Review article

Bone biology, signaling pathways, and therapeutic targets for osteoporosis

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ABSTRACT

Major advances have occurred recently in the treatment of osteoporosis in recent years. Most patients are currently treated with bisphosphonates, denosumab, raloxifene, or teriparatide, and in some countries, strontium ranelate. Strontium ranelate and calcitonin have recently had their use restricted due to cardiovascular concerns and malignancy, respectively. The available agents have generally provided excellent options that effectively reduce fracture risk. New targets are being sought based on appreciation of the bone biology and signaling pathways involved in bone formation and resorption. These agents will directly target these signaling pathways, and further expand the options available for treatment of osteoporosis.

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1. Introduction

Discovery of new therapeutic targets for treatment of osteoporosis is important because of the limited efficacy and variable toxicities of currently approved agents. These agents, while significantly more effective than estrogen, have a relatively moderate ability to reduce hip, vertebral, and non-vertebral fractures. Current treatments reduce vertebral fracture risk significantly more than hip or nonvertebral fracture risk.

Currently approved agents in the U.S. include bisphosphonates, hormone therapy, raloxifene, calcitonin, teriparatide, or denosumab. Calcitonin was recently removed from the market in several countries due to concerns regarding cancer risk with an oral formulation used in clinical trials, and the FDA recently recommended against long-term treatment of osteoporosis with calcitonin. Teriparatide (PTH 1–34) and PTH 1–84 remain the only approved agents for stimulating new bone formation in some countries.

This narrative review will briefly summarize the bone biology relevant for understanding osteoporosis, describe the critical signaling pathways involved in bone resorption and bone formation and discuss potential therapeutic targets, and review data available for some of the newer agents developed against these targets.

2. Bone biology

2.1. Bone modeling and remodeling

The skeleton undergoes longitudinal and radial growth, modeling, and remodeling during life. Longitudinal and radial growth occurs during childhood and adolescence. Growth plates cause longitudinal growth due to cartilage proliferation in the epiphyseal and metaphyseal regions of long bones. Newly-produced cartilage mineralizes to form primary new bone. Radial growth occurs due to periosteal apposition during and after puberty, and also later in life with normal aging.

Modeling results in bones changing shape due to mechanical forces exerted on the skeleton, which leads to gradual adjustment in the shape of bone due to the physical forces encountered. Bones widen or change their axis by the removal or addition of bone to the appropriate surfaces by the action of osteoblasts and osteoclasts in response to biomechanical forces. Bones normally widen with aging in response to periosteal apposition of new bone and endosteal resorption of old bone. Bone formation and resorption are less tightly coupled during modeling than during remodeling. Bone modeling occurs less frequently than remodeling in adults [1]. Modeling increases in hypoparathyroidism [2], chronic kidney disease [3], or treatment with anabolic agents such as teriparatide [4].

Bone remodeling occurs throughout life in order to renew bone, and to maintain bone strength and preserve mineral homeostasis. Remodeling removes discrete packets of old bone continuously, and replaces these with packets of newly synthesized proteinaceous matrix which subsequently mineralize to form new bone. Remodeling thereby prevents the accumulation of bone microdamage that could lead to fracture.

The bone remodeling unit is a tightly-coupled group of osteoclasts and osteoblasts that sequentially resorbs old bone and forms new bone. Bone remodeling activity increases around the time of menopause, and continues at an accelerated rate for the first 5–10 years after menopause. After this period of rapid activity, bone remodeling slows down, but continues at a faster rate than in premenopausal women. Bone remodeling also increases mildly with aging in men.

The remodeling cycle has four sequential phases. Recruitment and activation of bone resorbing osteoclasts occurs first, followed by bone resorption for several weeks. Bone resorption then stops, with subsequent reversal, followed by an extended period of bone formation. Remodeling sites may develop randomly at a low rate, but seem to concentrate in areas of microdamage requiring repair [5,6].

Activation of bone resorption involves recruitment and activation of mononuclear monocyte–macrophage osteoclast precursors from the circulation [7], lifting of the endosteal membrane containing lining cells off the bone surface, and fusion of multiple mononuclear cells to form multinucleated preosteoclasts on the surface of the exposed bone. Preosteoclasts bind to the bone matrix via interactions between integrin receptors in their cell membranes and RGD (arginine, glycine, and asparagine)-containing peptides in matrix proteins, and form sealing zones around the bone-resorbing compartments beneath multinucleated osteoclasts.

