Update on Bone Anabolics in Osteoporosis Treatment: Rationale, Current Status, and Perspectives

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Osteoporosis is defined as low bone mineral density associated with skeletal fractures secondary to minimal or no trauma, most often involving the spine, the hip, and the forearm. The decrease in bone mineral density is the consequence of an unbalanced bone remodeling process, with higher bone resorption than bone formation. Osteoporosis affects predominantly postmenopausal women, but also older men. This chronic disease represents a considerable medical and socioeconomic burden for modern societies. The therapeutic options for the treatment of osteoporosis have so far comprised mostly antiresorptive drugs, in particular bisphosphonates and more recently denosumab, but also calcitonin and, for women, estrogens or selective estrogen receptor modulators. These drugs have limitations, however, in particular the fact that they lead to a low turnover state where bone formation decreases with the decrease in bone-remodeling activity. In this review, we discuss the alternative class of osteoporosis drugs, *i.e.* bone anabolics, their biology, and the perspectives they offer for our therapeutic armamentarium. We focus on the two main osteoanabolic pathways identified as of today: PTH, the only anabolic drug currently on the market; and activation of canonical Wnt signaling through inhibition of the endogenous inhibitors sclerostin and dickkopf1. Each approach is based on a different molecular mechanism, but most recent evidence suggests that these two pathways may actually converge, at least in part. Whereas recombinant human PTH treatment is being revisited with different formulations and attempts to regulate endogenous PTH secretion via the calcium-sensing receptor, antibodies to sclerostin and dickkopf1 are currently in clinical trials and may prove to be even more efficient at increasing bone mass, possibly independent of bone turnover. Each of these anabolic approaches has its own limitations and safety issues, but the prospects of effective anabolic therapy for osteoporosis are indeed bright. (***J Clin Endocrinol Metab* 97: 311–325, 2012**)

Osteoporosis is the result of a dysfunction of endocrine and/or autocrine/paracrine factors and/or their target cells in bone, leading to the inability to reach a proper peak bone mass and/or to maintain skeletal homeostasis. These alterations, together with genetic determinants and mechanical and nutritional cues, cause a decrease in bone density, alterations in bone microarchitecture, and ultimately fractures. Osteoporosis is predominantly a disease of aging, affecting particularly postmenopausal women but also older men. The coordinated actions of bone cells that become disturbed in osteoporosis occur according to two main biological principles, bone modeling and remodeling.

Bone Modeling and Remodeling

The development and maintenance of mammalian bones depends on the coordinated actions of matrix-resorbing

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2011-2332 Received August 18, 2011. Accepted October 14, 2011.
First Published Online January 11, 2012

Abbreviations: ActR, Activin receptor; BFR, bone formation rate; BMD, bone mineral density; BMU, basic multicellular unit; CaSR, calcium-sensing receptor; Dkk1, Dickkopf homolog 1; GSK3β, glycogen synthase kinase 3β; HBM, high bone mass; Lrp, low-density lipoprotein receptor-related protein; MAR, mineral apposition rate; OPG, osteoprotegerin; PLC, phospholipase C; P1NP, procollagen type 1 amino-terminal propeptide; PTH1R, PTH receptor; RANKL, receptor activator of nuclear factor-κB ligand; rhPTH, recombinant human PTH; sFrp, secreted frizzled-related protein; Wnt, wingless-int.

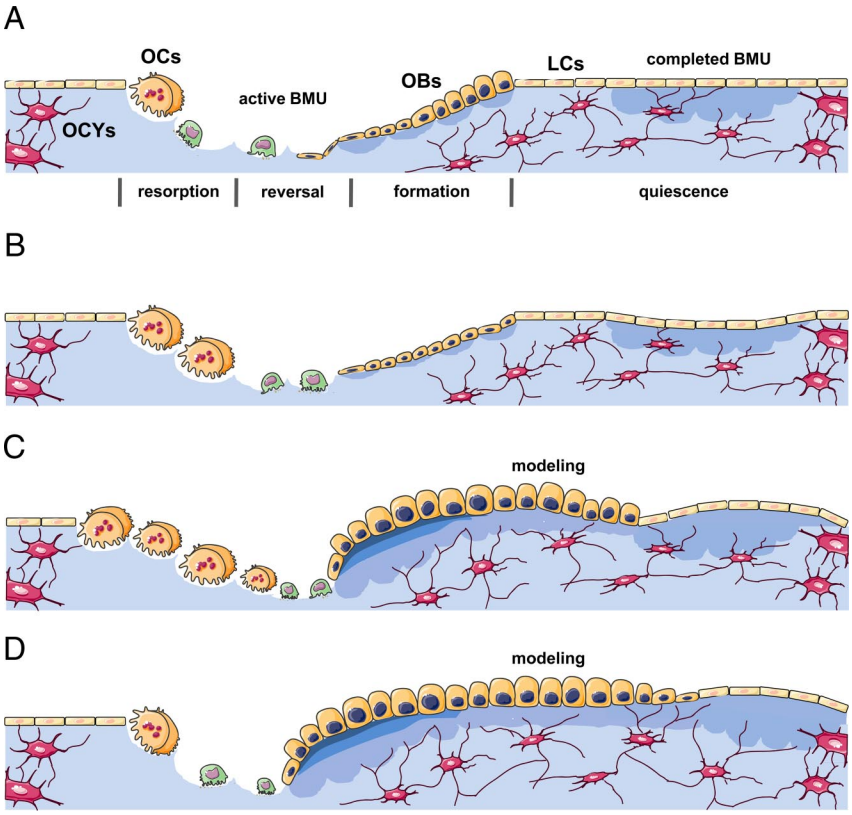

FIG. 1. Schematic of the remodeling and modeling activities under physiological conditions, in osteoporosis, and during anabolic treatment. A, Within an active BMU under physiological conditions, bone is constantly removed by osteoclasts (OCs) during the resorption phase of the remodeling cycle. After the reversal phase, new bone matrix is produced by osteoblasts (OBs) during the formation phase at sites where bone resorption has occurred with the amount of bone formed being equal of the amount of bone resorbed, thereby maintaining bone mass. Once the BMU is completed, osteoblasts become entrapped as osteocytes (OCYs) into the newly formed matrix, remain on the bone surface as lining cells (LCs), or undergo apoptosis. Bone then remains in the quiescence phase until a new BMU is initiated. B, In osteoporosis, bone resorption is increased and bone formation is decreased, resulting in a loss of bone. C, Administration of recombinant human PTH (rhPTH) stimulates both osteoclast-mediated bone resorption and osteoblast-mediated bone formation, resulting in a high bone turnover with a net gain in bone mass. In addition to its remodeling-based bone anabolic effect, rhPTH also induces bone formation on surfaces around the resorption sites that were not previously subject to bone resorption (modeling). D, Activation of the canonical Wnt signaling pathway tends to decrease bone resorption but mostly increases both remodeling-based and modeling-based bone formation, thereby causing a striking increase in bone formation, particularly in areas that were not previously resorbed (modeling).

