Emerging Therapeutic Opportunities for Skeletal Restoration

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Preface

Osteoporosis, a syndrome characterized by thin bones and fractures, has become more prevalent in both women and men. Established therapies for this disorder consist primarily of drugs that prevent bone loss, such as the bisphosphonates and selective estrogen receptor modulators. Although these drugs have been shown to reduce fractures in randomized trials, there is an urgent need for treatments that could lower fracture risk further without additional adverse effects. The introduction of parathyroid hormone (teriparatide), which significantly increases bone mineral density, albeit for a relatively short duration, raised expectations that drugs which stimulate bone formation might cure osteoporosis. After outlining current approaches to treating osteoporosis, this review focuses on emerging therapeutic opportunities for osteoporosis that are based on recent insights into skeletal physiology. Such novel strategies offer promise for not only reducing age-related bone loss and the associated risk of fractures, but restoring bone mineral density to healthy levels.

Introduction

Osteoporosis affects almost 44 millions of men and women aged 50 and older in the US, and evidence indicates its prevalence is rising, particularly as life expectancy continues to increase (http://www.nof.org/). Similar statistics have been noted in Europe, Asia and Australia. Osteoporosis is manifested by fractures and enhanced skeletal fragility due to micro-architectural changes in the trabecular and cortical skeleton. Osteoporotic fractures cause significant morbidity and in many individuals leads to loss of productivity and reduced quality of life. The mortality rate in the first year after a hip fracture is 20–30% and more than half of those individuals sustaining a hip fracture will not return to their previous lifestyle[1]. Primarily as a result of these fractures, health care costs in the U.S. alone from osteoporosis exceed 15 billion dollars annually.[2]

There is a long latency period until the clinical onset of osteoporosis, and during this time there are profound alterations in bone remodeling, the basic homeostatic process that controls calcium balance and skeletal integrity [3]. Imbalances in the 'bone remodeling unit', often due to accelerated bone turnover, are caused by increased bone resorption or reduced bone formation relative to resorption. The end result is a net loss of bone mass and

qualitative changes in skeletal architecture, both of which greatly increase an individual's risk of fracture.

Until recently, treatment options for osteoporosis were limited to agents that prevented bone loss and partially reduced fracture risk by suppressing bone resorption, thereby recalibrating the remodeling unit to a new steady state in which bone turnover slowed and bone mineral density was maintained. Although the number of approved therapies for osteoporosis has more than doubled in the last 15 years, the level of fracture-risk reduction has not improved dramatically. Indeed, the currently available treatments for osteoporosis, assuming optimal compliance, reduce the risk of non-vertebral fractures by at best only 30–40% [3,4,5,6].

Newer pharmacological agents offer possibilities for building on earlier treatment successes through actions on bone formation. This has led some to speculate that these drugs can restore old skeletons to more youthful states. Such guarded optimism is due in part to a greater understanding of the molecular and cellular events that occur during acquisition of peak bone mass in adolescence and bone remodeling in adults. Emerging from these findings is a new therapeutic paradigm in osteoporosis medicine; i.e. a targeted molecular approach to enhance the inherent capacity of the skeleton to augment bone mass regardless of age.

In this review, we will outline the physiology of the skeleton and discuss recent discoveries that provide new insight into bone remodeling and its relationship to energy and metabolic status. We will briefly overview the evidence supporting current therapeutic approaches to osteoporosis, but then focus on pharmacological targeting of specific pathways that offer new possibilities for intervention, especially those that could stimulate bone formation. Finally, we will discuss the potential commercial and regulatory challenges that must be overcome before final approval and marketing of these agents

Overview of skeletal physiology

Trauma is the principal cause of fractures at any stage of life, but factors that alter the quality or quantity of bone mass further predispose a susceptible individual to fractures. The bone remodeling sequence is a critical determinant of both the amount and type of bone that is laid down across the lifespan. Remodeling is essential for preserving serum calcium and maintaining bone strength during adulthood. This cyclic process occurs within microscopic elements called remodeling or *basic multicellular units* (BMU)[5]. Remodeling begins with bone dissolution or resorption and ends with bone formation, although the precise sequence of initiation is still not fully understood.

Bone resorption is carried out by osteoclasts, which originate from the hematopoietic monocyte-macrophage lineage. New bone is formed by osteoblasts, cells of the fibroblast-stromal lineage that produce bone matrix proteins and synthesize a lattice for subsequent mineralization. Stromal cells are found in the marrow and are destined to become either fat, bone or cartilage under the influence of differentiation factors within the marrow milieu. Osteocytes are osteoblasts that have completed their task of bone formation and are entombed within the bone matrix. Although these cells were originally considered inert, it is now clear that they communicate with resting surface osteoblasts (bone lining cells) and, under the proper circumstances (e.g. micro cracks, gravitary forces, fluid shifts), initiate bone remodeling. Micro canaliculi connect the osteoblast to the osteocyte and likely serve as the conduit for signals that originate from osteocytes in response to gravitational forces[5]. Remodeling begins when osteocytes are activated by local factors (as noted in Figure 1) or systemic modulators such as PTH, interleukins, estrogen withdrawal, or hormones. Marrow stromal cells synthesize and release two cytokines, mCSF and RANKL, which enhance the recruitment and differentiation of osteoclasts. These cells cause bone resorption and in the

process release growth factors such as TGF-β from the matrix to recruit more osteoblasts[5a] The outcome of these events is resorption followed by formation in a coupled remodeling sequence.

In adulthood, each remodeling unit is balanced—resorption equals formation—and the process lasts between 90 and 130 days. Maintenance of bone density during remodeling ensures a ready source of calcium for the body and a persistent reservoir of stored calcium. Prior to adult remodeling, acquisition of peak bone mass occurs, usually between 12 and 15 years of age. During this time, there is a tremendous increase in bone mass as a result of growth and modeling of the skeleton.. Pubertal surges of gonadal steroids and growth hormone are considered critical for the increase in bone mass during adolescence. Longitudinal studies suggest that several factors regulate peak bone density, including dietary intake of specific nutrients, physical activity, and, most importantly, genetic determinants. This view was established in both human and mouse models. However, despite intense efforts over the past decade to identify heritable determinants of bone mass, progress has been slow in part because it has been recognized that polymorphic differences in many genes, including the vitamin D and estrogen receptors, parathyroid hormone (PTH), collagen A1a, receptor activator of NF-κB ligand (RANKL) and others, all contribute to skeletal acquisition.

Bone loss occurs after peak acquisition when resorption exceeds formation. During the early menopause estrogen deficiency contributes to rapid skeletal loss. In addition, with advanced age, bone formation is often compromised by multiple factors including further loss of gonadal steroids, reduced vitamin D production and action, increased PTH secretion, and local generation of cytokines and growth factors that are inhibitory to osteoblasts. Less work has focused on the genetics of bone loss, although this is likely to be the major pathogenic process in the development of osteoporosis.

Current therapies for osteoporosis

Current therapies for osteoporosis target adult bone loss and are centered on restoring the balance between formation and resorption. Estrogens, selective estrogen receptor modulators (SERMs), bisphosphonates, calcitonin and denosumab are all anti-resorptives, although their sites of action may differ. Only one anabolic agent for the treatment of osteoporosis has been approved by American regulatory agencies: teriparatide, a recombinant form of the human parathyroid hormone. European agencies have also approved the use of PTH (1–84) for postmenopausal osteoporosis. Below, we provide a brief overview of the these therapies, and a summary is also provided in Tables 1,2.

Estrogen

Estrogen was extensively used for the prevention and treatment of osteoporosis prior to the development of bisphosphonates, because it was effective in improving bone mineral density (BMD). However, the most definitive data for the anti-fracture efficacy of estrogen only came in 2002, published from the Women's Health Initiative (WHI) [7]. In that study, combined therapy (0.625 mg/d conjugated equine estrogen, plus medroxyprogesterone acetate, 2.5 mg/d) was associated with a significant reduction in hip fractures as well as all osteoporotic fractures[7]. Ironically, although the WHI was the most definitive trial on the anti-fracture efficacy of estrogen therapy, this study also led to a marked reduction in the use of estrogen for fracture risk reduction due to the increased risk of cardiovascular events and breast cancer[7,8].