Osteoclast-mediated bone resorption takes about 2–4 weeks during each remodeling cycle. Osteoclast formation, activation, and resorption are regulated by the ratio of receptor activator of nuclear factor κB ligand (RANKL) to osteoprotegerin (OPG), and exposure of preosteoclasts to interleukin (IL)-1 and IL-6, macrophage colony stimulating factor (M-CSF), parathyroid hormone (PTH), 1,25-dihydroxyvitamin D, and calcitonin [8,9]. Activated multinucleated osteoclasts secrete hydrogen ions via H⁺-ATPase proton pumps and chloride channels within their ruffled border cell membranes into the resorption pits beneath them to lower the pH within the bone-resorbing compartment to as low as 4.5, which helps solubilize bone mineral [10]. Resorbing osteoclasts secrete trarate-resistant acid phosphatase (TRAP), cathepsin K, matrix metalloproteinase-9 (MMP-9), and gelatinase from cytoplasmic lysosomes [11] to digest organic matrix, resulting in formation of saucer-shaped Howship’s lacunae on the surface of trabecular bone and resorption tunnels in cortical bone. The resorption phase is completed when the multinucleated osteoclasts stop functioning and undergo apoptosis [12,13].

During the reversal phase, bone resorption converts to bone formation. At the completion of bone resorption, resorption cavities contain several types of mononuclear cells, including monocytes, osteocytes released from resorbed bone matrix, and pre-osteoblasts recruited to begin new bone formation. Coupling signals linking completion of bone resorption to initiation of bone formation remain an area of active investigation. Coupling signal candidates include bone matrix-derived factors such as
transforming growth factor (TGF)-β, insulin-like growth factor (IGF)-1, IGF-2, bone morphogenetic proteins (BMPs), platelet-derived growth factors (PDGFs), and fibroblast growth factors (FGFs) [14–16]. TGF-β released from bone matrix decreases osteoclast bone resorption by inhibiting RANKL production by osteoblasts. Osteoclasts also secrete ephrins and other factors that directly influence activation of osteoblast precursors and osteoblasts [17]. Multiple other molecules likely also play a role in recruiting osteoblast precursors to resorption lacunae.

The reversal phase may also be mediated by the strain gradient in the cutting cone in the cortical resorption tunnel or Howship’s lacunae [18,19]. As osteoclasts resorb cortical bone in a cutting cone within a cortical resorption tunnel, strain is reduced in front and increased behind, and in Howship’s lacunae, strain is highest at the base and less in surrounding bone at the edges of the lacunae. This strain gradient may influence sequential activation of osteoclasts and osteoblasts, with osteoclasts activated by reduced strain, and osteoblasts by increased strain.

Bone formation typically takes 4–6 months to complete. Osteoblasts synthesize new collagenous organic matrix, and regulate mineralization of the matrix by releasing small membrane-bound matrix vesicles that concentrate calcium and phosphate and enzymatically destroy mineralization inhibitors such as pyrophosphate or proteoglycans via alkaline phosphatase [20]. Osteoblasts surrounded by and buried within matrix become osteocytes, with an extensive canalicular network connecting them to bone surface lining cells, osteoblasts, and other osteocytes, maintained by gap junctions between the cytoplasmic processes extending from the osteocytes [21]. The osteocyte network within bone functions as a syncytium. At the completion of bone formation, about 50–70% of osteoblasts undergo apoptosis, while remaining osteoblasts become osteocytes or bone lining cells. Bone lining cells may regulate influx and efflux of mineral ions into and out of bone extracellular fluid, thereby providing a blood-bone barrier, but also retain the ability to redifferentiate into osteoblasts upon exposure to PTH or mechanical forces [22]. Bone lining cells within the endosteum lift off the surface of bone prior to bone resorption to form discrete bone remodeling compartments with a specialized microenvironment [23].

The end result of each bone remodeling cycle is a new osteon. Remodeling is essentially the same in cortical and trabecular bone, with bone remodeling units in trabecular bone equivalent to cortical bone remodeling units divided in half longitudinally [24]. Bone balance is the difference between the old bone resorbed and new bone formed. During adult life, periosteal bone balance is mildly positive, whereas endosteal and trabecular bone balance are slightly more negative. Endosteal resorption normally occurs faster than periosteal formation during aging.

Bone remodeling is critical for maintenance and restoration of bone mechanical strength by replacing older microdamaged bone with newer healthy bone, and maintenance of calcium and phosphate homeostasis. The relatively low adult cortical bone turnover rate of 2–3%/year is adequate to maintain biomechanical strength of bone. The rate of trabecular bone turnover is higher than required for maintenance of mechanical strength, indicating that it plays a more significant role in mineral metabolism. Bone remodeling units appear to be mostly randomly distributed throughout the skeleton, but may be focused in areas of microcrack formation or osteocyte apoptosis. The bone remodeling space is the sum of all the active bone remodeling units in the skeleton at a given time.

2.2. Osteoclasts

Osteoclasts are the only recognized cells capable of resorbing bone. Activated multinucleated osteoclasts are derived from mononuclear precursor cells of the monocyte-macrophage lineage in bone marrow [25]. Bone marrow monocyte–macrophage precursor cells give rise to osteoclasts, and arrive in the bone remodeling unit compartment via the circulation.

