetic analysis of certain rare syndromes with skeletal phenotypes (5-8).

The discussion in this *Perspective* stems from those discoveries, and will include some osteoporosis treatment options at the trial stage and some very early in preclinical evaluation. The pressure within universities and granting bodies to "apply" our research is so great now that it is uncommon to read a paper or grant application that does not identify ways in which "this research could lead to new treatments for osteoporosis." Although it is difficult to escape the view that this is usually more a hope than an expectation, nevertheless our considerations here are unlikely to be comprehensive enough to satisfy everyone. A number of currently studied mechanisms and candidate drugs will be considered.

#### **New Anti-resorptives in Early and Late Development**

Although there have been great advances in osteoporosis treatment with anti-resorptive drugs that achieve fracture risk reductions of 30-50%, their real and potential limitations are such that there is a continued search for new approaches. There is probably a limit to the fracture risk reduction that can be



**Fig. 1.** Some current anti-resorptive drug development targets (highlighted as 1 to 7) indicated in the osteoclast. Targeting RANKL or its signaling (1 and 2) inhibits osteoclast formation and activity. Drug approaches through any of pathways 4 to 7 might inhibit bone resorption without an accompanying inhibition of bone formation.

achieved with drug therapy, but the aims will be to improve efficacy without long-term deleterious effects on the skeleton or other side effects, and to find drugs whose effects cease with cessation of therapy. We will also consider the possibility that is being canvassed at present, of developing resorption inhibitors that do not inhibit bone formation.

#### Neutralization of RANKL

The discovery a decade ago of osteoclast regulatory mechanisms has had a profound influence on therapeutic approaches. The common and essential factor mediating osteoclast formation in response to all known stimuli is nuclear factor  $\kappa$ B ligand (RANKL) (2) that binds to its receptor, RANK, on hemopoietic precursors to promote osteoclast differentiation as well as

osteoclast survival and activity. The decoy receptor, osteoprotegerin (OPG), is an essential paracrine regulator of osteoclast formation, produced by the osteoblasts and binding RANKL to prevent its promotion of osteoclast formation through its receptor, RANK. Thus, regulated production of RANKL and of its local "brake" mechanism, OPG, are essential for the maintenance of normal bone turnover.

These discoveries revealed a pathway that was obviously rich in targets for pharmaceutical development, many of which have been explored in the last few years in preclinical and some early clinical studies. For example, recombinant OPG was effective in preventing the bone loss resulting from a lack of estrogen, and the increased resorption associated with bone metastases, humoral hypercalcemia of

cancer, and adjuvant-induced arthritis (9;10). The formation of significant antibody titers in a patient given OPG brought that development to an end, replacing it with a fully human anti-RANKL. This point of targeting is indicated in Figure 1. Other routes to anti-resorptive drug development in this pathway that continue to be explored include small molecule compounds that inhibit RANK signaling (Fig. 1), or that promote production of OPG (11).

Denosumab (Amgen) is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL to inhibit its action. In a phase II study, subcutaneous injections of denosumab to postmenopausal women with low bone density every three or six months for 12 months significantly decreased bone resorption and increased BMD at the lumbar spine, hip and distal third of radius (12). Bone resorption marker indices decreased rapidly following denosumab injection, as did markers of bone formation, reflecting the coupling of formation to resorption. Extension of the phase II study to 48 months, reported in abstract form (13), showed a BMD increase of 13.4% with continuous denosumab over placebo treatment. Any discontinuation of treatment was associated with a return of BMD to baseline, and BMD increased again upon rechallenging with treatment. Discontinuation also resulted in a rapid rise in resorption markers, indeed overshooting above control levels. This also corrected with resumed treatment.

This proof-of-concept that substantial bone resorption inhibition can be achieved by neutralizing RANKL reveals an exceptionally prolonged and powerful action, which is being investigated in a phase III study that uses subcutaneous injection of denosumab every 6 months. The striking suppression of bone turnover with denosumab is even more marked than with the most effective bisphosphonates. Further study of this drug may help us find out whether achieving an ever-greater inhibition of resorption will further improve fracture reduction and make for better bone.

### *Integrin antagonists*

The  $\alpha v \beta 3$  integrin receptor, which is produced in osteoclasts (Fig. 1) as well as in budding blood vessels and leukocytes, is a relatively selective osteoclast-specific structure. It plays a rate-limiting role in osteoclast activity. Treatment of rats with the disintegrins, echistatin or kistrin, which bind with high affinity to  $\alpha v \beta 3$ , inhibit bone resorption stimulated by PTH or estrogen deficiency. Moreover, small molecular weight compounds that mimic the tripeptide RGD sequence, recognized by the integrin, were shown to have similar effects (14). One such compound, MK0429 (Merck), which had proven efficacy in animal models of estrogen-deficiency bone loss, was used in a 12-month, randomized, double-blind, placebo-controlled study in 227 women with low BMD. Treatment was associated with significantly increased lumbar spine BMD and decreases in markers of bone resorption and formation (15). Recently, the same compound was shown to be effective in preventing the establishment of bone tumor deposits in nude rats undergoing intra-tibial engraftment of human breast cancer or melanoma cells (16). The validity of this target having been established, the next step requires commitment to development.