Given the risks associated with conventional doses of estrogen (0.625 mg/d of equine conjugated estrogens [equivalent to approximately 1.0 mg/d of oral micronized 17β-estradiol

or 0.05 mg/d of transdermal 17β-estradiol]), a number of studies have examined the use of very low dose estrogen in women at risk for fractures. Doses of 0.25 mg/d of oral micronized 17β-estradiol or unopposed transdermal estradiol, 0.014 mg/d increased BMD at multiple sites and reduced markers of bone resorption [9,10]. Importantly, for doses of 0.25 mg/d of 17β-estradiol[6], the adverse effect profile was similar in the estrogen and placebo groups, with no statistically significant differences in breast tenderness, abnormal mammograms, or changes in endometrial thickness. Thus low dose estrogen therapy, which keeps serum estradiol levels well below the premenopausal range, is effective at improving BMD. However, it remains to be seen whether large scale studies examining fracture and safety endpoints (breast cancer, cardiovascular events, others) will ever be initiated using this approach, given the growing list of alternative agents to prevent and treat osteoporosis.

Selective Estrogen Receptor Modulators (SERMs)

There has long been interest in identifying compounds with beneficial skeletal effects, but without the adverse breast, endometrial, and cardiovascular effects associated with estrogen treatment. SERMs are non-steroidal compounds with tissue-specific actions, which are believed to be due to the fact that these drugs induce a different conformation of the ER than estradiol [11]. Raloxifene at a dose of 60 mg/d is the only SERM currently approved by the US FDA for the treatment of postmenopausal osteoporosis. It has been shown to reduce vertebral fracture risk by 30–50% relative to placebo in postmenopausal women with osteoporosis, but has not been shown to protect against non-vertebral or hip fractures[12]. Raloxifene also reduces the risk of developing breast cancer [13], is neutral with regards to cardiovascular events, but does lead to an increase in the risk of venous thromboembolic disease, fatal stroke, and hot flushes[14].

Several additional SERMs (idoxifene, levormeloxifene, arzoxifene) have been studied but their clinical development abandoned due either to lack of efficacy for reducing non-vertebral fractures or to increases in endometrial thickness. More recently, a large, randomized placebo-controlled trial with lasofoxifene demonstrated that this drug significantly reduced the risk of vertebral as well as non-vertebral fractures, ER-positive breast cancer, coronary heart disease, and stroke[15]. However, lasofoxifene was also associated with an increased risk of venous thromboembolic events and hot flushes. In addition, one of the two doses tested (0.25 mg/d) resulted in a 37% increase in all cause mortality; thus, the future status of this drug remains uncertain.

Another SERM, basedoxifene, has been tested in a somewhat novel approach in combination with conjugated equine estrogens. The rationale was that such a combination would improve BMD and reduce hot flushes, but without some of the other adverse effects on the endometrium and breast associated with estrogen therapy alone. In a study of 3997 postmenopausal women, Lindsay et al. [16] found that the combination of basedoxifene and estrogen increased BMD at multiple sites over two years, reduced the number of hot flushes, and improved measures of vaginal atrophy, lipid parameters, homocysteine levels, with a similar incidence of breast pain and other adverse events compared to placebo [17]. However, in the three year fracture trial, new vertebral fractures were reduced with basedoxifene, but non-vertebral fractures were not relative.

Bisphosphonates

Bisphosphonates are currently the most widely used drugs for the prevention and treatment of osteoporosis due to their potent effects in inhibiting bone resorption, ease of frequency of administration, and lack of serious long term non-skeletal effects. Structurally, they are chemically stable derivatives of inorganic pyrophosphate (PPi) and like PPi, bisphosphonates have a very high affinity for bone mineral because they bind to

hydroxyapatite crystals [18]. These drugs are potent inhibitors of bone resorption, and they do so by binding to and inhibiting the activity of farnesyl pyrophosphate synthetase, a key regulatory enzyme in the mevalonic acid pathway critical to the production of cholesterol, other sterols, and isoprenoid lipids [19]. As a result, the isoprenylation of proteins (including the small guanosine triphosphate-binding proteins Rab, Rac, and Rho, which regulate key osteoclast cellular activities such as stress fiber assembly, membrane ruffling, and survival) is inhibited, resulting in varying degrees of apoptosis of osteoclasts [20].

There are currently four bisphosphonates approved in the US for the prevention and/or treatment of osteoporosis: alendronate, risedronate, ibandronate, and zolendronic acid [18]. Alendronate, risedronate, and ibandronate are administered orally (daily, weekly, or monthly), whereas ibandronate can also be administered intravenously every 3 months and zolendronic acid (5 mg) is administered intravenously either annually (for treatment) or every other year (for prevention). All of these drugs are potent inhibitors of bone resorption and reduce the risk of vertebral fractures by 40–70% and hip fractures by 40–50%. Overall non-vertebral fractures (i.e. fractures that do not include spine or hip) are suppressed by only 20–40% [18] with the use of alendronate, risedronate or zoledronate. Ibandronate has not been shown to have non-vertebral fracture efficacy. In respect to cost, yearly zoledronic acid is much more expensive than generic alendronate whereas risedronate and ibandronate are intermediate in price.

While the bisphosphonates are extremely useful agents for reducing fracture risk, concerns have been raised regarding their long term safety. Osteonecrosis of the jaw, a painful condition with exposed bone in the oral cavity, has received perhaps the greatest attention [21]. The risk of this complication appears to be greatest in patients with cancer receiving high monthly doses of zolendronic acid or pamidronate, but is in the range of 1 in 10,000 to 1 in 100,000 per patient treatment-years in patients receiving doses of bisphosphonates for the treatment of osteoporosis. More recently, a specific type of femoral fracture (subtrochanteric) has been associated with bisphosphonate use [22]. The risk of this complication remains uncertain, [23], and further studies are needed to define its true incidence as well as the pathogenesis of both osteonecrosis of the jaw and sub-trochanteric femoral fractures. Notwithstanding the rare occurrence of these complications, significant concerns have been raised about long term treatment with the bisphosphonates. This in turn has led to a greater urgency for considering alternative approaches to osteoporosis therapy.

Denosumab

Osteoclast maturation is dependent on on receptor activator of NF-κB ligand (RANKL) [24,25], which is expressed on the surface of bone marrow stromal/osteoblast precursor cells, T-cells, as well as B-cells [26] (Figure 1). RANKL binds its cognate receptor, RANK, on osteoclast lineage cells, and is neutralized by the soluble, decoy receptor, osteoprotegerin (OPG), which is also produced by osteoblastic lineage cells [27,28]. Denosumab is a fully human monoclonal antibody that binds to RANKL, thereby reducing osteoclast differentiation, activity and survival, and leading to a decrease in bone resorption. Multiple trials using denosumab have been done in humans, the largest being the phase III FREEDOM trial [29], in which treatment with denosumab resulted in a relative decrease in vertebral fractures of 68%, in hip fractures of 40%, and in non-vertebral fractures of 20% compared with placebo.

Since oral bisphosphonates are currently the first-line therapy for osteoporosis, Brown et al. compared the efficacy and safety of denosumab versus oral alendronate in the DECIDE study [30]. Postmenopausal women receiving denosumab treatment had significantly greater increases in BMD at all skeletal sites assessed (total hip, lumbar spine, femoral neck, trochanter and the distal one-third radius). Decreases in levels of bone-turnover markers

were also significantly greater in denosumab- versus alendronate-treated patients. Analysis by high-resolution peripheral quantitative CT (HR-pQCT) demonstrated that while alendronate reduced bone loss, denosumab partially restored cortical bone density [31]. These findings suggest that denosumab may reduce cortical porosity by allowing filling or partial filling of cortical pores. A subsequent study, the STAND trial, included postmenopausal women that were previously treated with alendronate and switched to denosumab [32]. In subjects transitioning to denosumab, total hip BMD increased by 1.90% compared to a 1.05% increase in subjects continuing on alendronate. Serum CTX levels decreased further in the subjects transitioning to denosumab versus continuous alendronate treatment. In sum denosumab suppresses bone resorption to a greater degree than the bisphosphonates, thereby leading to a bigger increase in bone mineral density.

Based on all the available data, the FDA recently approved denosumab (Prolia, Amgen) for the treatment of postmenopausal women who have a high risk of osteoporotic fractures, including those with a history of fracture or multiple risk factors for fracture, or those who have failed or are intolerant to other osteoporosis therapies. Like bisphosphonates [18], denosumab has also been used to reduce pain and other skeletal events in patients with metastatic cancer [33]. Of interest, a recent study by Thomas et al. found that high, frequent doses of denosumab (120 mg monthly) resulted in near complete or complete elimination of giant-cell tumors as evaluated by histology, suggesting that at least for this tumor type, denosumab may have direct anti-tumor effects[34]. Recently Denosumab gained approval from the U.S. FDA for the prevention of skeletal related events in patients with bone metastases from solid tumors.