RANKL and M-CSF are critical for osteoclast formation. Both RANKL and M-CSF are produced mainly by bone marrow stromal cells and osteoblasts in membrane-bound and soluble forms, and osteoclastogenesis requires the presence of stromal cells and osteoblasts in bone marrow [26]. RANKL belongs to the Tumor Necrosis Factor (TNF) superfamily. M-CSF is required for the proliferation, survival, and differentiation of osteoclast precursors, as well as osteoclast survival and cytoskeletal rearrangement required for bone resorption. Osteoprotegerin (OPG) is an osteoblast membrane-bound and secreted protein that binds RANKL with high affinity to interfere with its interaction with the RANK receptor [27].

Bone resorption depends on osteoclast secretion of hydrogen ions and cathepsin K and other digestive enzymes across the ruffled border facing the resorption pit. While H+ ions acidify the resorption compartment beneath osteoclasts to dissolve the mineral component of bone matrix, cathepsin K and other enzymes digest the proteinaceous matrix, which is largely composed of type I collagen [25].

Osteoclasts bind to bone matrix via osteoclast membrane-bound integrin receptors that link to bone matrix peptides. The β1 family of integrin receptors in osteoclasts binds to collagen, fibronectin, and laminin in the matrix, but the main integrin receptor facilitating bone resorption is the αvβ3 integrin, which binds to osteopontin and bone sialoprotein [28].

Binding of osteoclasts to bone matrix causes them to become polarized. The bone resorbing surface develops a ruffled border when acidified vesicles containing matrix metalloproteinases and cathepsin K fuse to the membrane after intracellular transport to the membrane by microtubules. The ruffled border secretes H+ ions via H+-ATPase and chloride channels, and releases cathepsin K and other enzymes in the acidified vesicles [29].

After osteoclasts contact with bone matrix, the fibrillar actin cytoskeleton of the osteoclast organizes into an actin ring, promoting formation of the sealing zone around the periphery of the osteoclast attachment to matrix. The sealing zone surrounds and isolates the acidified bone resorption compartment from the surrounding bone surface [30]. Actively resorbing osteoclasts form podosomes that attach to bone matrix, with podosomes composed of an actin core surrounded by αvβ3 integrins and associated cytoskeletal proteins.

2.3. Osteoblasts

Osteoprogenitor cells give rise to and maintain the osteoblasts that form new bone matrix on bone-forming surfaces, osteocytes within bone matrix that sense bone biomechanical forces and support bone structure, and lining cells that cover the surface of quiescent bone. Self-renewing, pluripotent stem cells give rise to osteoprogenitor cells in various tissues under the right environmental conditions. Bone marrow contains a small population of mesenchymal stem cells capable of giving rise to bone, cartilage, fat, or fibrous connective tissue, distinct from the hematopoietic stem cell population that gives rise to blood cells [31]. Multipotential myogenic cells have been identified that are capable of differentiating into bone, muscle, or adipocytes. Mesenchymal cells committed to one phenotype may dedifferentiate during proliferation and develop into another phenotype, depending on the local tissue environment. Blood vessel pericytes may develop an osteoblastic phenotype during dedifferentiation under the right circumstances [32].

Commitment of mesenchymal stem cells to the osteoblast lineage requires activity of the canonical Wnt/β-catenin pathway.
and associated proteins [33]. Identification of kindreds with a high bone mass phenotype associated with activating mutations of LDL receptor-related protein 5 (LRP-5) highlights the importance of the canonical Wnt/β-catenin pathway in embryonic skeletal patterning, fetal skeletal development, and adult skeletal remodeling [34,35]. The Wnt system is also important in chondrogenesis and hematopoiesis, and may be stimulatory or inhibitory at different stages of osteoblast differentiation.

Flattened bone lining cells are believed to be quiescent osteoblasts forming the endostem on trabecular and endosteal surfaces, and underlying the periosteum on the mineralized surface. Osteoblasts and lining cells are found in close proximity, and joined by adherens gap junctions. Cadherins are calcium-dependent transmembrane proteins that are integral components of adherens junctions, and together with tight junctions and desmosomes, join cells together by linking their cytoskeletons [36].

Osteoblast precursors change shape from spindle-shaped osteoprogenitors to large cuboidal differentiated osteoblasts on bone matrix surfaces after pre-osteoblasts stop proliferating. Preosteoblasts found near functioning osteoblasts in the bone remodeling unit usually express alkaline phosphatase. Active mature osteoblasts synthesizing bone matrix have large nuclei, enlarged Golgi structures, and extensive endoplasmic reticulum. Active osteoblasts secrete type I collagen and other matrix proteins vectorially toward the bone formation surface.

Populations of osteoblasts are heterogeneous, with different osteoblasts expressing slightly different sets of genes that may explain the heterogeneity of trabecular microarchitecture seen at different skeletal sites. Variation in gene expression also likely explains anatomic site-specific differences in multiple disease states, and regional variation in the ability of osteoblasts to respond to agents used to treat bone disease.