### *Cathepsin K inhibitors*

A commitment to drug development has indeed been made in the case of a cathepsin K inhibitor, with a phase II study completed and a phase III planned for odanacatib (Merck). Cathepsin K is selectively expressed in osteoclasts and is the predominant cysteine protease in these cells. It accumulates in lysosomal vesicles, and is localized at the ruffled border in actively resorbing osteoclasts, discharging into the acidified, sealed resorption space beneath the osteoclast when the lysosomal vesicles fuse with the cell membrane (Figure 1). Defects in the gene encoding cathepsin K are linked to pycnodysostosis, an autosomal recessive dysplasia characterized by skeletal defects including dense, brittle bones, short stature and poor bone remodeling (17). Similarly, the deletion

of the cathepsin K gene in mice results in osteopetrosis (18). The substantial body of evidence indicating that cathepsin K plays an important role in bone resorption makes it a target for inhibition of resorption.

Peptide inhibitors designed to inhibit cysteine proteases by binding at the substrate site to mimic a cathepsin K-substrate complex are in development (19; 20). A 12-month study with an orally bioavailable specific inhibitor of human cathepsin K, balicatib (compound AAE581, Novartis), in postmenopausal women showed an increase in lumbar spine and hip BMD associated with statistically significant decrease in markers of bone resorption but not in those of bone formation (21). Other potent cathepsin K inhibitors are currently under investigation by GlaxoSmithKline and Medivir AB (22). A study of odanacatib in the oophorectomized rabbit showed that resorption was inhibited without decreased bone formation (23). This potentially interesting property was not maintained in oophorectomized monkeys, where the drug inhibited bone loss without preserving bone formation (24). In a 12-month double-blind, placebo-controlled, dose-ranging study in postmenopausal women with low BMD, odanacatib treatment resulted in significantly increased BMD and decreased bone resorption markers (25), and a phase III study is planned.

*Can resorption inhibitors be developed that do not inhibit formation?*

This is an intriguing question that arises from the current interest in the mechanism of coupling between bone resorption and formation. With existing resorption inhibitors, e.g., bisphosphonates, estrogens and SERMs, the coupling of bone formation to resorption is illustrated by the decrease in bone formation that accompanies treatment. This appears very likely to be the case also with the anti-RANKL, denosumab. Will it be possible to develop drugs that inhibit bone resorption without inhibiting formation – in other words, uncoupling bone formation from resorption? The data to the present time with cathepsin K inhibition is conflicting

on this question, but there are other candidates.

Acidification of the resorption lacuna is essential for resorption, reducing the pH to about 4 in order to dissolve the bone mineral. Passive transport of chloride through chloride channels preserves electroneutrality in the course of the acidification process, and preventing chloride transport will lead to a rapid hyperpolarization of the membrane, preventing further secretion of protons, thus resulting in an inhibition of further bone resorption. A vacuolar H<sup>+</sup>ATPase in the osteoclast membrane plays a key role in this process by mediating the active transport of protons (Figure 1). Inhibitors of this enzyme, for example, bafilomycin, have been shown to inhibit osteoclastic bone resorption *in vitro* and *in vivo*. A relatively osteoclast-selective H<sup>+</sup>ATPase inhibitor has been shown to inhibit ovariectomy-induced bone loss in rats (26). Of particular interest is the fact that v-ATPase V0 subunit d2-deficient mice are osteopetrotic because impaired osteoclast fusion reduces the resorptive capacity of osteoclasts (27). The increased numbers of osteoclast precursors may be related to the increased bone formation in these mice.

In *Src(-/-)* mice (28), increased numbers of osteoclasts were present in bone, but they failed to resorb bone because they lacked the protein tyrosine kinase, Src, and could not form ruffled borders (29). In the case of chloride channel 7 (*CICN7*) (30;31), osteoclasts of *CICN7(-/-)* mice survive better than those of wild-type mice but do not resorb bone because acidification fails. Bone resorption is inhibited without any inhibition of the rate or extent of formation. In each of these mouse mutations, osteoclast numbers are maintained, but the osteoclasts are unable to resorb bone although they look healthy. This is the case also in human subjects with inactivating mutations either of *CICN7* (32) or the vacuolar H<sup>+</sup>ATPase (33).