As noted above, the use of bisphosphonates, particularly in cancer patients administered high doses, has been associated with the development of osteonecrosis of the jaw [21]. Similarly, several cases of osteonecrosis of the jaw have now also been reported with denosumab use [35]. Perhaps the major concern about long-term denosumab use relates to its possible effects on the skin and immune system, since TRAIL, (TNF-related apoptosis-inducing ligand) is suppressed by denosumab and is produced by not just on osteoblasts but also immune cells [24]. In the large denosumab clinical trial, compared to placebo, the active treatment group had statistically significant increases in the rates of eczema and hospitalizations for cellulitus [29]. In addition, it has been shown that patients treated with denosumab have a slightly greater risk of recurrent neoplasms [36], which, although not statistically significant, supports ongoing surveillance of such patients, particularly when used in the community setting in patients with co-morbidities that might have excluded them from participating in clinical trials.

Adherence of patients to prescribed drugs and the cost of treatment are important considerations in the selection of treatment options and response to therapy. In a recent study, 77% of patients preferred denosumab injections every 6 months over weekly oral alendronate [37]. The annual cost for the use of denosumab (two injections per year) is approximately $1650, which is very comparable to the cost for the annual infusion of the bisphosphonate, zolendronic acid but much less than generic alendronate.

Strontium ranelate

Strontium ranelate is a chemical compound originally developed as an anabolic agent for the treatment of postmenopausal osteoporosis. Strontium is taken up by bone, primarily through a physical mechanism, and associated with limited calcium exchange in hydroxyapatite. Although its mechanism of action is not fully elucidated it has both anti-resorptive and anabolic properties. Strontium ranelate (2 grams/daily) was found to reduce vertebral fractures by 40% in a phase III trial and non-vertebral fractures by 16% in a second large

randomized study It is well tolerated with few adverse events [37a,b]. It is approved in Europe and Asia but not in the United States for the treatment of postmenopausal osteoporosis.

Teriparatide and PTH (1–84)

A new class of anti-osteoporosis drugs was introduced in 2002 with the approval of Forteo (teriparatide, a recombinant version of human PTH$_{1-34}$) for the treatment of postmenopausal osteoporosis. PTH is a naturally occurring hormone secreted by the parathyroid glands in response to low circulating calcium levels. It acts directly on bone to stimulate resorption and preserve serum calcium, but also indirectly influence calcium metabolism by acting on the kidney to enhance the activity of the 1 alpha hydroxylase enzyme that converts 25OH vitamin D to 1,25 dihydroxyvitamin D, thereby enhancing absorption of calcium and phosphate from the intestine. Thus, PTH has both direct and indirect activity on calcium metabolism (i.e. direct on bone to mobilize calcium; indirect through the kidney to promote calcium reabsorption and 1, alpha hydroxylase activity, and through the gut to promote calcium absorption via 1,25 dihydroxyvitamin D) [38].

However, the mechanism of the anabolic action of PTH has been debated for nearly as long as the first animal studies were completed with PTH in the 1930s. The ultimate paradox of PTH relates to the longstanding observation that primary and secondary hyperparathyroidism is associated with cortical bone loss and fractures, whereas intermittent PTH administration enhances trabecular bone mass and reduces fractures with very modest effects on cortical bone. PTH targets lining cells and osteoblasts generating a number of growth factors including IGF-I and RANKL [38]. RANKL is a critical modulator of osteoclastogenesis and is almost certainly responsible for the coupling of resorption with formation during PTH therapy. Recent studies have shown that PTH also targets osteocytes and inhibits sclerostin thereby further promoting bone formation through the Wnt/β-catenin signaling pathway [39].

In clinical studies, intermittent subcutaneous injections of PTH not only increased bone mineral density but also reduce fractures of the spine and non-vertebral bones. In the largest randomized, placebo-controlled clinical trial using teriparatide in postmenopausal women with severe osteoporosis, 20 μg/day of PTH, administered subcutaneously, reduced spinal and nonvertebral fractures but not hip fractures by more than 50% while it substantially increased (i.e., 8%/year) lumbar BMD [40]. Similar findings were noted in men with osteoporosis who were treated for 11 months. Unfortunately, the PTH trial in postmenopausal women was stopped after 20 months because of concerns related to the development of osteosarcoma in rats treated with high doses of PTH$_{1-34}$. However, retrospective studies have found no association between osteosarcoma and primary or secondary hyperparathyroidism, and only 2 cases of osteosarcoma in PTH-treated patients have been reported in more than 9 years of market use[40a].

More recently, recombinant human full-length PTH (PTH$_{1-84}$) has shown similar benefits on the spine and hip and is approved in most European countries but not in the United States. The risk profile is identical to teriparatide except for a slightly greater risk of hypercalcemia [41]. Currently, it is recommended that PTH therapy should be limited to those individuals with moderate to severe osteoporosis, and then only for 2 years. PTH is also approved for the treatment of glucocorticoid-induced osteoporosis. In general, PTH is well tolerated, although nausea, flushing, hypotension, and mild but asymptomatic hypercalcemia (i.e., serum calcium <11.0 mg/dL) can occur. Unlike the bisphosphonates, discontinuation of PTH can result in bone loss of 3 to 4% in the first year after PTH cessation [42]. This post-treatment effect is prevented by adding an antiresorptive drug after PTH is stopped. Despite the appeal of using an anabolic with an antiresorptive, no evidence so far indicates that combinations of the current classes of drugs have additive or synergistic effects [41,42],

although this certainly does not preclude the possibility that more efficacious combinations of antiresorptive and anabolic agents may be found in the future. Finally it should be noted that the marked increase in bone mineral density with intermittent PTH use plateaus after two years of treatment. Whether there is resistance at the osteoblast level to continued administration of PTH, or a reset remodeling balance based on the increase in bone resorption is not clear, but is important when considering new anabolic approaches to osteoporosis treatment. Cost and compliance with daily injections have been major limiting factors of for PTH therapies for osteoporosis.

Newer formulations of PTH, including transdermal patches, have been studied in short-term dose-ranging phase II trials. Also, oral agents that provoke intermittent PTH release by acting on the calcium sensing receptor in the parathyroids, i.e. calciolytics, are also under investigation as a means of capitalizing on the anabolic window produced by PTH. However, preliminary reports suggest that the administration of calciolytics do not fully recapitulate the pharmacokinetics of subcutaneously administered PTH. Another potential anabolic osteoporosis agent that is closely related to PTH, is PTH related protein, PTHrp, a peptide hormone that also activates the PTH receptor and regulates a number of homeostatic processes including chondrocyte proliferation Intermittent PTHrp administration has been shown to increase bone mass in postmenopausal women, and one pharmaceutical group has completed a Phase II study with daily subcutaneous PTHrp for 12 months. Bone mineral density increased significantly in the spine and hip at both 6 and 12 months in that study. These findings were comparable to results in women from the same study treated with teriparatide for 12 months.

Future Anabolic Therapies for Osteoporosis

There is still a need for therapies that reduce fracture risk beyond that achievable with bone-resorbing agents, particularly since virtually all the currently available drugs do not eliminate the possibility of future fractures. Moreover, concern has grown about rare but important adverse effects of the bisphosphonates on skeletal sites such as the jaw (i.e. ONJ) and the proximal femur (i.e. subtrochanteric fractures). Drug discovery and development for osteoporosis is currently focused on several potential anabolic agents, with one major exception: cathepsin K inhibitors, one of which, odanacatib, is in Phase III trials (Box 2). The remainder of this article therefore focuses on advances in the understanding of osteoblast regulation and function that have revealed new opportunities for developing drugs to stimulate bone formation. These opportunities stem directly from ongoing benchand translational studies that have identified new pathways for regulating osteoblast differentiation and bone formation.

Box 2

Cathepsin K inhibitors
A new class of drugs to treat osteoporosis is cathepsin inhibitors. Cathepsin K is the most abundantly expressed cysteine protease in osteoclasts and exhibits collagenolytic activity in bone (mainly type 1 collagen) under acidic conditions; however, it is also expressed in skin and skin-derived cells. The most specific cathepsin K inhibitor developed so far is odanacatib. In a Phase II trial [166], the 50 mg dose of odanacatib was associated with an increase in spine (5.5%) and total hip (3.2%) bone mineral density (BMD) at 24 months of treatment. This study has now been extended for an additional 12 months [Odanacatib] was not associated with any increase in skin or upper respiratory tract infections during the treatment period, a difference from earlier cathepsin K inhibitors. A Phase III fracture trial of odanacatib including 16,200 postmenopausal osteoporotic women is currently underway (http://clinicaltrials.gov/ct2/results?term=odanacatib+) - NCT00529373. Of

interest, the decreases in bone formation markers following odanacatib therapy were modest and transient as compared to those seen with other antiresorptive therapies (Unpublished data, McClung et al. the 37th European Symposium on Calcified Tissues, 2010). These results were consistent with findings in bone biopsies that were available from a subset of the study participants [171] in which there was a non-significant decrease in bone formation rate and mineralizing surface. In addition, TRAP5b, a serum marker thought to reflect osteoclast numbers, did not change even with the highest dose of odanacatib. These data reflect a potentially important difference between odanacatib and other anti-resorptive agents. Odanacatib selectively inhibits the removal of matrix proteins, but it permits persistent osteoclast viability and cellular activity, including acid secretion. In general, cathepsin K inhibitors do not result in apoptosis of osteoclasts; therefore, osteoclasts are available to produce chemokines and growth factors such as the Wnts that are responsible for coupling with osteoblasts to maintain bone formation [115]. Also, the dramatic decrease in bone turnover with other anti-resorptive treatments may carry long-term risks, including the accumulation of microcracks that cannot be repaired and over time leading to an accumulation of microfractures that weaken bone and could result in fractures. Whether odanacatib would have a similar effect can only be demonstrated in long term clinical trials.