hematopoietic lineage-derived osteoclasts and matrix-producing mesenchyme-derived osteoblasts. During bone modeling, the process that shapes skeletal elements at developmental stages but also at a low pace throughout life, bone resorption and formation occur in an uncoupled manner and on separate surfaces. In contrast, bone remodeling, the mechanism that ensures tissue turnover while maintaining bone mass in the adult, is based on the coupled and balanced activities of bone resorption and formation within each basic multicellular unit (BMU). BMUs are constituted of cells of both lineages, which are active at specific times during the remodeling cycle. These packages of cells are located along the bone surface, mostly at the interface with the hematopoietic bone marrow (endosteum) but also at the surface of bones (periosteum). BMUs are initiated through the activation of bone resorption, which is followed by bone formation. Within each BMU, activities are "coupled" if bone formation follows bone resorption, and activities are "balanced" if the amount of bone formed by osteoblasts equals and compensates for the amount of bone that was previously resorbed by osteoclasts (Fig. 1A). Stimulating bone remodeling increases bone turnover through an increase in the number of BMUs per bone surface area, also called activation frequency (1). In osteoporosis, within a BMU both coupled but unbalanced or uncoupled bone remodeling can cause severe alterations in bone mass, which will increase in severity proportionally to the activation frequency, *i.e.* with the turnover rate (Fig. 1B).

During a remodeling cycle, preosteoclasts are activated, migrate, and fuse to mature osteoclasts at sites where bone matrix needs to be replaced due to diminished matrix quality, cell viability/metabolism, or microfractures. At the end of the resorption phase (approximately 1–2 wk in humans), osteoclasts recruit and are replaced by osteoblasts through active cross talk between these two cell lineages, and bone formation begins. During the bone formation phase (approximately 2–3 months in humans), osteoblasts lay down bone matrix, which then mineralizes. The rate at which this occurs is the mineral apposition rate (MAR), which reflects the activity of individual osteoblasts. The bone formation rate (BFR) is the MAR multiplied by the surfaces undergoing bone formation. Both are true measures of the bone-forming activity in an individual (1). At the end of the bone formation phase, osteoblasts become quiescent as bone-lining cells on the surface of the newly formed bone, die by apoptosis, or become included within the matrix as osteocytes (Fig. 1A). Osteocytes are not merely "old" osteoblasts but have emerged as key cells that contribute to the regulation of calcium (Ca^{2+}) and phosphorus metabolism through the control of bone remodeling and Ca^{2+} fluxes and the secretion of fibroblast growth factor 23, respectively. Osteocytes also secrete

sclerostin, a protein that inhibits bone formation, and sense compromised bone matrix, thereby stimulating osteoclast recruitment and the generation of a new remodeling cycle. Furthermore, two recent studies demonstrate that osteocytes are an important source of receptor activator of NF-κB ligand (RANKL). RANKL binds to the RANK receptor on osteoclast precursors and mature osteoclasts and stimulates osteoclastogenesis and bone resorption (101, 102). Thus, osteocytes regulate bone resorption and formation in the context of both bone modeling and remodeling (2).

Osteoporosis

Osteoporosis is a systemic skeletal disease characterized by an unbalanced and/or uncoupled bone-remodeling activity leading to bone loss (Fig. 1B), microarchitectural deterioration of bone, and ultimately fractures at typical sites such as the lumbar spine, the femoral neck, and the distal radius. These fractures are often associated with an increase in morbidity and mortality. Because of its widespread nature, with a 50% fracture risk in all women after the age of 50 yr and a 25% risk in men, osteoporosis is a global public health concern and a great socioeconomic burden (3).

The goal of any osteoporosis therapy is the prevention of both vertebral (mostly dependent on trabecular bone density and architecture) and nonvertebral (mostly dependent on cortical thickness and porosity) fractures, which in principle can be achieved by inhibiting bone resorption and/or by stimulating bone formation. Yet, the dependence of trabecular and cortical bone on remodeling or modeling activity is different, with cortical bone being more susceptible to modeling activity, particularly along its periosteal surface. This difference may in part be responsible for the relative lack of efficacy of antiresorptive drugs on nonvertebral fractures because their effects are restricted to remodeling-based activities. Current antiresorptive drugs decrease the activation frequency, thereby causing a secondary decrease in BFR. This culminates in a low bone turnover, which in turn limits further increases in trabecular bone mass. In addition, questions have been raised about the association of long-term treatment in osteoporosis and high-dose use of these agents in oncology and clinical complications such as osteonecrosis of the jaw and so-called "atypical" subtrochanteric fractures (4).

Anabolic therapies are dependent on increasing the activation frequency and favoring bone formation within the BMU, on directly stimulating bone formation through activation of bone modeling, or on a combination of both (Fig. 1, C and D). Thus, true bone anabolics are defined by their ability to increase bone formation, as measured by biochemical markers procollagen type 1 amino-terminal propeptide (P1NP) and bone-specific alkaline phosphatase, and histomorphometric parameters (MAR and BFR) on bone biopsies.

The two main bone anabolic pathways are PTH signaling and canonical wingless-int (Wnt) signaling. Of the two, the canonical Wnt pathway might be more dependent on increasing bone modeling, potentially increasing bone mass in patients independent of bone resorption and activation frequency/bone turnover. In contrast, PTH anabolic function is more dependent on increasing the activation frequency, which may in part limit its therapeutic window (see below).

Given the limitations of current antiosteoporosis drugs, a search for new therapeutics has focused in the last few years on also identifying novel antiresorptives that prevent the decrease in activation frequency and bone formation and on bone anabolics that increase bone formation directly without affecting bone resorption. In this review, we will focus on bone anabolics and discuss their mode of action, limitations, and promises for the near future.

Although other biological agents, such as bone morphogenetic proteins (5) or IGF (6), are theoretically capable of increasing bone formation, either locally or systemically, practical limitations of their use and/or systemic effects outside of the skeleton have so far prevented their development in osteoporosis. In this review, we will focus only on the approaches that are currently in the clinic or in clinical trials.

Intermittent PTH: the Current Anabolic Option

PTH is secreted by the parathyroid glands in response to a reduced serum Ca^{2+} concentration and normalizes the Ca^{2+} levels by enhancing Ca^{2+} uptake in the intestine, Ca^{2+} re-absorption in the kidney, and by stimulating the osteoclast- and osteocyte-mediated Ca^{2+} release from bone. Mechanistically, PTH binds to the PTH receptor (PTH1R), a class II G protein-coupled receptor that activates several signaling pathways, including the Gsα-linked cAMP-dependent protein kinase A signaling pathway and the Gq/11-linked phosphatidyl inositol-specific phospholipase C (PLC)-protein kinase C signaling pathway. *In vivo* studies have been conducted to determine the specific role of these distinct PTH1R signaling pathways in bone. For instance, Guo *et al.* (7) reported a mouse that expresses a mutant PTH1R (DSEL), which stimulates adenylyl cyclase but is unable to activate PLC signaling. At 10 wk of age, DSEL-mutant mice demonstrate a low trabecular