2.4. Bone loss

As a result of the age-related changes in osteoclast and osteoblast function described, bone density and structure deteriorate over time and lead to increased fracture risk in both women and men. Distal forearm (Colles’) fractures increase rapidly in women after menopause, and then remain relatively constant from about 10 to 15 years after menopause until the end of life. In contrast, vertebral fractures increase more slowly after menopause, but continue to increase exponentially during later life. Hip fractures in women increase more slowly than vertebral fractures after menopause, but continue to increase throughout life also, and increase rapidly in later life. In men, however, distal forearm fractures do not appear to increase with normal aging, probably because larger bone size protects against them. Vertebral and hip fractures increase gradually with aging in men, beginning about a decade later compared to women, likely due to their relative preservation of gonadal steroid production until later in life.

It is estimated that 40% of Caucasian women aged 50 years or older will develop a vertebral, hip, or wrist fracture sometime during the remainder of their lives, and that this risk increases to about 50% if vertebral fractures detected only by radiological imaging are included in the estimate [37]. It is estimated that about 13% of Caucasian men will sustain similar fractures. The risk of these fractures is somewhat lower in non-Caucasian women and men. It is estimated that osteoporotic fractures cost the U.S. between $12.2 to $17.9 billion each year, as measured in 2002 dollars [38].

3. Signaling pathways

Signaling pathways are critical in regulation of how osteoclasts and osteoblasts control bone turnover leading to bone loss after menopause and during normal aging. Activation of pathways that stimulate osteoclast recruitment and activation lead to bone loss unless other factors prevent this, and those that stimulate osteoblast recruitment and activation lead to bone formation, unless other factors prevent this. Whereas currently available agents used to treat osteoporosis mostly inhibit bone resorption, newer agents will largely focus on targets in signaling pathways that stimulate osteoblast function.

4. Antiresorptive pathways

Antiresorptive agents reduce bone turnover relatively rapidly, by reducing bone resorption initially, followed by a slower reduction in bone formation. These changes lead to an altered balance between bone resorption and formation at a lower rate of bone turnover that favors an increase in BMD, preservation or strengthening of structural and material properties of bone, increase in bone strength, and reduction in fractures [39].

4.1. Bisphosphonates and denosumab

Nitrogen-containing bisphosphonates block osteoclast function by inhibiting farnesyl pyrophosphate synthase, an enzyme in the mevalonate cholesterol synthesis pathway which is critical for membrane protein prenylation, thereby disrupting the osteoclast cytoskeleton and leading to osteoclast detachment from bone, reduced bone resorption, and osteoclast apoptosis [40]. Denosumab inhibits RANKL produced by osteoblasts from stimulating osteoclast activation, thereby leading to reduced differentiation and activation of osteoclasts, as well as apoptosis [41].

4.2. Cathepsin K

Recognition of signaling pathway targets has led to the development of the newer antiresorptive agents. Cathepsin K is a cysteine protease that cleaves the triple helix of type I collagen, in addition to cleaving collagen telopeptides from the N- and C-termini of this collagen. These actions contribute to resorption of the proteinaceous bone matrix containing type I collagen [42]. Cathepsin K inhibitors have been developed to block the action of this enzyme and limit bone resorption.

5. Anabolic pathways

Anabolic agents stimulate bone turnover significantly and relatively rapidly by stimulating bone formation initially, followed by slower stimulation of bone resorption. These changes lead to an altered balance between bone formation and resorption, at a higher rate of bone turnover, which leads to an increase in BMD, preservation or strengthening of structural and material properties of bone, increase in bone strength, and reduction in fractures.

5.1. PTH

Teriparatide (rhPTH 1–34) is the only anabolic agent approved for treatment of osteoporosis in the U.S. Recombinant human PTH 1–84 is approved for treatment of osteoporosis in Europe and other countries. Both of these PTH peptides stimulate osteoblast function by binding to the PTH/PTHrP type 1 receptor and activating several signaling pathways, including the canonical Wnt-signaling pathway. These pathways are stimulated, in part, by PTH actions expressed through PTH/PTHrP type 1 receptors on osteocytes [43].
5.2. PTHrP

PTHrP peptides such as PTHrP 1–36 or PTHrP 1–34 (abaloparatide) bind to the PTH/PTHrP type 1 receptor also, and activate signal transduction with equal potency to PTH. In a clinical trial, PTHrP 1–36 appeared to stimulate bone formation with equal potency to PTH, but led to reduced increases in bone resorption [44].

5.3. Wnt signaling pathway

Stimulators of the Wnt-signaling pathway are being actively explored for their anabolic effects on bone [45] (Fig. 1). Sclerostin is produced by osteocytes during late stages in their differentiation, after the start of mineralization of the surrounding matrix [46]. Newly synthesized sclerostin is transported to the bone surface by the canalicular network of osteocytes within bone, and inhibits osteoblast function and stimulates osteoblast apoptosis [47,48]. Sclerostin also plays an autocrine role and upregulates RANKL synthesis in osteocytes, leading to stimulation of osteoclastogenesis [49]. Sclerostin antagonizes the Wnt-signaling pathway in osteoblasts leading to reduced osteoblast proliferation, differentiation, and survival [50–52].