Targeting the Wnt/β-catenin pathway

The identification of patients with high bone mass due to mutations in the Wnt signaling pathway in 2002 led to an explosion of interest in modulating this pathway as a novel approach for treating for osteoporosis. Wnts are secreted glycoproteins crucial for the development and homeostatic renewal of many tissues, including bone. Figure 2 depicts the central role played by the canonical Wnt pathway in regulating osteoblast development [43,44]. Wnts stimulate several signaling pathways by binding a receptor complex consisting of LRP5/6 and one of ten Frizzled (Fz) molecules [45]. The canonical Wnt signaling pathway, which involves stabilization of β-catenin and regulation of Lef/Tcf transcription factors, has been the most extensively studied. The Wnt pathway in osteoblasts is active in all osteoblast lineage cells, including pre-osteoblasts, lining cells and osteocytes[46]. Notably, Wnt/β-catenin signaling may be a normal physiological response to mechanical loading [47] and is certain to be participate in the fracture healing process [48]. Wnt signaling has three major functions in osteoblast lineage cells: dictating osteoblast specification from osteo/chondro-progenitors, stimulating osteoblast proliferation, and enhancing osteoblast and osteocyte survival. Within the BMU, Wnts also suppress bone resorption through suppression of osteoclast formation at least in part by regulating RANKL levels in pre-osteoblasts [49]. Wnt ligands are ubiquitous and can have significant pro-proliferative effects on both normal and neoplastic cells. Presumably owing to concerns about potential carcinogenicity there has been no effort to develop experimental Wnt-like ligands for stimulating bone formation. However, other aspects of the Wnt/β-catenin pathway have been targeted, and selected examples are discussed below.

Sclerostin

A target in the Wnt pathway for which drug development efforts are the most advanced is sclerostin, a secreted Wnt antagonist produced almost exclusively by osteocytes. It regulates bone mass by binding to LRP5 and LRP6 to inhibit the canonical Wnt/β-catenin signaling pathway [50–54]. The biological importance of sclerostin in humans is highlighted by sclerosteosis and van Buchem's disease—two genetic diseases caused by loss of sclerostin function— which are associated with markedly increased bone mass [55–58]. Since sclerostin is expressed almost exclusively in osteocytes, inhibitors are anticipated to stimulate bone formation through Wnt pathway activation while having reduced carcinogenic potential

compared with broader Wnt pathway activators. Moreover, in sclerostin related genetic disorders bone formation is high but bone resorption is also reduced suggesting that agents which suppress sclerostin might have dual properties in the remodeling unit.

Sclerostin-neutralizing monoclonal antibodies have been developed to inhibit the binding of sclerostin to the LRP5 receptor, resulting in the activation of the canonical Wnt signaling pathway [59]. Initial studies in ovariectomized rats as a model of postmenopausal osteoporosis showed strong anabolic effects of the sclerostin antibody, with marked increases in bone formation on trabecular, periosteal, endocortical, and intracortical surfaces following short-term treatment (5 weeks) [60] (Figure 3). A subsequent study in adolescent gonad-intact, non-human primates for 2 months using three different doses of the sclerostin-neutralizing monoclonal antibody demonstrated a significant increase in bone mineral content (BMC) and BMD at several skeletal sites (femoral neck, radial metaphysis, and tibial metaphysis). Overall, there was an 11 to 29% increase in BMD from baseline compared to the vehicle-treated animals. There were also significant increases in trabecular thickness and bone strength at the lumbar vertebrae in the highest-dose group [61].

A Phase I trial was conducted with 74 healthy men and postmenopausal women in a randomized, double-blind, placebo controlled ascending single-dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of the sclerostin antibody [62]. The study demonstrated a dose-related increase in BMD of up to 5.3% at the lumbar spine and 2.8% at the total hip with a single subcutaneous dose of 10 mg/kg compared to placebo. Furthermore, the sclerostin-neutralizing antibody not only increased bone formation (P1NP, bone specific alkaline phosphatase, and osteocalcin) but also decreased bone resorption (sCTx) markers, resulting in a large anabolic window. Osteoblast and osteoclast activity was uncoupled following the treatment with the sclerostin-neutralizing antibody, a finding similar to sclerostosis or von Buchem's disease. A Phase II dose escalation trial has recently been initiated with a total of 419 postmenopausal women with a T-score between −2 and −3.5 by DXA. In this study, different doses will be given once a month or once every 3 months. The primary endpoint is the percentage change from baseline in spine BMD. This study will be particularly informative since investigators will compare the sclerostin antibody with alendronate and teriparatide treatment. However, the long term safety of a sclerostin monoclonal antibody, particularly in respect to the development of neoplasms will await larger phase III trials.

Wnt binding proteins

Other secreted Wnt antagonists whose action might be blocked to stimulate the Wnt signaling pathway and increase bone mass include secreted frizzled-related proteins (sFRPs) and Wnt inhibitory factor 1 (Wif1), since both bind directly to Wnt proteins and inhibit Wnt signaling. Mesenchymal cells express endogenous Wif1 and its expression level decreases during osteoblast differentiation, but increases during adipogenesis. Furthermore, over-expression of Wif1 in mesenchymal cells resulted in a significant decrease in alkaline phosphatase activity and an increase in adipogenesis. By contrast, complete knockdown of Wif1 leads to an increase in alkaline phosphatase activity *in vitro* [63]. Thus, either monoclonal antibodies or antisense RNA molecules against Wif1 or sFRPs would be expected to lead to an increase in Wnt activity and result in an anabolic bone response.

However, much like the Wnts, attention needs to be paid to the possible oncogenic effects of pharmacological stimulation of the Wnt pathway by inhibiting these inhibitors. For example, Kansara et al. showed that Wif1 suppressed β-catenin levels, indirectly though its binding to the Wnts, and inhibited growth of mouse and human osteosarcoma cells [64]. Deletion of Wif1 accelerated the development of radiation-induced osteosarcomas *in vivo*. In primary osteosarcomas, silencing of WIF1 by promoter hypermethylation was associated with loss of

differentiation, increased β-catenin levels, and increased cell proliferation. Compared with normal human osteobasts, Wif1 mRNA and protein levels were significantly down-regulated in several osteosarcoma cell lines. The down-regulation of WIF1 mRNA expression was also associated with its promoter hypermethylation in several cell lines [65]. Therefore, the development and use of Wnt antagonists, as well as all agents that target the Wnt/β-catenin pathway must proceed with extreme caution due to the potential for carcinogenicity. Further studies will be required to fully uncover the regulatory mechanisms involving Wnts and tumor development.

DKK1 antagonists

DKK1 is another negative regulator of the Wnt signaling pathway, which acts by direct binding to Lrp5/6, similar to sclerostin. Blocking the receptor leads to inhibition of osteoblastogenesis in various osteogenic cell lines and *in vivo*, mice deficient in the DKK1 gene develop extra digits [66]. The loss of a single allele of DKK1 resulted in an overall increase in bone mass [67]. Conversely, mice over-expressing DKK1 develop osteopenia [68]. To date, most studies inhibiting DKK1 have been done in association with different types of cancer cell lines or cancer mouse models. DKK1 is highly expressed in patients with multiple myeloma (MM) as well as in patients with breast cancer, and this contributes to the development and progression of myeloma and breast osteolytic bone metastases. In a study by Bu et al. [69], while conditioned media from breast cancer cells blocked Wnt-3a-induced C2C12 osteoblast differentiation and OPG expression, conditioned media from cells in which DKK1 expression had been silenced was unable to do so. Serum from patients with MM inhibited osteoblast differentiation in vitro, and this effect was blocked using a neutralizing antibody to DKK1. *In vivo* experiments treating SCID-rab mice transplanted with myeloma cells with an anti-DKK1 antibody stimulated bone formation and reduced bone loss and tumor growth [70]. In addition, an increase in bone mineral density was observed in the control (i.e., not transplanted with the myeloma cells) mice treated with the DKK1 antibody. The neutralizing DKK1 antibody (BHQ880, which can neutralize both human and murine DKK1) prevented suppression of osteoblast numbers but did not prevent increases in osteoclast numbers. In addition, the anti-DKK1 antibody prevented osteolytic bone disease, but did not reduce overall tumor burden [71].