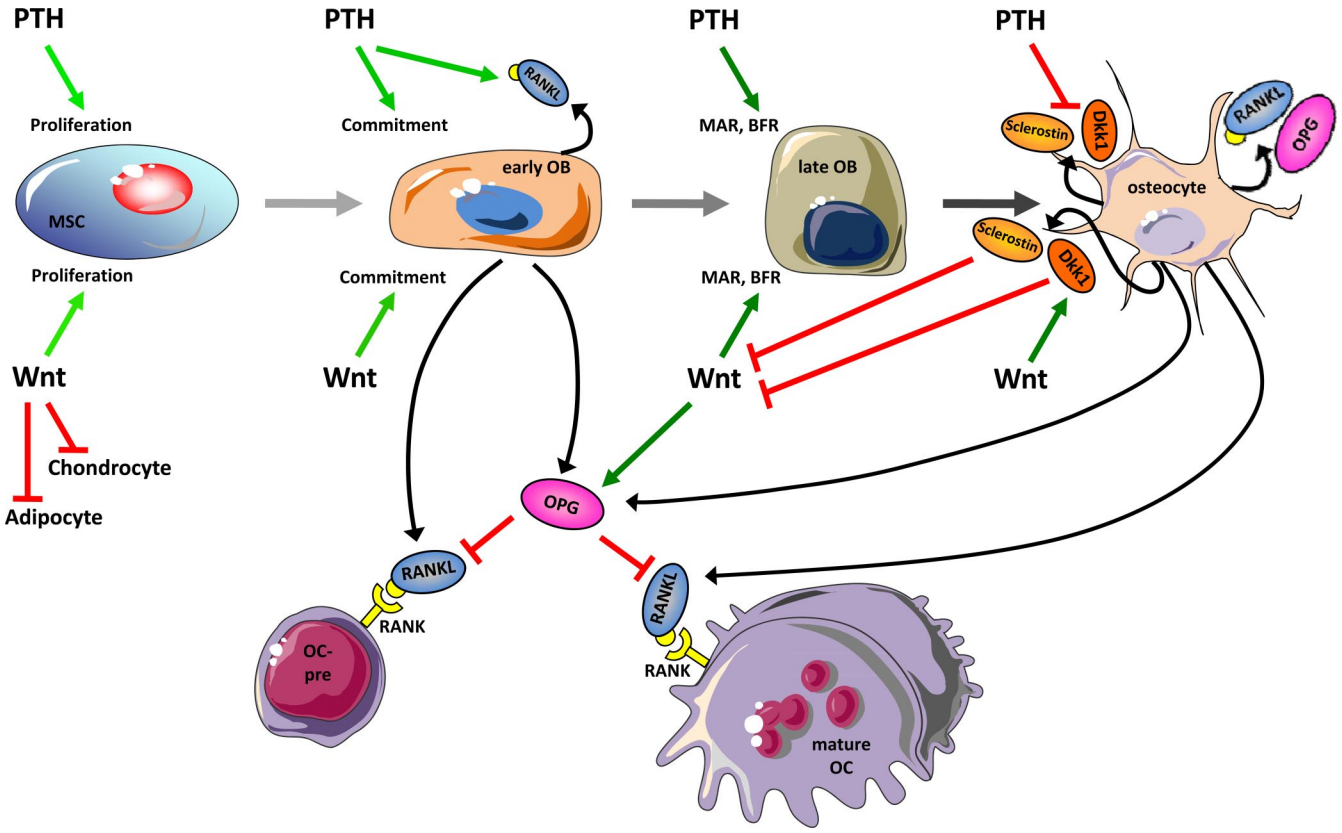

FIG. 2. Effects of the two main anabolic pathways, PTH and Wnt signaling, on osteoblasts, and indirectly on osteoclasts. PTH and Wnt both stimulate the proliferation of mesenchymal stem cells (MSCs) and the commitment of these cells into the osteoblast (OB) lineage, whereas the differentiation into chondrocytes and adipocytes is prevented by canonical Wnt signaling. In the late OB, both pathways increase the mineral apposition and bone formations rate (MAR, BFR). In addition, the Wnt pathway stimulates the production of osteoprotegerin (OPG), a soluble decoy receptor for the RANKL, preventing osteoclast (OC) differentiation and function. In contrast, PTH stimulates the secretion of RANKL, which binds to its receptor RANK on OC precursor cells (OC-pre) and mature OCs, thereby stimulating OC differentiation, function, and ultimately bone resorption. Wnt activity is inhibited by sclerostin and Dkk1, both secreted by late OBs and osteocytes. PTH represses the expression of both sclerostin and Dkk1, whereas Dkk1 expression is increased by Wnt activity, establishing a negative feedback loop. Thus, PTH and Wnt signaling pathways increase bone formation through several mechanisms, but only the Wnt pathway represses bone resorption, whereas PTH stimulates OCs via the induction of RANKL production by OBs.

bone mass, showing the importance of the PTH1R-mediated activation of the PLC signaling pathway for normal bone homeostasis (7). As revealed recently, binding of PTH to the PTH1R also activates the canonical Wnt-signaling pathway, which is discussed below in greater detail. In addition, PTH increases the commitment of mesenchymal precursor cells to the osteoblast lineage, promotes osteoblast maturation, and inhibits osteoblast apoptosis, thereby increasing osteoblast number and function (Fig. 2) (8).

Analysis of biopsies from patients with primary hyperparathyroidism called the attention of the field to the effects of PTH on bone remodeling. Bone exposed to sustained high levels of PTH show a marked increase in activation frequency and bone resorption but also in osteoblast numbers and BFR. Although trabecular bone density is often unchanged or even slightly increased, the enhanced bone resorption leads to an increased cortical porosity. Osteoblasts, but not osteoclasts, were found to express the PTH1R and to respond with an increase not only in proliferation and differentiation, but also in the secretion of RANKL. RANKL binds to the RANK receptor on osteoclast precursors and mature osteoclasts and stimulates osteoclastogenesis and bone resorption. This led to the conclusion that the mechanism of action is primarily an increase in bone formation and only secondarily, through the cross talk between osteoblasts and osteoclasts, an increase in bone resorption. Animal studies then demonstrated that short exposures to recombinant human PTH (rhPTH), as opposed to sustained increases, could dissociate the positive bone anabolic response from the negative bone catabolic response, and this led to the development and marketing of rhPTH.

To date, injectable forms of rhPTH are the only approved osteoanabolic drugs on the market for the treatment of osteoporosis. Although the intact hormone rhPTH 1–84 (Preotact) is approved only by European regulatory agencies, the bioactive N-terminal 34-amino acid fragment rhPTH 1–34 (teriparatide, Forsteo, Forteo) is available in the United States and Europe. Upon sc injection, both forms rapidly reach peak concentrations and

are degraded in about 1 h. rhPTH reduces the fracture risk of the spine much greater than that of nonvertebral bones, perhaps owing to site-specific differences, with cortical bone being less positively affected than trabecular bone (9). Indeed, rhPTH typically does not increase bone mineral density (BMD), which even often decreases, in the distal 1/3 of the forearm. This is due to an increase in cortical porosity secondary to enhanced endocortical remodeling (9, 10). In contrast, rhPTH increases bone formation along the periosteum, a primarily bone modeling surface, perhaps contributing to improving the trabecular and cortical architecture (11). Thus, in addition to the remodeling-based increase in bone formation, rhPTH also induces modeling-based bone formation, and this also occurs on surfaces adjacent to the BMU (Fig. 1C) (12). Clinical approval of teriparatide by the U.S. Food and Drug Administration was based on clinical trials including more than 2800 osteoporosis patients. In the pivotal clinical trial of Neer *et al.* (9), rhPTH (1–34) lowered the risk of vertebral fractures by 65% and that of nonvertebral fractures by 40% compared with placebo during the 19 months of treatment.