Wnt ligands normally bind to LRP5/6 and Frizzled co-receptors at the cell surface to cause transduction of a signal that stimulates activation and accumulation of intracellular beta-catenin, which translocates to the nucleus and stimulates transcription of target genes in osteoblasts (Fig. 2). Sclerostin binds to the first propeller domain of the LRP5/6 receptor, inhibiting the formation of the LRP5/6–Frizzled co-receptor complex, which results in inhibition of the Wnt-signaling pathway [51]. Sclerostin may require LRP4 as a cofactor to be fully functional, similar to Kremen, which is necessary for another Wnt pathway inhibitor, Dkk1, to fully inhibit the Wnt signaling pathway. Biomechanical strain and high PTH levels cause downregulation of sclerostin production in osteocytes, and lead to stimulation of bone formation [53].

Dickkopf 1 (Dkk1) also binds to the extracellular domains of LRP5/6 to prevent interaction with Wnts, which leads to inhibition of the formation of the LRP5/6–Frizzled co-receptor complex, resulting in inhibition of the Wnt-signaling pathway [54]. Dkk1 is produced by osteocytes, but also by other cells in the body, and may not be expressed in the bones of older animals. These observations may make Dkk1 inhibitors potentially less useful as treatments for osteoporosis. Because Dkk1 is secreted by multiple myeloma cells to block osteoblasts from filling in resorbed bone in lytic lesions, Dkk1 inhibitors are being explored as treatments for multiple myeloma.

Other more distal targets within the Wnt-signaling pathway may also be useful in treatment of osteoporosis. Glycogen synthase kinase (GSK)-3β is central in the Wnt-signaling pathway, with inhibition of GSK-3β stabilizing beta-catenin and activating later steps in the pathway. Lithium chloride inhibits GSK-3β, resulting in increased bone formation and bone mass in mice [55]. GSK-3β is also involved in other signaling pathways, however, and numerous growth factors regulate its activity, limiting its utility as a target for treatment of osteoporosis.

5.4. Activin A

Activin A is a member of the TGFβ superfamily that has been reported to inhibit bone mineralization and stimulate osteoclastogenesis [56]. Its mechanism of action is not yet fully understood. Soluble activin A type II receptor (ActRIIA) has been shown to block this signaling pathway and to prevent inhibition of bone formation.

5.5. Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) stimulate bone formation when given locally by injection for non-union of fractures. Unfortunately, BMP signaling occurs in many tissues, is mitogenic, and has a short half-life, making it less useful for treatment of systemic osteoporosis. Proteosome inhibitors are chemotherapeutic agents that stimulate BMP-2 expression, so these may have potential benefits in bone tissue, and could serve as anabolic therapies. Although growth hormone and insulin-like growth factor-I also stimulate bone formation, these hormones also act in many other tissues, limiting their applicability for treatment of osteoporosis.

5.6. Nitroglycerin

Nitroglycerin, which stimulates the nitric oxide pathway, has been shown to simultaneously stimulate bone formation and inhibit bone resorption. Epidemiologic studies have shown that patients using nitrate therapy have increased BMD compared to those not using it. Women treated with nitroglycerin ointment for two years had modestly increased bone mineral density, with a decrease in urinary NTX and non-significant increase in serum bone alkaline phosphatase [57], but the study was not powered to detect fractures.

6. Therapeutic targets for osteoporosis

Newer therapies for osteoporosis are targeted to molecules discovered during elucidation of signaling pathways in osteoclasts and osteoblasts (Table 1). The limitations of new therapies are mostly related to their specificity of action and safety. Agents that also affect tissues other than bone may result in off-target effects, which cause adverse events in clinical trials.

7. Antiresorptive agents

Most currently approved therapeutic agents primarily prevent bone loss. There is continued interest in development of new antiresorptive agents that offer selective advantages over existing agents. Because newer antiresorptive agents do not markedly suppress bone formation, and may uncouple bone formation from resorption, these compounds offer the potential of creating anabolic windows of variable magnitude and duration to stimulate new bone formation.

7.1. Cathepsin K inhibitors

The category of new antiresorptive agent farthest along in development is cathepsin K inhibitors [58,59]. Cathepsin K is a major digestive enzyme that breaks down type I collagen secreted by activated osteoclasts during bone resorption. Because cathepsin K inhibitors selectively target one of the main osteoclast digestive enzymes, but do not inhibit other digestive enzymes, or block acid secretion by actively resorbing osteoclasts, their antiresorptive effect is milder than that of more potent antiresorptive agents such as bisphosphonates or denosumab that markedly decrease both bone resorption and bone formation. Cathepsin K inhibitors, like most of the newer agents, have rapid offset of action, so patients taken off this type of drug will require addition of another medication to prevent loss of bone previously gained with cathepsin K inhibitor treatment.