Thus, a possible alternative strategy to prevent estrogen deficiency-induced osteoporosis would be to inhibit the function of DKK1, as was demonstrated by Wang et al. [72]. In that study, injection of sham or ovariectomized rats with end-capped phosphorothiate DKK1 antisense oligonucleotides resulted in an increase in bone volume and osteoblast numbers as well as a decrease in osteoclast numbers. In addition, DKK1 antisense oligonucleotide treatment decreased the expression of RANKL in the bone microenviroment. No studies in nonhuman primates or human studies using the DKK1 inhibitor have been initiated so far. Moreover, unlike sclerostin, which is expressed primarily in bone, DKK1 is expressed in multiple tissues [73–75]. Thus, there are greater concerns regarding the use of DKK1 antagonists and potential side effects, such as an increased incidence of tumors in, for example, the colon, where increased Wnt signaling is associated with tumorigenesis [76–79].

Lithium

Lithium has been used for decades to treat psychiatric disorders and clearly is not an emerging therapy for osteoporosis. But due to recent clinical and basic studies of the Wnt/β-catenin pathway, lithium has been re-examined as an anabolic skeletal factor. Osteoporosis is prevalent in patients with bipolar disorders and other psychiatric conditions. The first choice of long-term treatment for these disorders has been lithium salts. However, lithium has a complex effect on calcium homeostasis and it is unclear whether lithium is protective or detrimental to the skeleton. For example, lithium can stimulate PTH release and clinically

it has been shown that lithium therapy may be associated with a mild, reversible hyperparathyroid state. [80–85] Lithium was recently shown to activate the canonical Wnt signaling pathway both *in vitro* and *in vivo*. Lithium inhibits glycogen synthase kinase-3β (GSK-3β), an enzyme that phosphorylates β-catenin in the cytoplasm, targeting it for ubiquitination and degradation. Using Lrp5 knock-out and normal C57Bl/6 mice, Clement-Lacroix et al. demonstrated that lithium activated Wnt signaling independent of the Wnt receptor and resulted in significant increased bone formation and bone mass in different lines of mice [86]. However, administration of lithium carbonate caused bone loss in healthy sexually mature Wistar rats [87]. Even though lithium treatment may lead to hyperparathyroidism, a recent study by Zamani et al. found a significant decrease in bone formation and resorption markers and a significant increase in bone density in treated patients compared to normal women [88]. Consistent with these findings, evaluation of data from large cohort studies demonstrated a decreasing relative risk of any fracture with an increasing accumulated dose of lithium [89,90]. These findings were confirmed by Wilting et al. who also found a significant increased risk of fractures after discontinuation of lithium treatment [91]. Nevertheless, lithium has non-skeletal side effects that undoubtedly would limit its promise as an anabolic agent for osteoporosis.

Hormones, growth factors and cytokines

One approach to treat osteoporosis is by targeting extracellular molecules such as growth factors and cytokines. A number of extracellular factors have identified as potential anabolic factors for skeletal metabolism and some of them have been tested in clinical settings. In this section we will summarize the recent findings of extracellular factors which are involved in skeletal metabolism and discuss the potential usefulness for the treatment of osteoporosis.

IGF-I/IGF-II, IGFBPs/insulin

Regulatory systems governed by insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are operative in most tissues and exert profound effects on a wide range of metabolic processes [92–94]. Accumulating evidence both in human and animal studies demonstrate the anabolic role of IGF-I and IGF-II on the skeleton in part through stimulating bone formation. For example, IGF-I serum levels are robustly increased at the periods of puberty when bone acquisition is maximized, then decline with age and are associated with bone loss in human. In genetically engineered animal models, the anabolic effect of circulating and skeletal IGF-I have been implicated in the physiological process of skeletal accrual, particularly for the cortical skeleton [95–103]. The effects of IGF-I on stromal cell differentiation appear to be bimodal, with IGF-I exhibiting mitogenic properties during early osteoblast development. On the other hand, IGF-I also promotes terminal osteoblast differentiation (e.g. mineralization),

IGFBPs principally function as carriers for IGFs and regulate bone turnover by maintaining the proper IGFs concentration in the skeletal micro-environment[104–106]. Molar excess of the IGFBPs compared to the IGFs reduces bone mass in part through inhibiting the access of IGFs to their receptor, whereas the lack of IGFBPs relative to IGFs leads to the osteoporotic phenotype by increasing the turnover of IGFs in the pericellular space. Indeed, administration of IGF-II together with IGFBP2 has anabolic properties on the skeleton [107,108]. However, IGFBPs may have intrinsic biological activity independent of bound IGFs and this may occur through their unique motifs including their heparin-binding domain and RGD sequence. For example, the heparin-binding domain of IGFBP2 has been implicated in the down-regulation of PTEN expression, which may be beneficial for IGF-I signaling.

Given the anabolic properties of the IGFs/IGFBPs system, these peptides have been considered as potential therapeutic agents for the treatment for osteoporosis. In line with this, several small studies suggest that administration of recombinant human IGF-I (rhIGF-I) could positively affect bone mass [109, 110]. Ghiron et al. evaluated the bone formation and resorption markers in 16 healthy elderly women (71.9 +/− 1.3 years of age) administered a high dose of rhIGF-I (60ug/kg/day) or a low dose of rhIGF-I (30ug/kg/day) for 28 days [110]. Bone formation markers, including osteocalcin, skeletal alkaline phosphatase (sALP), and type I procollagen carboxy-terminal extension peptide (CICP), were increased by rhIGF-I treatment, whereas bone resorption markers such as urine hydroxyproline (OHP), total pyridinolines (PYD), and N-telopeptide showed dose-dependent differences. High-dose rhIGF-I increased these resorption markers, but low-dose rhIGF-I did not appreciably affect bone resorption. These data are suggestive that low-dose rhIGF-I might be considered as an anabolic treatment since it increases bone formation with only minimal increases in bone resorption.

rhIGF-I has also been studied in patients with anorexia nervosa which is characterized by amenorrhea, low BMD, reduced body weight, low serum IGF-I levels, and GH resistance. Grinspoon et al. reported that rhIGF-I caused an increase in BMD in these patients and when combined with oral contraceptive pills rhIGF-I showed a greater increase in BMD than oral contraceptives alone [109]. rhIGF-I has a very short half life, and hence combination therapy with its carrier such as rhIGFBP3 has been proposed[111]. Boonen et al. evaluated the effect of rhIGF-I/rhIGFBP3 combination therapy on the recovery of bone mass in patients with recent hip fracture after surgery (aged 65–90 yr). Interestingly, bone loss in the contralateral hip was recovered in patients with combinational therapy, but placebo-treated patients failed to regain lost bone. However, IGF-I also stimulates bone resorption, and hence it seems unlikely that therapy with this peptide could be continued indefinitely. In addition, metabolic adverse effects such as hypoglycemia and hypophosphatemia caused by the activation of the IGF receptor in other tissues may limit the widespread application of this therapy.

Insulin has been used as a treatment for Type I and II diabetes mellitus for nearly 90 years. However, two recent studies suggest that insulin has novel activity on the skeletal remodeling unit that cannot be duplicated by IGF-I. The Clemens laboratory demonstrated that absence of the insulin receptor in osteoblasts leads to markedly decreased bone formation and an obesity phenotype [112] The Karsenty laboratory showed that insulin mediates bone remodeling by suppressing Twist 2 and OPG, thereby leading to higher bone turnover [113]. Moreover as noted below, osteocalcin, a bone specific protein, enhances insulin sensitivity and secretion, which in turn could lead to increased bone formation. Whether insulinotropic agents might be useful in the treatment of osteoporosis requires further studies.

TGF- β

TGF-β has complex effects on bone, but there has been considerable interest in this growth factor recently based on the demonstration by Tang et al. that active TGF-β1 released during bone resorption coordinates bone formation by inducing the migration of osteoblast precursors [114] This has led to the proposal that TGF-β1 is a factor that couples bone resorption to bone formation signaling Additional factors produced by osteoclasts that appear to serve a similar coupling function include sphingosine kinase I, Wnt10b, and BMP6 [115]. Collectively, these findings raise the possibility that regulating the production of TGF-β or these other coupling factors in the bone microenvironment may represent a novel approach to increasing osteoblast numbers and/or activity. However, like other growth factors, TGF-β is ubiquitously expressed and therefore using this peptide as a treatment modality might have adverse non-skeletal effects.