Several factors seem to limit the effectiveness of rhPTH. As mentioned above, in response to rhPTH, osteoblasts not only produce bone matrix but also secrete growth factors and cytokines including RANKL, thereby stimulating osteoclastogenesis. Thus, even if administered intermittently, chronic use of rhPTH increases bone formation in part through an increase in the activation frequency (remodeling-based anabolic), and this ultimately leads also to an increase in bone resorption. Although the net effect is still a gain in cancellous bone mass at early time points, it appears that bone resorption slowly catches up with bone formation, leading to a plateauing of the net anabolic effect after 18–24 months (9). Another possible reason that limits the use of rhPTH therapy is the progressive decrease in responsiveness secondary to tachyphylaxis, or a depletion of the pool of mesenchymal osteoblast precursors, or both (13).

Thus, administration of an antiresorptive drug combined with rhPTH could further increase bone mass by blunting the rhPTH-activated bone resorption. Although clinical studies have generated inconsistent results, with Black *et al.* (14) and Finkelstein *et al.* (15) reporting blunting of the anabolic response to rhPTH in patients under alendronate treatment, a recent report demonstrates that administration of a single dose of zoledronic acid combined with daily sc injections of rhPTH increased hip and spine BMD greater and more rapidly than either drug alone, suggesting that this combination therapy could be beneficial for patients with a high fracture risk (16). Furthermore, sequential administration of antiresorptive drugs after rhPTH is already an established treatment protocol because bone resorption continues after cessation of the treatment, causing a 4% bone loss in the first year after rhPTH withdrawal (17).

Potential Concerns

Other factors that have limited the use of rhPTH are its cost and concerns about its potential link to osteosarcoma. Indeed, treatment of osteoporosis with rhPTH is limited to 24 months in the United States and 18 months in Europe due to the risk of cancer because treatment of rats with high doses of rhPTH 1–34 increased the prevalence of osteosarcoma (18). It should, however, be noted that at present no connection has been demonstrated between elevated PTH serum levels in the context of hyperparathyroidism or rhPTH treatment and the occurrence of osteosarcoma in humans (19).

Although rhPTH is usually well tolerated, a few adverse effects are observed in patients, including hypercalcemia, nausea, headache, dizziness, and leg cramps (9). Both forms of rhPTH have the same adverse effects, but rhPTH 1–84 has been reported to have a lower risk of hypercalcemia (17). Despite all efforts made with rhPTH, the limited effect on nonvertebral fractures, the costs, the inconvenient route of administration, the activation of bone resorption, and the loss of efficacy with time suggest that rhPTH, although the best anabolic option today, will ultimately only partially meet the medical needs. Reducing the impact of some of these limitations constitutes the basis for current attempts to develop small molecules affecting the secretion of endogenous PTH and to use different routes of rhPTH administration.

New Approaches to PTH

Novel formulations

Transdermal application of rhPTH

One attractive option for the alternative delivery of rhPTH is transdermal self-administration using coated microneedle patches (20). In a randomized study, 165 postmenopausal women at a mean age of 64 yr were treated daily with either 20 μg rhPTH 1–34 sc or with a patch (Zosano Pharma) for the transdermal application of 20, 30, or 40 μg rhPTH 1–34 or placebo control over a period of 6 months. Interestingly, whereas the transdermal application of 40 μg rhPTH 1–34 increased the BMD in the lumbar spine to the same extent as the sc administered rhPTH 1–34, the gain of total hip BMD was much greater (20), suggesting that the pharmacokinetics of this formulation may be more beneficial to cortical bone than the daily injections.

Oral and inhaled delivery of rhPTH

A phase I randomized, placebo-controlled study reported that PTH serum concentrations rose in a dose-dependent manner upon oral administration of rhPTH 1–34 (1, 2.5, 5, and 10 mg) formulated with 100 or 200 mg of the absorption enhancer 5-CNAC [N-(5-chlorosalicyloyl)-8-aminocaprylic acid] and similarly to sc administered rhPTH. The pulmonary route of delivery is another option currently being explored (21). A phase I clinical trial has recently been performed to compare this mode of administration to sc rhPTH (www.clinicaltrials.gov).

Using an alternative protein: PTHrP

PTHrP is highly homologous to PTH in its first 36 amino acids, binds to and activates PTH1R, and increases bone mass to an extent similar to rhPTH in rats and in humans, improving mechanical strength of the spine, femur, and tibia in rats (22). In a double-blind, placebo-controlled, randomized clinical pilot study, 16 women between the ages of 50 and 75 yr were tested for the bone anabolic effect of PTHrP. Half of the study subjects were given placebo, whereas the others received approximately 400 μg PTHrP 1–36 sc daily. No adverse effects were observed and the BMD in the lumbar spine increased by 4.7% in response to the treatment (23).

A more recent study aimed to define the therapeutic window and the dose-limiting toxicities of PTHrP and to determine whether PTHrP acts as a pure anabolic agent (24). The study included 41 healthy postmenopausal women between the ages of 45 and 75 yr that were given either placebo or increasing doses of PTHrP 1–36. As reported previously, PTHrP did not cause severe adverse effects, despite a mild hypercalcemia in some subjects that were given the maximal tolerable dose of 750 μg/d. However, this was thought to be an indirect effect due to activation of 1,25-dihydroxyvitamin D production with a resulting increase in intestinal Ca^{2+} absorption rather than a direct effect of PTHrP on the bone-resorbing osteoclasts. Unlike rhPTH, PTHrP appeared to act as a pure bone anabolic agent without concomitant activation of bone resorption because no changes in the bone resorption markers C-telopeptide of type I collagen and N-telopeptide of type I collagen were found, but the markers of bone formation osteocalcin and P1NP were increased (24). This suggests distinct mechanisms of action for PTHrP and PTH, possibly related to differences in the on-off kinetics of the ligands on their common receptor, affecting different aspects of its downstream signaling (25). Although the effect of PTHrP on BMD was not investigated in this small study, these and previous data suggest that PTHrP might be a promising pure anabolic agent for the treatment of osteoporosis.

Based on a successful phase II, showing that sc injection of the PTHrP analog BA058 for 1 yr increased BMD at critical sites such as the spine and hip faster and greater than rhPTH, and with a reduced risk of hypercalcemia, BA058 is entering phase III. In addition, a patch for the transdermal delivery of BA058 using a microstructured transdermal system microneedle technology is currently being developed. This product is currently in phase I (www.radiuspharm.com). Whether the chronic use of PTHrP will, like rhPTH, lead to the same efficacy and/or safety limitations remains to be determined.

Increasing Endogenous PTH Secretion with Calcilytics

The costs and modes of administration of large peptides such as rhPTH and PTHrP have motivated a search for alternative approaches to the manipulation of this anabolic pathway with small molecules. Because the search for small molecule agonist of the PTH1R has so far proven unsuccessful, targeting the CaSR offers a possible alternative.