One possibility is that osteoclasts are able to generate a factor (or factors) that can contribute to bone formation, despite the fact that they do not resorb bone (27;34;35).

Early data with an orally delivered CICN7 inhibitor showed that it inhibited bone loss in the ovariectomized rat without inhibiting bone formation (30). In a phase I study in healthy males, AZD0530, a highly selective, dual-specific orally available inhibitor of Src and Abl kinases, was shown to suppress bone resorption markers reversibly with variable changes in bone formation markers (36).

It is possible that such inhibitors of resorption, with candidates at the moment being CICN7, vacuolar H<sup>+</sup>ATPase, Src and cathepsin K (Figure 1), might conceivably provide a new class of resorption inhibitory drug that does not inhibit bone formation. If they are safe and at least as effective in fracture reduction as other inhibitors, they could provide a real advance. For example, they might more effectively be combined with anabolic therapy than those resorption inhibitors (e.g., bisphosphonates, and likely anti-RANKL agents) that lead to inhibition of bone formation.

### **Anabolics**

The establishment of intermittent PTH as the first therapy capable of putting new bone back where it has been lost has provoked a major interest in the development of anabolic treatments for the skeleton, with excitement in this area enhanced through the potential drug targets revealed from human and mouse genetics. At the same time, this potential presents real challenges. Thus far, the fracture reduction with anabolic treatment with PTH differs little from that with adequately studied resorption inhibitors (37;38). The fact is, though, that PTH and most likely any new anabolics are doing something more than anti-resorptives are, by building new bone rather than simply preventing further loss. How valuable is this? Can we devise ways of measuring this "benefit" and giving it a weighting for cost-effectiveness? If we are unable to do that, the various regulatory authorities will most likely continue to look at fracture data and say: "This (anabolic) drug reduces fractures by 50-60%; so too does this (anti-resorptive) drug. Tell us, what is the difference?"

In this *Perspective*, we will not consider PTH and its various possible forms of delivery, but will confine comments to early attempts to develop anabolic therapies through new pathways.

### *Possible drugs for bone in the Wnt signaling pathway*

The canonical Wnt signaling pathway is crucial for the specification of cell fates, and the regulation of cell growth, differentiation and apoptosis (39-41). The first link between Wnt signaling and human bone disease came from observations that inactivating mutations in the gene encoding low density lipoprotein receptor-related protein 5 (*LRP5*) cause the osteoporosis-pseudoglioma syndrome characterized by severely decreased bone mass (6). Conversely, a syndrome of high bone mass was found to be caused by a gain-of-function mutation of *LRP5* (7). These genetic syndromes were reproduced with the appropriate genetic manipulations in mice (42;43).

With these as a guide, the Wnt/ $\beta$ -catenin signaling pathway offers several targets that may be suitable for pharmacological intervention aimed at increasing osteoblast differentiation and bone formation. These include extracellular agonists and the points of interaction of antagonists, especially the secreted frizzled-related proteins, Dickkopf (DKK) proteins and sclerostin, as well as regulation within the cell of glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ), the enzyme that is crucial in determining the availability of  $\beta$ -catenin for the transcriptional effects that are the essential requirement of Wnt signaling (44-46, also see <http://www.stanford.edu/~rnusse/wntwindow.html>). The primary aim of these interventions is to increase Wnt/ $\beta$ -catenin canonical signaling in order to increase bone mass. Initial promise in animal models has been offered through the use of anti-DKK-1, GSK-3 $\beta$  inhibition and anti-sclerostin approaches. Discussion will be confined to these, although there are a number of other potential targets.

(a). *Inhibition of Dickkopf 1 (DKK-1) action*

LRP5 can form a ternary complex with DKK-1 and Kremen (a receptor for DKK), which triggers rapid internalization and depletion of LRP5, leading to inhibition of the canonical Wnt signaling pathway. Inhibition of interactions between DKK-1 and LRP5 would release LRP5 to activate the Wnt pathway. Genetic studies with mice lacking a single allele of *DKK-1* showed a marked increased trabecular bone volume and elevated trabecular bone formation rate (47), while transgenic over-expression of *DKK-1* under the control of *Col1A1* promoters caused severe osteopenia (48). The production of DKK-1 by multiple myeloma cells has been invoked as a contributing factor to the reduced bone formation in the lytic bone lesions of myeloma (49). A study using antibodies raised against DKK-1 in the treatment of a mouse model of multiple myeloma showed increased numbers of osteoblasts, a reduced number of osteoclasts, and reduced myeloma burden in the antibody-treated mice (50). Drug development attempts, initially, are with monoclonal anti-DKK-1 reagents in osteoporosis also, but the possibility of directing small molecules to prevent the DKK-1-LRP5 interaction is also being explored.