Osteocalcin

Osteocalcin (OCN) is an osteoblast-specific secretory protein which is the major non-collagenous protein in the extracellular matrix of bone [116]. OCN undergoes γ-carboxylation which occurs on glutamic-acid residues post-translationally and converts under-carboxylated OCN to γ-carboxyglutamic acid (Gla). This process increases the affinity of OCN for the extracellular matrices, especially hydroxyapatite [117,118]. OCN has been widely used as a marker for bone turnover and bone formation [119]; however, the exact role of OCN still remains unknown [120]. For example, OCN null mice are obese and insulin resistant suggesting that bone plays an integral role in energy metabolism in part through OCN [121–122] Un-carboxylated and under-carboxylated forms of OCN can circulate and function as endocrine factors increasing the production of insulin in the β-cells of pancreas and adiponectin in adipocytes [1116, 121, 122], thus exerting a systemic glucose-lowering effect. Interestingly, OCN bioactivity is regulated negatively by adipocytes through increased sympathetic nervous tone driven by leptin [116, 122,123], resulting in decreased insulin secretion from β-cells.

Recently, two independent laboratories have expanded our understanding of the metabolic and skeletal functions of OCN. Conditional deletion of the insulin receptor in osteoblasts leads to impaired bone formation and insulin resistance [112]. This occurs through modulation of the ratio of under-carboxylated OCN relative to total OCN. Under-carboxylated OCN is presumed to be the active metabolic form and its release from the skeletal matrix is triggered by bone resorption through insulin signaling and suppression of OPG [113]. Thus, modulating OCN activity could improve insulin sensitivity, which might also prove beneficial for skeletal health. However, whether this pathway is operative in humans still needs to be determined. Clinical studies have demonstrated that serum OCN and under-carboxylated OCN levels are inversely associated with glucose levels[124–129]. On the other hand, glucose metabolism in patients treated with warfarin, which blocks γ-carboxylation of several molecules including osteocalcin, has not been studied systematically. In addition, a receptor for osteocalcin has not been identified. Clearly, further clinical and translational studies are needed to clarify the effectiveness of OCN in the regulation of bone and glucose metabolism in humans. However, the possibility that one could administer an agent that enhances glucose sensitivity and is either neutral or anabolic for bone is certainly tantalizing.

Antagonists of PPARγ

Peroxisome proliferator-activated receptor-gamma (PPARγ) is a member of the PPAR family of transcriptional factors and nuclear receptors and plays a critical role in many aspects of cellular activities, including cell differentiation, lipid and glucose metabolism, and neoplasm development [130–132]. Alternative splicing generates PPARγ isoforms including PPARγ1 and PPARγ2, two major isoforms of PPARγ. PPARγ1 is expressed in most of the tissues, but expression of PPARγ2 is limited to adipogenic cells. PPARγ2 has been identified as a critical transcription factor regulating adipogenesis and adipocyte metabolic functions.

The synthetic class of compounds, the thiazolidinediones, TZDs, are ligands for PPARγ and have been used frequently for the treatment of type 2 diabetes mellitus because of their lipid and glucose lowering effects[133–137]. Recent data from the clinical and animal studies points to an adverse effects of these agents on skeletal metabolism such that bone loss and fractures may result[138–140]. In contrast, PPARγ heterozygous mice have high bone mass and reduced marrow adiposity[141]. Similarly, inhibition of a downstream target of PPARγ, nocturnin, results in enhanced osteogenesis and suppressed adipogenesis [142]. This occurs because PPARγ is a master regulator of mesenchymal cell fate, favoring the adipogenic lineage when activated and suppressing osteoblastogenesis. In addition to changes in marrow stromal cell

allocation, activation of PPARγ also increases osteoblast and osteocyte apoptosis, possibly through reduction of IGF-I and enhances osteoclastogenesis by stimulating c-fos expression in osteoclast precursors. Thus, inhibition of PPARγ activity could be a pharmacologic means of treating osteoporosis. This concept has been studied in experimental models. Krause et al. reported that the PPARγ inhibitor, GW9662, enhanced osteogenic markers in human mesenchymal stem cells[143] However, another PPARγ antagonist, bisphenol-A-diglycidyl ether (BADGE) treatment did not prevent bone loss in streptozoticin-induced type 1 diabetic mice, although BADGE inhibited the development of marrow adiposity in these mice [144]. Since PPARγ could be a positive regulator for osteoclastogenesis, it is possible that PPARγ antagonists increase skeletal mass in part through inhibiting bone resorption, although this concept has not been tested [145, 146]. These lines of evidence demonstrate that targeting PPARγ activity could be a promising strategy for the treatment of osteoporosis. Indeed, several companies are exploring this target, although further animal, clinical and translational studies will be required (Figure 4).

Serotonin

Serotonin is a neurotransmitter that affects appetite, energy balance and behavioral and emotional activity, and also functions to modulate gastrointestinal peristalsis, platelet contraction and hemostasis, depending on its site of synthesis [147–150]. Serotonin synthesis is catalyzed by the rate-limiting enzyme, tryptophan hydroxylase (Tph)[151]. Circulating serotonin, which accounts for the 95 % of the total serotonin in the body, is generated in the enterochromaffin cells of the duodenum by Tph1[152]. Circulating serotonin is taken up principally by platelets through the 5-hydroxytrophan (serotonin) transporter (5-HTT) and does not cross the blood brain barrier. In contrast, serotonin in the brain is mainly produced in the brainstem by Tph2[153].

Circulating and CNS serotonin may have opposing effects on skeletal metabolism. Yadav et al. reported that suppression of circulating serotonin by inhibiting Tph1 expression in the gut resulted in a high bone mass phenotype[154], suggesting that circulating serotonin functions as a hormone that suppresses bone acquisition. In contrast, suppression of brain-derived serotonin by targeting the *Tph2* gene decreased bone mass through the activation of the hypothalamus and stimulation of the sympathetic nervous system [155]. This would indicate that brain serotonin is a neurotransmitter regulating bone mass in a positive direction.

Given the effects of serotonin as a modulator of behavioral and emotional activity, a number of serotonin-targeted drugs have been developed to treat psychiatric disorders. Two classes of drugs, second-generation antipsychotic agents (SGAs) and the selective serotonin reuptake inhibitors (SSRIs), have been widely prescribed to treat psychosis. Unexpectedly, however, these agents have been shown to have a deleterious effect on skeletal mass [90, 156–163]. SGAs such as risperidone, which have antagonist activity at the 5-HT$_{2A}$ receptor, and to a lesser extent the dopamine D2 receptor[164], have been implicated in bone loss and higher fracture risk[156, 157, 163]. Hyperprolactinemia caused by the inhibition of dopamine D2 receptor signaling could in part account for the bone loss caused by SGAs because hyperprolactinemia can stimulate bone resorption by suppressing gonadotropin secretion [165]. However, it is still possible that the SGAs cause bone loss by inhibiting serotonin receptor signaling. Indeed, male mice treated with risperidone exhibit profound bone loss without affecting serum prolactin levels (personal communication, Rosen CJ).

SSRIs, which inhibit serotonin uptake by 5-HTT and are supposed to increase the local concentration of serotonin, have also been implicated in reduced bone mass and fractures [158–162]. Because SSRIs could increase pericellular serotonin concentrations both in the central nervous system and skeletal microenvironment, it is still unclear as to the exact

mechanisms whereby SSRIs reduce bone mass. Nevertheless, these lines of evidence clearly suggest that serotonin signaling is involved in skeletal metabolism and manipulating this pathway is a potential target for the treatment for osteoporosis. In fact, a small molecule which inhibits Tph1 (LP533401) has been shown to possess an anabolic effect on the skeleton of mice[166] at least in one preliminary study. Further translational and pre-clinical studies will be required to shed light on this novel approach for osteoporosis treatment.

Outlook and challenges for anabolic agents

Drug discovery and development for osteoporosis is now largely focused on anabolic agents (with the notable exception of the cathepsin K inhibitors discussed in Box 2), and several of the approaches discussed above could lead to marketed therapies in the next decade. PTHrP (Box 1) and a monoclonal antibody to sclerostin show particular potential for moving into Phase III trials. However, the road to approval of new osteoporosis therapies has several important obstacles. First, the only endpoint recognized by European and American regulatory agencies for establishing the efficacy of new osteoporosis drugs is a reduction in fractures over a minimum of three years. Low bone mineral density is a risk factor for fracture, but an increase in density is not sufficient to gain regulatory approval. Indeed, several agents have been shown to increase bone mineral density but not reduce the number of fractures. After completing the requisite animal studies (two models are required) and the toxicology work, sponsors must have dose ranging phase II studies ready before embarking on very large and expensive phase III fracture trials. With the exception of PTHrP, which could potentially be studied in a non-inferiority fracture trial due its resemblance in structure and function to PTH1–34, any of the other agents mentioned above would currently have to be tested through randomized placebo-controlled trials. Even assuming that sufficient efficacy is achieved, demonstrating acceptable safety will be a major challenge particularly for newer agents where non-skeletal tissues may be involved (e.g. Wnt/β-catenin and neoplastic growth). Not only does the design of the trial have to include assurances that there is adequate power to assess fracture risk reduction, but adjudication of events from these other possible non-skeletal effects needs to be a major component of the research design.