Cells of the parathyroid glands synthesize and store PTH and express on their surface the G protein-coupled seven-transmembrane-spanning CaSR, which inhibits PTH secretion when Ca^{2+} is bound. A low concentration of Ca^{2+} in the blood decreases the activity of the CaSR, thereby stimulating parathyroid glands to release PTH into the blood stream. The resulting rise in Ca^{2+} activates the CaSR and terminates the secretion of PTH by the parathyroid gland cells (26). This system is amenable to pharmacological manipulation using allosteric modulators of the CaSR, *i.e.* calcimimetics to treat hypercalcemia and secondary hyperparathyroidism or calcilytics to induce PTH secretion (27). Calcilytics mimic the state of hypocalcemia and, if short-acting, provoke a transient burst of endogenous PTH independently of extracellular Ca^{2+}. In addition to a rapid onset of action, calcilytics should have a sufficiently high clearance and a low volume of distribution, allowing a timely clearance of the drug to mimic the pharmacokinetics of injected PTH and thereby reduce the risk of severe adverse effects such as hypercalcemia and the onset of bone resorption. If administered orally, calcilytics might be more convenient than rhPTH and therefore a possible alternative to the prevailing treatment (28).

Daily delivery of NPS 2143, the first calcilytic, to ovariectomized rats for 5 wk caused a prolonged increase in PTH plasma concentration for at least 4 h due to its long half-life. This provoked a strong increase in bone turnover but no rise in BMD. Based on the concomitantly activated bone formation and bone resorption, a concurrent admin-

istration of the antiresorptive drug 17β-estradiol caused a net increase in bone mass by decreasing bone resorption (29, 30). Another study demonstrated the short-acting compound SB-423562 and its precursor SB-423557 to promote the transient release of endogenous PTH in rats, dogs, monkeys, and humans. Although human studies have been somewhat limited so far, a single dose of SB-423562 iv or of SB-423557 orally caused a rise of endogenous PTH with a kinetic profile similar to that of injected rhPTH (31). In ovariectomized rats, orally administered SB-423557 increased bone formation and improved bone strength. ATF936 is another calcilytic that efficiently triggered the rapid elevation of endogenous PTH in rats and dogs after a single oral dose (26). In aged rats, the daily oral administration of ATF936 for 8 wk increased BMD in the proximal tibia, including trabecular- and cortical thickness. In humans, ATF936 was also well tolerated and caused a transient increase in PTH levels similar to the profile seen after rhPTH administration (26).

The first randomized, placebo-controlled, multicenter, dose-ranging trial reporting the use of a calcilytic tested Ronacaleret, a molecule similar to NPS 2143 but with reduced off-target effects and half-life. The trial included 569 postmenopausal women with low BMD. Although the markers of bone turnover increased with both rhPTH and Ronacaleret and decreased in subjects treated with alendronate after 12 month, the Ronacaleret-induced rise in serum PTH was prolonged compared with that found in subjects treated with rhPTH. In turn, the gain in lumbar spine BMD after Ronacaleret treatment was significantly below the increase seen with rhPTH or alendronate. Moreover, total hip BMD was even slightly decreased after 12 months of treatment with Ronacaleret, compared with the increase seen with rhPTH or alendronate (32). These disappointing results have led to the interruption of the clinical development of this compound for osteoporosis treatment. JTT-305/MK-5442 is another calcilytic that increased lumbar BMD in a placebo-controlled trial. However, more studies are needed to determine its clinical usefulness (33).

Besides the need for an appropriate pharmacokinetic profile, additional challenges exist with the use of calcilytics. First, the therapeutic window between the desired effects on bone and the unwanted hypercalcemia is quite small. Second, off-target effects limit the usefulness of calcilytics because CaSR are also expressed in other organs besides the parathyroid glands, including kidney, brain, and endothelial cells. Third, the secretory vesicles of the parathyroid glands that are induced to release their content upon inactivation of the CaSR by calcilytics contain not only PTH but also other products, such as chromogranin, which may negatively affect PTH secretion (34) and have other adverse effects (35). Lastly, in addition to the indirect effects of calcilytics on bone homeostasis via the CaSR in the parathyroid gland, calcilytics and/or calcimimetics may also directly affect bone cells because CaSR are expressed by osteoblasts and osteoclasts and might regulate cell recruitment, differentiation, and survival (36). Thus, although the results of ongoing clinical trials are still pending, the use of calcilytics to activate the PTH pathway to induce bone anabolism has so far not been convincing.

Modulating the Canonical Wnt-Signaling Pathway

About a decade ago, the identification of human mutations in the low-density lipoprotein receptor-related protein 5 (Lrp5) linked to low bone mass (osteoporosis pseudoglioma syndrome) or high bone mass (HBM) (37–39) called the attention of the field to the Wnt pathway as a strong regulator of bone density and a potential alternative target to the PTH signaling pathway. At about the same time, the mutations causing HBM in sclerosteosis and van Buchem syndrome were identified and shown to affect the expression of another component of the Wnt signaling pathway, the Wnt antagonist sclerostin (40, 41). Interestingly, the single point HBM mutation initially identified in *lrp5* (G171V) was shown to decrease the affinity of Lrp5 for the inhibitors sclerostin (42) and dickkopf1 (Dkk1) (39). Thus, these rare human genetic mutations demonstrated that Wnt signaling is a dominant regulator of bone density in humans. This was then confirmed in mouse genetic studies and became a major focus in our field for the discovery of new bone anabolic therapeutics. More recently, large genome-wide association studies identified *lrp5* as one of the highly significant genes associated with BMD (43, 44), confirming in large populations the observations made in the rare genetic conditions described above, and validating the Wnt signaling pathway as a major regulator of bone mass.

Canonical Wnt activation occurs upon simultaneous binding of the secreted Wnt glycoproteins to one of the seven-helix-receptors of the frizzled family and the coreceptors Lrp5 or Lrp6. Although partially redundant and widely expressed, Lrp5 and Lrp6 are also expressed in cells of the osteoblast lineage (37, 45), and Lrp5 may be preferentially expressed and active in osteocytes (46, 47). In the absence of Wnt, intracellular Axin forms the destruction complex together with adenomatous polyposis coli, β-catenin, glycogen synthase kinase 3β (GSK3β), casein kinase 1a, and protein phosphatase 2A, leading to phosphorylation and subsequent degradation of β-catenin.

Binding of Wnt to Lrp5/6 causes a conformational change of the cytoplasmic receptor domain, followed by the recruitment of Axin2 and the disassembly of the destruction complex. This prevents the phosphorylation of β-catenin by GSK3β and the proteasomal degradation of β-catenin, which accumulates in the cytosol and translocates into the nucleus, stimulating the expression of Wnt target genes including several osteoblast marker genes and osteoprotegerin (OPG), an osteoblast-derived inhibitor of osteoclast differentiation (48–50), thus potentially activating bone formation and decreasing bone resorption at the same time (Figs. 2 and 3).