(b). *Inhibition of GSK-3 $\beta$*

Inhibition of GSK-3 would prevent the phosphorylation of  $\beta$ -catenin, leading to stabilization of  $\beta$ -catenin independently of Wnt interactions with the receptor complex. Mice treated with lithium chloride as a GSK-3 inhibitor showed increased bone formation and bone mass (51). Treatment of ovariectomized rats with an orally active dual GSK  $\alpha/\beta$  inhibitor, LY603281-31-8, for two months resulted in an increase in the number and connectivity of trabeculae as well as in trabecular area and thickness. Bone mineral density at cancellous and cortical sites was increased and this was associated with increased strength. The increased bone formation shown on histomorphometric analysis was associated with increased expression of mRNA markers of the osteoblast phenotype, such as bone

sialoprotein, type 1 collagen, osteocalcin, alkaline phosphate and Runx2 (52). When the same drug was compared with PTH in a cDNA microarray study, ovariectomy in 6-month-old rats resulted in decreased markers of osteogenesis and chondrogenesis, as well as increased adipogenesis, both of which were reversed by PTH and the GSK-3  $\alpha/\beta$  inhibitor (53).

These observations with GSK-3 inhibition are useful mainly as proof-of-concept that activation of the Wnt signaling pathway can be effective in promoting bone formation. This enzyme participates in so many cell functions that any plan to use it as a skeletal anabolic might require application of targeted therapy.

(c). *Inhibition of sclerostin*

Sclerostin, the protein product of *SOST*, is a circulating inhibitor of the Wnt-signaling pathway that acts by binding to LRP5 and LRP6 (54). High bone density in van Buchem disease is caused by an inactivating mutation in the *SOST* gene expressed in osteocytes (8;55). Inhibition of production or action of sclerostin resulting in enhanced Wnt canonical signaling would be predicted to lead to increased bone mass. Indeed, in preclinical studies reported in abstract form at the time of writing, monoclonal antibody against sclerostin has been shown to promote bone formation rapidly in monkeys and ovariectomized rats. Considerable increases in bone formation rates and in amounts of trabecular bone took place rapidly without increases in resorption parameters (56-58). In a single injection study in post-menopausal women, anti-sclerostin treatment was followed by significantly increased bone formation markers for more than 3 weeks and a trend to increased resorption marker, CTX (59).

Sclerostin is produced in bone virtually exclusively in osteocytes. Of particular interest is the fact that PTH rapidly reduces sclerostin mRNA and protein production by osteoblasts *in vitro* and in bone *in vivo* (60;61), raising the possibility that transient reduction of sclerostin output by osteocytes in response to intermittent PTH could

mediate enhanced osteoblast differentiation and bone formation (62) and reduced osteoblast apoptosis (63). Such a mechanism would offer real possibilities as a drug target, and the mechanism of this inhibition is all the more interesting with the recent finding (64) that the cyclic AMP-mediated effect of PTH to diminish sclerostin production operates through a long range enhancer, MEF2, the discovery of which came from the pursuit of the nature of the van Buchem disease mutation. There may be small molecule approaches amenable to sclerostin regulation, in addition to antibody neutralization of activity.

### Safety and Specificity

Any new therapy emerging from manipulation of the Wnt canonical signaling pathway will need to ensure, firstly, that it is safe, and secondly, that its action can be targeted specifically to bone. Wnt proteins are critical signaling proteins involved in developmental biology, with roles in early axis specification, brain patterning, intestinal development and limb development. In adults, Wnt proteins play a vital role in tissue maintenance, with aberrations in Wnt signaling leading to diseases such as adenomatous polyposis (45). Inhibition of GSK-3 results in increased cyclin D1, cyclin E, and c-Myc, and over-expression of these cell cycle regulators has been linked to tumor formation (65;66).

The need for caution in enhancing Wnt signaling arises also from recent evidence in mice that activation of this pathway is associated with aging, particularly with an age-dependent increase in muscle fibrogenesis at the expense of myogenesis (67;68). This may result from stem cell depletion, and provides an interesting contrast to evidence showing that Wnt signaling does the reverse during development – when it promotes myogenic lineage development (69).

### Summary

A selection of drug targets at various stages of development has been considered. This includes anti-resorptive and anabolic drug

candidates that have either reached a stage of commitment to clinical translation, or are likely to do so. Interest remains in the possibilities that might lie with new anti-resorptive drugs, perhaps especially if discovery reveals agents that are effective without inhibiting bone formation. The main focus of new developments in anabolic therapies is on the Wnt signaling pathway, the result of new insights provided by mouse and human genetics.

**Conflict of Interest:** The authors report that no conflicts of interest exist.

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