Box 1

Parathyroid hormone–related protein
Parathyroid hormone–related protein (PTHrP) is a naturally occurring polypeptide closely related to PTH that activates the same receptor as PTH (PTHR1)[168]. It is expressed in many tissues, but is found in high levels at the growth plate, in osteoblasts, and within mammary tissue. It was cloned from cancer cells and identified as the circulating cause of hypercalcemia of malignancy (HOM). Originally thought to be a potent stimulus of bone resorption and hence hypercalcemia, experimental studies using intermittent PTHrP have shown significant anabolic properties similar to PTH 1–34. PTHrP was first shown to be effective in treating low bone mass in laboratory animals, but later was studied for safety and efficacy in humans [169].

One study of PTHrP (1–34) was performed to determine if it could increase bone mass in postmenopausal women with osteoporosis when administered daily by subcutaneous injection for 3 months [165]. This double-blind, placebo-controlled, randomized clinical study enrolled 16 postmenopausal women with osteoporosis between 50 and 75 years of age [170]. All had been on hormone replacement therapy for an average of 8 years and still had osteoporosis. Women who had been taking any other type of osteoporosis medication were excluded from the study. Half self-administered PTHrP, 400 μg/d and the other half injected a placebo. The patients were followed up for 3 months and the participants tolerated the treatment without developing hypercalcemia, hypotension, nausea, flushing,

or other adverse effects. The lumbar spine BMD increased by 4.7% during the 3-month treatment period, a larger increase in bone mass than seen with either anti-resorptives or PTH 1–34. Subsequently, a 3 week dose ranging study to determine the maximum tolerable dose was performed. Hypercalcemia developed only at the highest dose (750 µg/d) and in 1 of 6 individuals [169]. Bone formation markers increased to 40% with the higher doses, and resorption indices at day 21 were only modestly increased for the 625 µg/d and 750 µg/d doses. A similar but longer phase II dose ranging study was performed by Radius Pharmaceuticals with PTHrP 1–34 (BA058) vs PTH 1–34 in more than 200 postmenopausal women. The results remain unpublished but at a meeting on anabolic treatments for osteoporosis, they reported increases in spine and hip BMD that equaled or exceeded teriparatide (i.e. PTH 1–34; Personal communication from Dr. O'Dea; see Figure 2). Thus, PTHrP remains a potentially important future anabolic treatment for osteoporosis, particularly if this agent increases bone formation more than resorption. However, the long-term safety and fracture efficacy will only be determined by a large phase III clinical trial.
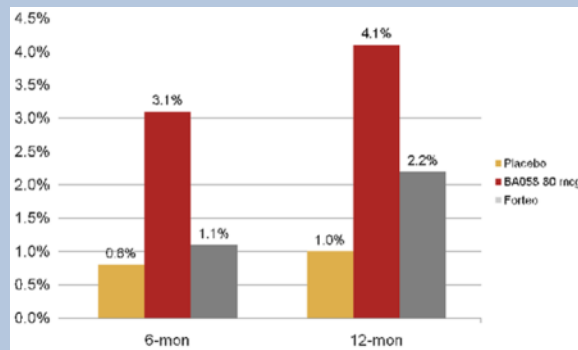
Second, there are now several drugs to treat osteoporosis. This has led some individuals to propose a halt to randomized placebo controlled trials for new osteoporosis agents. A discussion of those issues is beyond the scope of this paper, but is highlighted in a recent perspective in New England Journal of Medicine[167]. Suffice it to say, recruitment into a phase III randomized placebo controlled trial for a new osteoporosis drug will be difficult and require larger numbers of subjects, particularly if women at highest risk are excluded from participating. Improving recruitment and retention of subjects will require more careful consideration of individual risk profiles and may include a more personalized approach such as a FRAX analysis for 10 year fracture risk, at the time of informed consent.

Third, even if approval is gained, there is a major push at the regulatory level for more in depth post marketing trials that are more than just observational analyses. These would include multi-year extensions, either open label or blinded, in a phase III trial design, and further scrutiny of adverse event reporting. Nonetheless, there are major opportunities for new drug development in osteoporosis. The number of targets has increased substantially and as our knowledge of bone biology expands, this list is sure to grow. Hence, cautious optimism pervades the world of osteoporosis medicine in respect to newer therapeutic opportunities for skeletal restoration.

Acknowledgments

Abbreviations

Basic multicellular units	the single physiologic unit of skeletal remodeling including osteoblasts, osteoclasts and osteocytes
PTHrp	parathyroid hormone related peptide
WHI	women's health initiative- a large observational and randomized controlled trial performed in the United States
SERMs	selective estrogen receptor modulators
RANKL	receptor activator of NF-kB ligand
PPARγ	peroxisome proliferator activated receptor gamma
Stromal cells	connective tissue cells in the bone marrow that are multi-potent
Microcanaliculi	small channels that connect the osteocyte with resting cells on the endosteal surface
BMD, bone mineral density	areal bone mass as measured by dual energy x-ray absorptiometry
β-catenin	an intracellular transcription factor activated by extracellular ligands such as the Wnts as well as other intracellular signaling peptides
PTEN	phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a lipid phosphatase that opposes Insulin/IGF-I signaling by dephosphorylating phosphatidylinositol–3,4,5-trisphosaphate (PIP3) to phosphatidylinositol–4,5-diphosphate (PIP2)
Wnts	a family of growth factors that bind to LRP5,6 and their own frizzled receptor
Tph1	tryptophan hydoxylase enzyme 1, the rate limiting enzyme in the generation of serotonin
OCN	osteocalcin

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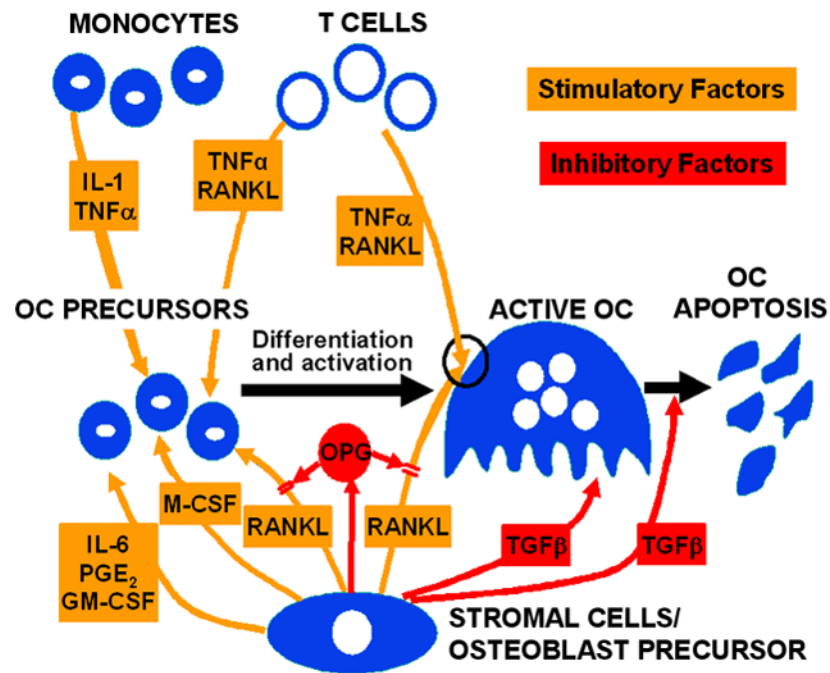
**Figure 1. Regulation of osteoclast development by RANKL and other cytokines in the bone marrow microenvironment**