The greatest therapeutic opportunity offered by the Wnt signaling pathway is the fact that it is under negative control by endogenous secreted factors like sclerostin and proteins of the Dkk family. Both bind to Lrp5/6 and interfere with Wnt binding (Fig. 3). In addition, Dkk1 interacts with Lrp5/6 and the Kremen receptors and initiate internalization of the receptor ligand complex (48). Other endogenous antagonists such as soluble frizzled receptor

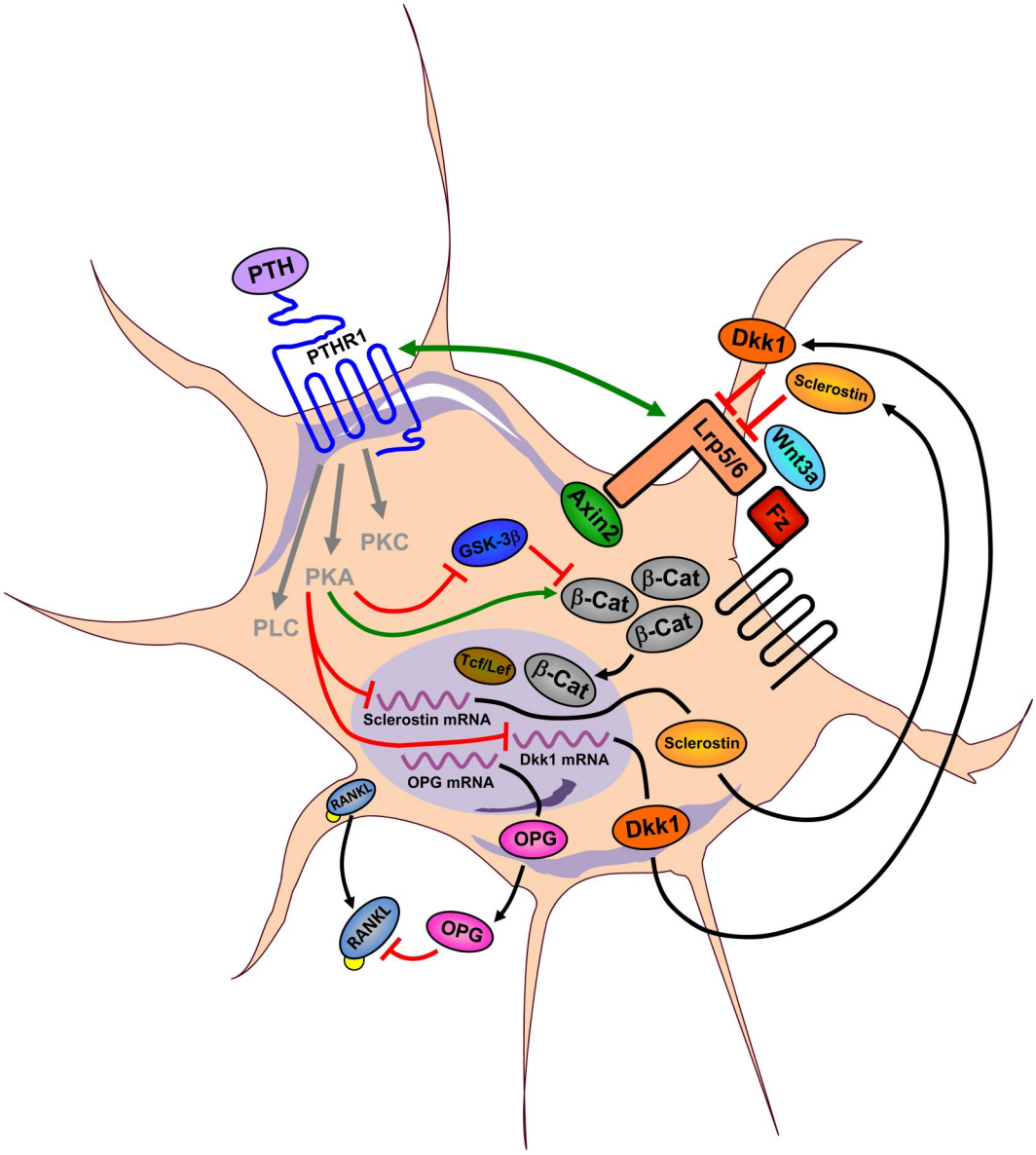

FIG. 3. Signaling and cross talk of the PTH and Wnt signaling pathways in osteocytes. In the osteocyte (and late osteoblasts), activation of the canonical Wnt signaling pathway occurs upon simultaneous binding of the secreted glycoprotein Wnt3a (or other Wnts like Wnt 10b for instance) to the seven-helix-receptor frizzled (Fz) family and the coreceptors Lrp 5/6. Binding of Wnt3a to Lrp5/6 changes the conformation of the cytoplasmic receptor domain, causing the recruitment of Axin2 and preventing the phosphorylation of β-catenin by GSK3β and its proteasomal degradation. β-Catenin accumulates in the cytosol and translocates into the nucleus, thereby stimulating the expression of the Lrp5/6 antagonists Dkk1 and sclerostin, and the RANKL inhibitor OPG, via the T-cell factor/lymphoid enhancer factor (Tcf/Lef). PTH binds to its seven-transmembrane-spanning receptor and activates phosphatidyl inositol-specific phospholipase C (PLC), cAMP-dependent protein kinase A (PKA), and the protein kinase C (PKC) downstream signaling cascades, all contributing to the bone anabolic effect of PTH. In addition, PTH signaling cross talks with the Wnt signaling pathway by associating with Lrp6, inhibiting GSK3β, stabilizing β-catenin, and inhibiting the expression of both sclerostin and Dkk1.

proteins (sFrp) or Wnt inhibitory factors bind the Wnt ligands, thereby decreasing not only canonical Wnt signaling, like sclerostin and Dkk1, but also its noncanonical signaling effects, the role of which is still poorly defined in bone.

Inhibiting the endogenous inhibitors

Although attractive, targeting Lrp5 or frizzled-receptors with small agonists or targeting intracellular components of the canonical Wnt pathway with small molecules could be challenging for bone anabolic therapies. Besides the molecular challenges, the lack of bone specificity has somewhat limited the enthusiasm for these approaches. In contrast, and possibly explaining the lack of extraskeletal side effects in patients with aberrant sclerostin expression, which has been shown to be exquisitely, albeit not entirely, restricted to late osteoblasts and osteocytes (51), pointing to its suitability as a therapeutic target of choice. Furthermore, any agent that would antagonize sclerostin would be active not only almost exclusively in bone but also only in areas of the skeleton where this inhibitor of bone formation is produced, possibly targeting therapy to specific areas of bone.