Odanacatib is a new selective cathepsin K inhibitor that causes a moderate sustained decrease in bone resorption, but a lesser and more transient decrease in bone formation in completed clinical trials in postmenopausal women [60–62]. Preclinical studies
in mice, rabbits, or monkeys showed that cathepsin K deficiency led to maintenance of, or an increase, in bone formation [63,64]. These changes resulted in a moderate increase in BMD, similar to what is seen with alendronate. Odanacatib has completed phase III clinical trials in postmenopausal women and older men in the U.S., and is undergoing adjudication of adverse events requested by the FDA. Odanacatib does not decrease osteoclast survival or limit bone formation, unlike bisphosphonates and other available antiresorptive agents, and appears to uncouple bone resorption from bone formation. This uncoupling leads to maintenance of bone formation with moderately decreased bone resorption, resulting in an anabolic effect.

ONO-5334 is another cathepsin K inhibitor that has completed phase I and II clinical trials [65,66]. The phase II OCEAN clinical trial [66] demonstrated that this agent decreased bone resorption similar to alendronate, with little or no change in bone formation, in a dose ranging study. Lumbar spine, femoral neck, and total hip BMD increased comparably to what was seen with alendronate. No clinically relevant safety concerns were identified in the trial. Early cathepsin K inhibitors demonstrated off-target effects including plaque-like skin thickening (morphea) that led to discontinuation of their clinical development. Balacatib was reported to cause morphea [67], perhaps because of cathepsin K inhibition not just in osteoclasts, but also in skin and pulmonary fibroblasts. Similar effects have not been seen to date with odanacatib, ONO-5334, or other cathepsin K inhibitors still under development.
Fig. 2. WNT signaling pathway members regulate bone mass: lessons from mouse genetics. Across all studies, increases in bone mass were observed as a result of pathway activation, and decreases in bone mass were observed as a result of pathway inhibition, although the relative impact on bone formation and resorption varied, reflecting the complex fine tuning of the WNT regulatory network. Alteration in the gene dosage of members of the pathway altered bone formation and resorption to varying degrees. Obl, osteoblast; Ocy, osteocyte; Obl/Ocy, osteoblast and osteocyte; TG, transgenic; gof, gain-of-function; O VX, ovariectomy; ECD, extracellular domain; Δ, deletion; BAC, bacterial artificial chromosome. Blue and red boxes indicate an effect on formation and resorption, respectively, and purple boxes indicate an effect on both. The direction of the arrows in the text boxes indicates an increase or decrease of bone resorption (red) or bone formation (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Source: Reproduced by permission from Baron and Kneissel [45].

8. Anabolic agents

Anabolic agents increase bone strength by directly stimulating new bone formation. A number of potential targets for anabolic agents have been identified [68]. New parathyroid hormone (PTH) and PTH-related protein (PTHrP) analogues remain under development. New anabolic targets being evaluated stimulate the Wnt-signaling pathway in osteoblasts. Monoclonal antibodies to these targets block inhibition of the pathway, leading to stimulation of new bone formation.

8.1. PTH analogues

The only anabolic therapies currently approved to treat osteoporosis are recombinant forms of human PTH. Human recombinant PTH 1–34 (teriparatide) [69] and PTH 1–84 [68] have been approved in many countries to treat osteoporosis. Stopping treatment with these agents leads to rapid bone loss within six months, so patients are usually switched to long-acting bisphosphonates or other antiresorptive agents to consolidate BMD gained during treatment [70].

PTH analogues are given by daily subcutaneous injection. Their most common side effects are mild asymptomatic hypercalcemia and hypercalciuria [70,71]. Teriparatide was given a black box warning by the FDA because of an increased risk of osteogenic sarcoma in the Fischer 344 rat in one preclinical study. There have been no human osteogenic sarcoma cases to date known to be caused by teriparatide or other PTH analogues [72]. PTH analogues are expensive to produce, and cost more than other agents used to treat osteoporosis.

A relatively recent clinical trial in Japan showed that higher-dose teriparatide given by once weekly subcutaneous injection significantly increased BMD [73]. Once monthly and once yearly administration of PTH fused to a collagen-binding domain fusion protein has been shown to lengthen its anabolic activity in mice [74,75]. The pharmacokinetic profile of a single dose of a novel oral PTH formulation has been evaluated in healthy postmenopausal women [76]. Tablet and transdermal patch formulations are being developed.