Modified from Riggs et al. with permission [22]. Remodeling begins with the elaboration of cytokines from osteoblast precursors leading to the recruitment and differentiation of multinucleated osteoclasts that subsequently attach to the endosteal bone surface. Resorption of the skeletal matrix releases growth factors such as IGF-I and TFG-beta which recruit lining cells and early osteoblast precursors that eventually form new bone. Osteoblasts that do not undergo programmed cell death are entombed within the skeletal matrix and become osteocytes that can signal lining cells and respond to gravitary forces to initiate remodeling.]
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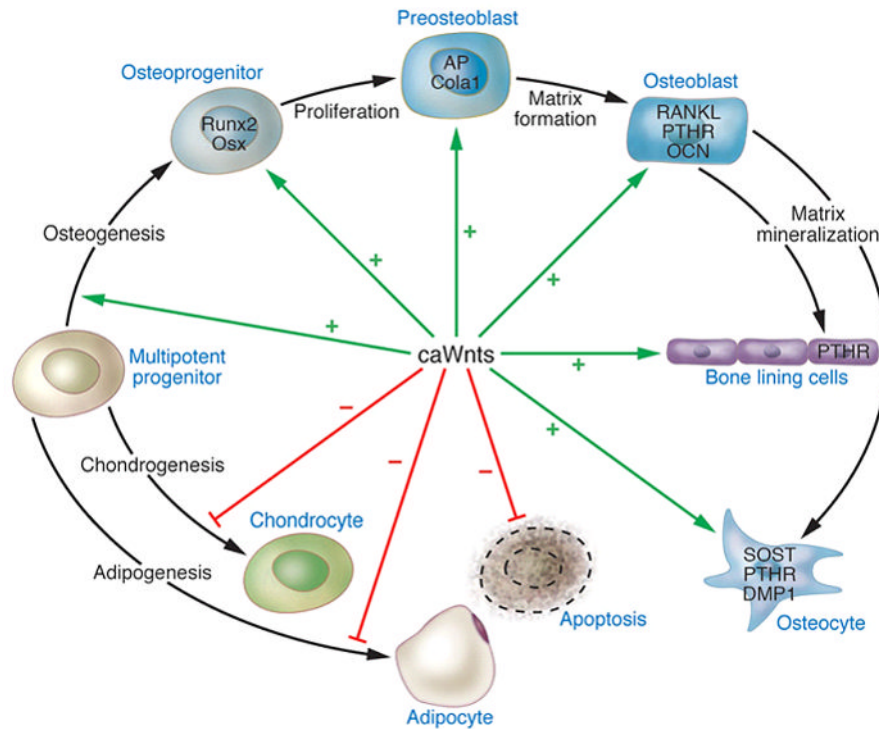
Figure 2. Osteoblast lineage specification, expansion, and terminal differentiation as well as the central role of canonical (ca) Wnts in regulating these processes
Osteoblasts are derived from multi-potent mesodermal or neural crest progenitors. Activation of the caWnt pathway through β-catenin stabilization prevents chondrogenesis. Wnt10b prevents adipogenesis. The caWnt pathway promotes survival of all cells of the osteoblast lineage and induces proliferation of preosteoblasts. Reproduced from Khosla et al. with permission [39].

REGION OF ANALYSIS SHAM

OVX + VEHICLE OVX + Scl-AbII
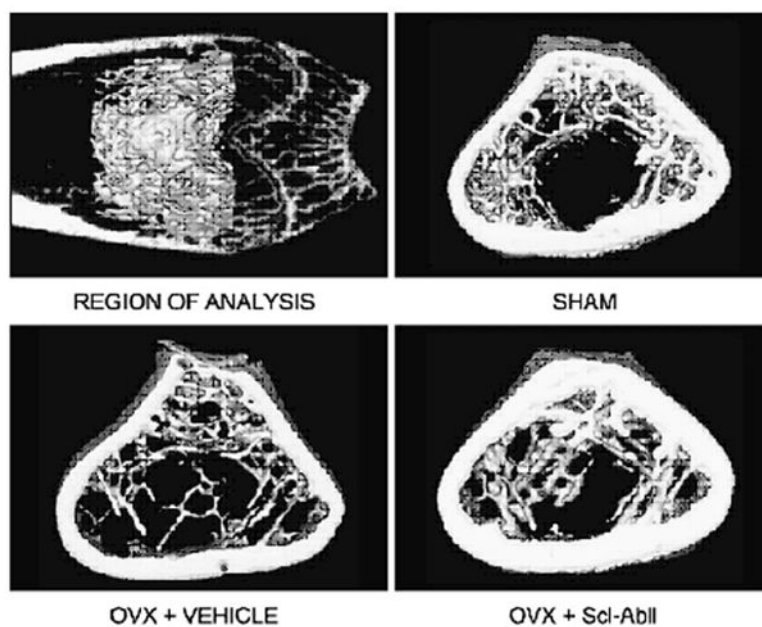
Figure 3. Sclerostin monoclonal antibody treatment in osteopenic rats restores trabecular BMD and bone volume back to sham levels at the distal femur
Shown is the distal femur region of analysis (top left panel) and representative 3D μCT images of a 1-mm central section (with attached cortices) from sham operated, ovariectomized rates treated with vehicle, and ovariectomized rats treated with the sclerostin antibody. Reproduced from Li et al. with permission [55].
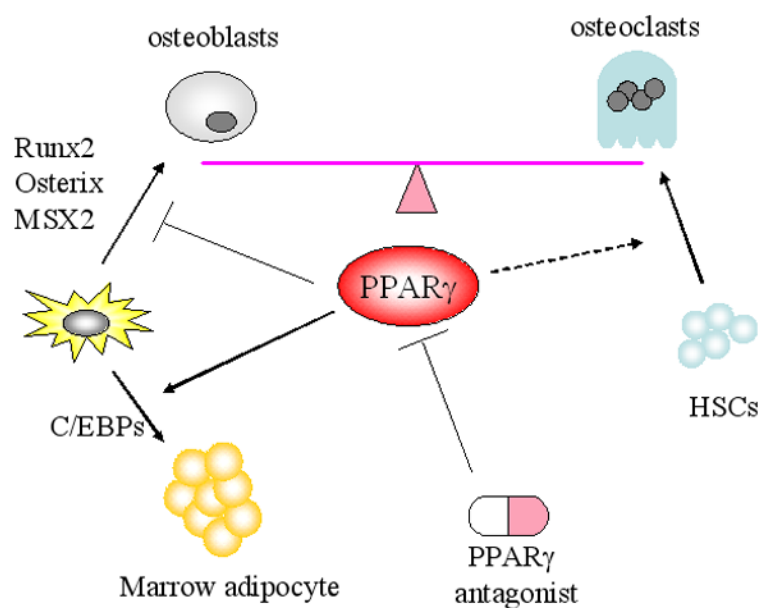
Figure 4. Proposed effect of PPARγ antagonist in the bone marrow milieu
Bone remodeling is fine tuned by the balance between bone formation by osteoblasts and bone resorption by osteoclasts. PPARγ is involved in the cell fate determination of mesenchymal stem cells (MSCs) toward the adipogenic lineage and away from osteogenic lineage. In addition, PPARγ may be the positive regulator for osteoclastogenesis although this tenet needs to be clarified. Therefore, PPARγ antagonist may increase bone mass by switching the cell fate of MSCs toward osteogenic lineage and suppressing osteoclastogenesis. HSCs: hematopoietic stem cells, PPARγ: Peroxisome proliferator-activated receptor-gamma. C/EBP: CCAAT enhancer binding protein. Runx2: Runt-related transcription factor 2. Msx2: Muscle segment homeobox homolog of 2.

Table 1

Mechanism of action of current therapies for osteoporosis

Drug class	Examples	Mechanism of action	Advantages	Disadvantages	Refs
Anti-resorptive agents					
Oestrogen		Inhibit cytokine release	Hip fracture risk reduction	Non skeletal adverse events	8,9
SERMs	Raloxifene	Inhibit cytokine release	Breast cancer risk reduction	Hot flashes	12,16
Bisphosphonates	Alendronate, risedronate, ibandronate, zolendronic acid	Inhibits farnesyl pyrophosphate synthetase	Hip and spine fracture reduction	Long term risk unknown but rare ONJ and sub-troch fractures possible	18,21,22
Antibodies	Denosumab	Inhibits RANKL	Significant fracture risk reduction	Long term safety not known	29,30
Anabolic agents					
PTH	Teriparatide	Recombinant PTH	Fracture risk reduction	Compliance, cost	40,41

Table 2

Clinical aspects of current therapies for osteoporosis. All these agents show vertebral fracture risk reduction.

Drug	Examples	Dosage regimen	Increase in BMD (%)	Fracture risk reduction-non vertebral	Refs
Anti-resorptive agents					
Oestrogen	Conjugated equine estrogen	0.625 mg	3–4%/yr	20–30%	7,8,9
SERMs	Raloxifene	60 mg	2%/yr	10% not significant	11,12,13,16
Bisphosphonates	Alendronate, risedronate, ibandronate, zolendronic acid	70 once wk 35 once wk 150 once mo 5mg IV/yr	4%/yr 3%/yr 3%/yr 4%/yr	20–30% non vert for all except ibandronate	18,20
Antibodies	Denosumab	60 mg SC/6mos	5%	20 non vert	32
Anabolic agents					
PTH	Teriparatide	20 ug SC/day	6–8%/yr	20–40% non vert	40–42