Sclerostin antibodies

Mouse genetics demonstrated that targeted deletion of sclerostin leads to HBM, due to a massive increase in BFR affecting not only trabecular but also cortical bone (52). In female rats, antibody-based sclerostin inhibition not only increased bone mass and strength in healthy animals but also reversed ovariectomy-induced bone loss (53). Furthermore, injection of sclerostin antibodies in aged male rats caused an increase in bone formation, bone mass, and strength of the long bones and the lumbar spine (54). In a model of hindlimb immobilization, antibody-mediated blockade of sclerostin in adult female rats resulted in a rapid increase in cortical and trabecular bone mass in both ambulated and immobilized bones. This effect was dominated by high bone formation and a decrease in bone resorption, suggesting that inhibition of sclerostin might be useful for the treatment of immobilization-induced osteopenia (55). In a model of bone healing, sclerostin-neutralizing antibodies increased the amount of bone and mechanical strength (56). Furthermore, sclerostin antibodies significantly improved the healing of fractures in rats and of osteotomies in monkeys accompanied with improvements in bone formation, bone mass, and bone strength at nonfractured cortical and trabecular sites in both models (57). Consistent with these data, injection of humanized sclerostin-neutralizing antibodies once a month for 2 months in gonad-intact female monkeys had a marked dose-dependent effect on bone formation (51). This increase was predominantly due to direct stimulation of bone formation along resting surfaces (modeling effect) with no increase in bone resorption but a stimulation of remodeling-based bone formation (58). Similar findings were made in sclerostin knockout mice and in ovariectomized rats treated with sclerostin antibodies (53, 54). Thus, the use of antagonists to endogenous inhibitors of the Wnt pathway seems to stimulate bone formation directly, through bone modeling, *i.e.* at least in part independent of bone remodeling and activation frequency and therefore without prior activation of bone resorption. This mode of action may open interesting therapeutic applications not only in osteoporosis but also in bone repair and in low turnover conditions where increasing bone mass is desired.

Recently, Padhi *et al.* (59) reported the first human phase I randomized, double-blind, placebo-controlled clinical trial testing ascending single doses of AMG 785, a humanized monoclonal sclerostin antibody, in healthy men and postmenopausal women. Bone formation markers increased within 1 month after a single sc dose of 10 mg/kg AMG 785 to levels similar to daily injections of rhPTH for 6 months, and markers of bone resorption decreased. Although this antiresorptive effect was expected due to the known Wnt-mediated increase in OPG expression in mice and due to the preclinical data, it remained a surprising finding in humans, particularly in its amplitude. Likewise, the gain in BMD at the lumbar spine and total hip was comparable or even greater than with rhPTH (59).

More recently Amgen/UCB reported in a press release (www.amgen.com) some of the results from the phase II study comparing the sclerostin-antibody AMG 785/CDP7851 to placebo in approximately 400 postmenopausal women with low BMD for the treatment of postmenopausal osteoporosis. AMG 785/CDP7851 was given at 70, 140, and 210 mg sc once a month and at 140 and 210 mg every 3 months. At 12 months, AMG 785/CDP7851 significantly increased BMD in the lumbar spine compared with placebo and teriparatide. Injection site reactions were the most frequently reported adverse events. These studies point to the promising future of sclerostin antibodies for the treatment of low bone mass diseases.

Dkk1 antagonists

Dkk1 is also an endogenous inhibitor of Wnt signaling, and the HBM mutations in Lrp5 negatively affect not only sclerostin but also Dkk1 affinity (39). Confirmation that Dkk1 plays a critical role in the regulation of bone mass was provided using mice in which Dkk1 was overexpressed or deleted, displaying severe loss (60) or gain (61) of bone mass, respectively. Ovariectomy-induced loss of bone and of mechanical strength were abrogated by Dkk1

antisense oligonucleotides (62), and inhibiting Dkk1 expression retards glucocorticoid-induced osteopenia (63). Antibody-mediated Dkk1 neutralization also protected against inflammatory bone loss in mice overexpressing TNF by preventing TNF-mediated impaired bone formation, osteocyte death, and enhanced osteoclast activity (64). In addition, Dkk1 inhibition reversed rheumatoid arthritis-associated bone destruction into a bone-forming osteoarthritis, with the formation of osteophytes, attributed to an enhanced bone formation when Wnt signaling is stimulated locally (65). In multiple myeloma, Dkk1 serum level correlated with focal bone lesions, and its inhibition increased osteoblast number and cancellous bone mass (66).

Based on this information, and although sclerostin antibodies are probably the preferred and most advanced therapeutic option for osteoporosis, antibodies to Dkk1 are also being developed, in particular for the treatment of multiple myeloma. If proven safe and efficacious, these antibodies could also find their way to a more general indication in low bone mass diseases, although the possibility that Dkk1 is less restricted to the bone microenvironment than sclerostin may raise more concerns about off-target effects.

Other potential approaches to enhance Wnt signaling

Neutralizing sFrps

Secreted frizzled-related proteins are a group of physiological antagonists of Wnt signaling. Effective inhibition of these Wnt antagonists causes a net activation of the Wnt pathway, both canonical and noncanonical, thereby potentially increasing bone mass in osteoporotic patients. This concept is supported by the presence of polymorphisms in the *Sfrp1* gene that are associated with BMD and bone mineral content (67). In addition, fracture repair was accelerated in adult mice germline-deleted of *Sfrp1* (68), and systemic overexpression of sFrp1 inhibited osteoblast function and the anabolic effect of rhPTH (69). Furthermore, mice overexpressing sFrp4 also exhibit a low BFR and bone mass phenotype (70). sFrps may therefore also be valid targets for enhancement of Wnt signaling, but the fact that antagonists would most likely activate both the canonical and noncanonical Wnt signaling pathways may lead to different clinical responses, both in terms of bone density and side effects.

Lithium and GSK3β inhibitors

Wnt activation by GSK3β inhibition could be useful for bone anabolic treatment, although the lack of skeletal specificity would be a challenge because it is expressed and functional in all cell types. Lithium is a nonspecific GSK3β inhibitor commonly used for the treatment of bipolar disease. Lithium activates Wnt signaling in a receptor-independent manner and increases bone mass in mice (71). Interestingly, in humans lithium decreased markers of bone formation and resorption but resulted in a net increase in bone density (72). Furthermore, lithium lowered the risk of fracture (73, 74), and the fracture risk increased after lithium withdrawal (75). It is not clear, however, whether this can be attributed to the direct effect of lithium on bone or to its effect on the mental disorder for which it was primarily given (75). Lithium also has a complex influence on Ca^{2+} homeostasis and exerts nonskeletal adverse effects. Thus, the potential of lithium as a bone anabolic agent may be limited, and it is not clear whether it is currently considered as a therapeutic option for osteoporosis treatment.

GSK3β inactivation can also be achieved using other, more specific small molecule inhibitors such as 603281-31-8, which increased bone formation markers, femoral bone mass, and bone strength in ovariectomized rats (76). Another study demonstrated the abrogation of glucocorticoid-induced bone loss by the GSK3β inhibitor 6-bromoindirubin-3′-oxim (77). Furthermore, the novel GSK3β inhibitor AR28 induced β-catenin nuclear translocation and increased bone mass after 2 wk, possibly due to an amplification of mesenchymal stem cells that became osteoblasts at the expense of adipocytes (78).

Potential concerns

Although activation of the Wnt signaling pathway is a very promising approach for the development of bone anabolic drugs, safety concerns exist, in particular regarding possible oncogenic effects and uncontrolled formation of bone leading to increased intracranial pressure, osteophyte formation, and/or closure of skeletal foramen, affecting hearing or vision for instance. The oncogenic concerns are based upon the fact that there is abundant literature linking aberrant activation of Wnt signaling to a variety of tumors, in particular colon cancer (79). Furthermore, recent studies have indicated that deletion of some of the endogenous inhibitors of Wnt signaling, such as Wnt inhibitory factor 1, can lead to the development of osteosarcoma (80). It is, however, reassuring that so far no increase in tumors has been reported in *sost*, *dkk1*, or *Sfrp* knockout mice. Similarly, the pharmaceutical companies that have been testing sclerostin or Dkk1 antibodies in clinical trials have performed extensive preclinical safety studies, which did not show any obvious oncogenicity. Finally, after long-term treatment with LiCl and HBM (sclerosteosis or van Buchem's disease), patients have not been noted to have an increased susceptibility to cancer (37–39, 41). Oncogenicity will nevertheless have to be one

of the major adverse effects that will need to be closely monitored in clinical trials and in postmarketing studies.