Because intermittent subcutaneous administration of PTH causes anabolic effects on bone, short-term intermittent antagonism of the parathyroid calcium-sensing receptor (CaSR) by calcilytic compounds may result in short bursts of endogenous PTH secretion. Ronacaler, an oral CaSR antagonist, transiently stimulated PTH secretion, but PTH release was prolonged enough to cause bone loss, similar to what is seen in primary hyperparathyroidism [77]. A preclinical study showed that JTT-305, another oral CaSR antagonist, stimulated transient PTH secretion and new bone formation in ovariectomized rats [78].

8.2. PTH-related protein analogues

PTHrP was initially identified to be the main cause of hypercalcemia of malignancy. Intermittent injection of recombinant analogues of PTHrP is being investigated to see if these might improve BMD and reduce fractures. In a previous clinical trial,
intravenous rhPTH 1–36 was shown to stimulate bone formation effectively, with a reduced increase in bone resorption, compared to rhPTH 1–34 [44]. Human recombinant PTHrP 1–34 (abaloparatide) was previously shown to have similar effects to teriparatide in a small clinical trial. In a larger clinical trial, the same agent was recently shown to cause significant increases in lumbar spine, femoral neck, and total hip BMD in a dose-dependent fashion with daily subcutaneous injection for 24 weeks [79]. The increase seen at the total hip was greater than seen with the teriparatide control.

### 8.3. Activators of the Wnt/β-catenin signaling pathway

Binding of Wnt to its 7-transmembrane receptor and LRP 5/6 in osteoblasts causes inhibition of formation of intracellular GSK-3β. Inhibition of Wnt binding leads to increased GSK-3β, which leads to breakdown of β-catenin. Decreased GSK-3β activity leads to increased translocation of β-catenin to the nucleus, causing transcriptional co-activation of genes integral to bone formation [80].

An anti-sclerostin monoclonal antibody (AMG 785) stimulated bone formation and increased BMD and bone strength in a rat preclinical study [81]. A randomized, placebo-controlled phase I study in 72 healthy adults showed that a single subcutaneous dose of anti-sclerostin antibody (romosozumab) increased BMD [82]. Subcutaneous doses ranging from 0.1 to 10 mg/kg were studied in 56 subjects, with intravenous doses of 1 or 5 mg/kg studied in 16 subjects. A single dose of romosozumab by either the subcutaneous or intravenous route caused a dose-dependent increase in markers of bone formation, and a dose-dependent decrease in a marker of bone resorption. A dose-dependent increase in BMD was seen as early as one month after administration. The largest BMD increases seen were 5.3% at the lumbar spine, and 2.8% at the total hip, on day 85 after a single 10 mg/kg subcutaneous dose. Romosozumab was well tolerated at all doses. Adverse events were mild, except for one subject who received the 10 mg/kg dose, who developed severe non-specific hepatitis. Liver enzymes were increased by the first day after dosing, but normalized by day 26.

Efficacy and safety of romosozumab was evaluated in a phase 2 randomized, placebo-controlled, parallel-group study over 12 months in 419 postmenopausal women who had low BMD [83]. Participants were randomly assigned to receive subcutaneous romosozumab monthly or every 3 months, subcutaneous placebo, open-label active comparator with oral alendronate

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**Table 1**

Newer biologic agents being developed for treatment of osteoporosis.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
<th>Dose and frequency</th>
<th>Treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denosumab</td>
<td>Anti-RANKL monoclonal antibody, ↓ bone resorption and ↑ BMD</td>
<td>60 mg SC q 6 months</td>
<td>- FREEDOM trial showed ↓ risk of new radiographic vertebral fractures by 68%, hip fractures by 40%, and nonvertebral fractures by 20% - 8 years of continuous treatment: LS BMD ↑ 16.5%, and TH ↑ 6.8% Reversible after stopping treatment</td>
</tr>
<tr>
<td>Teriparatide (rhPTH 1–34)</td>
<td>↑ Bone formation more than</td>
<td>20 mcg SC q day</td>
<td>- ↑ Bone formation and ↑ bone resorption - In phase 3 study, 20 mcg dose ↑ LS BMD by 9%, and ↑ FN by 3% by 21 months - ↓ Vertebra and nonvertebral fractures. - Offset of action occurs rapidly - Use limited to 2 years in U.S., 18 months in Europe</td>
</tr>
<tr>
<td>Anti-sclerostin antibodies:</td>
<td>Sclerostin inhibition</td>
<td></td>
<td>- Phase 3 fracture efficacy trial completed</td>
</tr>
<tr>
<td>Romosozumab</td>
<td>↑ Bone formation on quiescent surfaces, ↓ bone resorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blosozumab</td>
<td>↑ Bone formation and ↑ bone resorption, ↑ BMD</td>
<td>80 mcg SC q day maximum dose</td>
<td>- In phase 2 study, 80 mcg dose ↑ LS BMD by 6.7%, ↑ FN by 3.1%, and TH by 2.6% at 24 weeks - Offset of action occurs rapidly - Phase 3 fracture efficacy trials ongoing</td>
</tr>
<tr>
<td>Anti-DKK1 antibodies: BHQ880</td>
<td>Dkk1 inhibition</td>
<td>BHQ880 10 mg/kg dose for phase 2 trial</td>
<td>- Phase 1 trial completed for treatment of lytic bone lesions in multiple myeloma - Dkk1 production by multiple myeloma cells ↓ bone formation in lytic bone lesions - Dkk1 expression not restricted to bone</td>
</tr>
</tbody>
</table>