If uncontrolled bone formation leads to adverse effects in homozygous sclerosteosis or van Buchem patients (intracranial pressure, loss of hearing or vision), it is most reassuring that heterozygous carriers of these mutations do not demonstrate any adverse effects but exhibit significant increases in bone density (81). It is therefore important to mitigate these potential concerns with the fact that therapeutic intervention will not eliminate entirely the endogenous inhibitor and will occur only over a limited period of time.

The PTH and Wnt Pathways Converge on the Same Anabolic Mechanism

In the last few years, it has become apparent that Wnt signaling contributes significantly to the anabolic effects of PTH, raising the question whether we are really activating two different osteoanabolic pathways or whether these two pathways converge to eventually become only one. Indeed, it has been reported that PTH inactivates GSK3β (82) and stabilizes β-catenin (83). In addition, PTH1R and Lrp6 can form a complex when PTH binds to its receptor, leading to the disassembly of the destruction complex (84). PTH also targets the osteocytes and reduces the expression of the Wnt inhibitors sclerostin (47) and Dkk1 (Fig. 3) (85). This local decrease in endogenous inhibitors leads to enhancement of an autocrine Wnt signaling loop in these cells, and deletion of *lrp5* in mice blunts the osteoanabolic effect of PTH (47).

Thus, whereas other aspects of PTH signaling may also contribute to its effects on activation frequency and anabolic responses, the role of Wnt signaling downstream of the PTH1R has clearly emerged as a very significant contributor to the observed effects of PTH on bone formation.

Antagonizing activin: another approach for bone anabolic therapy

Activin elicits its signaling by binding to two type I receptors (ActRIA and -IB) and two type II receptors (ActRIIA and -IIB). Preclinical studies have shown that activin acts as an antagonist to human osteoblast differentiation (86) and as an agonist to osteoclast formation and bone resorption (87). These findings indicated that blocking the endogenous effects of activin could favor bone formation and block bone resorption. ACE-011, a soluble form of the extracellular domain of the ActRIIA fused to the Fc domain of murine IgG (ActRIIA-IgG1-Fc), acts as an activin decoy receptor and causes bone anabolic effects in healthy and ovariectomized mice (88). Furthermore, sc injection of ACE-011 for 3 months in monkeys promoted bone formation and inhibited bone resorption, thereby acting as a dual anabolic-antiresorptive compound (89). In a phase I trial, ACE-011 was well tolerated and increased markers of bone formation (90). Thus, in principle this soluble form of the ActRIIa receptor could be developed for the anabolic treatment of osteoporosis, provided its safety profile is acceptable, especially because it increases the hematocrit (91), and its efficacy is comparable to rhPTH or to antagonists of Wnt signaling inhibitors.

Can novel antiresorptives reveal an "anabolic" component?

During bone remodeling, osteoclasts and/or the resorption they exert on bone matrix have the capacity to activate osteoblasts and bone formation, a process called coupling. This process ensures the succession of bone formation to bone resorption in the remodeling cycle within each BMU. In the last few years, a few coupling factors have been identified, and analysis of osteopetrotic mutants has illustrated the fact that it is possible to reduce the activity of osteoclasts without automatically reducing bone formation. Indeed, osteopetrotic conditions in animals and humans revealed that an impaired osteoclast activity due to mutations in or deletions of chloride channels, components of the vacuolar ATPase, or of cathepsin K, decreases bone resorption with a paradoxical increase in the number of osteoclasts and activation frequency and with a maintained or even increased BFR (92, 93).

This has led to the search for compounds that could indeed mimic such conditions. Such antiresorptive compounds could in principle meet the criteria for being classified as bone anabolics, provided they increase bone formation markers (P1NP and bone-specific alkaline phosphatase), MAR, and/or BFR while reducing markers of bone resorption (C-telopeptide of type I collagen, N-telopeptide of type I collagen). Although not meeting these criteria in humans, pharmacological inhibition of cathepsin K in animals has been reported to reduce bone resorption markers to an extent comparable to oral bisphosphonates (approximately 40%) while increasing bone formation markers in rabbits and monkeys (94, 95). In human clinical trials, bone formation markers returned to near baseline (96) or were unchanged (97, 98). Thus, although not yet fully supported by current human studies, the possibility exists that novel antiresorptives may also fulfill the criteria of bone anabolics. Furthermore, ongoing research on the mechanisms regulating the cross talk between osteoclasts and osteoblasts has identified some bone formation-stimulating osteoclast-derived factors ("clastokines") and matrix-derived growth factors

(99, 100), which may in the future help to design novel osteoanabolic compounds.

Conclusion and Perspectives

The future of osteoporosis therapy is full of exciting prospects. In addition to the extraordinary improvement in our ability to reduce the incidence of fractures since the introduction of the first bisphosphonates on the market, new and more potent bisphosphonates have allowed the use of less frequent administrations and safer routes. Moreover, the identification of RANKL as an essential cytokine in osteoclast differentiation and the development of efficient antibodies to block its action have further improved our ability to counter the devastating effects of uncontrolled bone resorption. If both bisphosphonates and RANKL antibodies also decrease bone formation, new antiresorptives may succeed at avoiding this antianabolic action, thereby possibly improving the fracture outcomes.

Yet, the identification of agents that can stimulate bone formation has been recognized as a priority in our field to treat severe osteoporosis cases and to potentially improve upon the limited efficacy of antiresorptive drugs on nonvertebral fractures. rhPTH has definitely proven its ability to increase bone formation and significantly increase bone density in severe patients, but its ability to reduce nonvertebral fractures has also been modest and its effects are limited in time, possibly because its mode of action is mostly based on bone remodeling. In this context, the discovery of Wnt signaling as a major osteoanabolic pathway, which seems to exert its effects mostly through a bone remodeling-independent mechanism (modeling-based) opens tremendous possibilities to improve bone density not only in trabecular bone but also in cortical bone, which should reduce the incidence of nonvertebral fractures.

Several clinical trials for osteoporosis treatment are ongoing, testing new antiresorptives, different forms of rhPTH, or agents that activate Wnt signaling. In many of these trials combinations and sequences of these agents with various antiresorptives are also being tested. The next few years will therefore be very exciting for osteoporosis treatment.

Acknowledgments

We are grateful to Sundeep Khosla for critical reading of the manuscript.

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Roland Baron acknowledges the support of National Institutes of Health (National Institute of Arthritis and Musculoskeletal and Skin Diseases and National Institute of Dental and Craniofacial Research).

Disclosure Summary: R.B. has served as consultant for Merck, Novartis, Amgen, Takeda, Chugai, Teijin, Japan Tobacco, Ono, Servier, and GSK and is on the scientific advisory boards of Bone Therapeutics and Arcarios. E.H. has nothing to disclose.

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