**Abbreviations**: LS, lumbar spine; FN, femoral neck; TH, total hip; BMD, bone mineral density.
(70 mg weekly), or subcutaneous teriparatide (20 mcg daily). All dose levels of romosozumab caused significant increases in BMD at the lumbar spine, including an increase of 11.3% with the 210-mg monthly dose, as compared with a decrease of 0.1% with placebo, and increases of 4.1% with alendronate and 7.1% with teriparatide. Romosozumab was also associated with large increases in bone mineral density at the total hip and femoral neck, as well as transitory increases in bone-formation markers and sustained decreases in a bone-resorption marker. Except for mild, generally non-recurring injection site reactions with romosozumab, adverse events were similar among groups.

Blosozumab, another anti-sclerostin antibody, was evaluated in a randomized, double-blind, placebo-controlled phase 2 clinical trial in postmenopausal women with low BMD [84]. The study randomized 120 subjects to subcutaneous blosozumab 180 mg every 4 weeks, 180 mg every 2 weeks, 270 mg every 2 weeks, or matching placebo for 1 year, with calcium and vitamin D supplementation. Blosozumab treatment resulted in statistically significant dose-related increases in spine, femoral neck, and total hip BMD as compared with placebo. In the highest dose group, BMD increases from baseline reached 17.7% at the spine, and 6.2% at the total hip. Biochemical markers of bone formation increased rapidly during blosozumab treatment, and trended toward pretreatment levels by the end of the study. However, bone specific alkaline phosphatase remained higher than placebo at study end in the highest-dose group. CTx, a biochemical marker of bone resorption, decreased early in blosozumab treatment to a concentration less than that of the placebo group by 2 weeks, and remained reduced throughout blosozumab treatment. Mild injection site reactions were reported more frequently with blosozumab than placebo.

To evaluate the effect of discontinuing blosozumab, women enrolled in the 1-year randomized, placebo-controlled phase 2 trial were followed for an additional year [85]. At the end of follow-up, lumbar spine and total hip BMD decreased, but remained significantly greater than placebo in women initially treated with blosozumab 270 mg every 2 weeks, and blosozumab 180 mg every 2 weeks. During follow-up, median serum P1NP was not consistently different between the prior blosozumab groups and placebo. A similar pattern was apparent for median serum CTx levels, with more variability. Mean serum total sclerostin concentration increased with blosozumab, indicating target engagement, and declined to baseline after discontinuation. Anti-drug antibodies generally declined in patients who had detectable levels during prior treatment.

Monoclonal antibodies to Dickkopf-1 are being developed. One of these, RH2-18, was given subcutaneously to ovariectomized mice and rhesus macaques, and caused increased BMD, especially at trabecular sites [86]. Eight weeks of weekly injections of RH2-18 in ovariectomized mice increased femoral BMD to the level of controls without ovariectomy, and partially restored lumbar spine BMD toward that of controls. RH2-18 given to mice every two weeks for 9 months increased lumbar spine BMD by 5.0% [88]. There have been no randomized controlled trials in humans evaluating the use of these agents for treatment of osteoporosis.

9. Conclusions

Major advances have occurred recently in the treatment of osteoporosis. Patients are most often treated currently with bisphosphonates, denosumab, raloxifene, or teriparatide, and in some countries, strontium ranelate. Calcitonin has been removed from the market in some countries, and its use restricted in others, due to risk of malignancy. Use of strontium ranelate has been reduced due to cardiovascular concerns. These agents have generally provided excellent options that effectively reduce fracture risk. The signaling pathways described in this review, with the new agents targeting these signaling pathways, will further expand the options available for treatment of osteoporosis.

Practice points

- Until the newer anabolic or antiresorptive agents are approved, physicians should continue to prescribe the current best agent uniquely suited for each patient.
- Use of the currently available agents should not preclude the use of new agents when these become available.
- Physicians and patients should be aware that, unlike the longer-acting bisphosphonates, all the newer agents are relatively shorter-acting, and have reasonably rapid offset of action.
- Physicians should consider using longer-acting bisphosphonates or denosumab once treatment with the new shorter-acting agents is completed.

Research agenda

- Continue clinical trials of the available new antiresorptive and anabolic agents.
- Continue development of newer therapeutic agents currently undergoing preclinical evaluation.
- Continue preclinical research on agents targeting anabolic pathways other than the Wnt/β-catenin signaling pathway to expand options for treatment.

Conflict of interest

The authors declare no conflict of interest.

Authors’ contribution

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