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The osteocyte as a therapeutic target in the treatment of osteoporosis

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***Abstract*:** Osteoporosis is characterized by a low bone-mineral density associated with skeletal fractures. The decrease in bone-mineral density is the consequence of an unbalanced bone-remodeling process, with higher bone resorption than bone formation. The orchestration of the bone-remodeling process is under the control of the most abundant cell in bone, the osteocyte. Functioning as an endocrine cell, osteocytes are also a source of soluble factors that not only target cells on the bone surface, but also target distant organs. Therefore, any drugs targeting the osteocyte functions and signaling pathways will have a major impact on the bone-remodeling process. This review discusses potential advances in drug therapy for osteoporosis, including novel osteocyte-related antiresorptive and anabolic agents that may become available in the coming years.

Keywords: drug therapy, osteocyte, osteoporosis

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Introduction

The mammalian skeleton is a greatly active tissue that undergoes continuous remodeling throughout childhood and adult life. Bone remodeling is needed for microfracture consolidation, and skeleton adaptation to mechanical use, and also for calcium homeostasis [Dallas *et al.* 2013]. This bone remodeling implicates the coupling of osteoclastic bone resorption and osteoblastic bone formation. Common diseases, such as osteoporosis, multiple myeloma, Paget's disease, and other bone-metastasized cancers, are characterized by imbalances between the formation and resorption processes [Devogelaer, 2000; Daci *et al.* 2002; Shankar *et al.* 2013]. As these pathologies contribute to increase morbidity and mortality worldwide, there is great concern towards improving our understanding of the processes that regulate bone remodeling [Shoback, 2007]. Osteoporosis, which occurs mainly in postmenopausal women, is characterized by excessive bone resorption compared with the formation of new bone. Osteoporosis is thus characterized by a loss of bone strength, a decrease in bone mass, and a worsening in bone quality, leading to an increased risk of fracture [Mosley, 2000].

In addition to the well-known behavior of mature osteoblasts and osteoclasts, and their respective precursor cells, on the bone-remodeling process, there is increasing evidence that osteocytes play important roles in detecting imperfections or microfractures and initiating a targeted bone remodeling [Verborgt *et al.* 2000; Kogianni and Noble, 2007; Heino *et al.* 2009]. Osteocytes originate from mesenchymal stem cells through osteoblast lineage differentiation, with only 10–20% of osteoblasts differentiating into osteocytes [Aubin and Turksen, 1996]. During this differentiation, osteocytes become embedded in the bone matrix during the modeling and/or remodeling processes where the bone matrix is synthesized [Rochefort *et al.* 2010]. Osteocytes remain active in the bone-remodeling process by maintaining connections to the bone surface, to osteoblasts and osteoclasts, and to other osteocytes through an extensive canalicular network. Osteocytes are able to release nitric oxide, prostaglandin E_2, and adenosine-triphosphate that activate bone formation, and sclerostin, Dickkopf-related protein 1 (DKK1), and frizzled-related protein 1 that inhibit bone formation. They are also able to release the receptor activator of the nuclear factor kappa-B ligand (RANKL) to support osteoclastogenesis, but also to secrete the bone-formation inhibitor sclerostin [Winkler *et al.* 2003; Van Bezooijen *et al.* 2004; Mulcahy *et al.* 2011; Nakashima *et al.* 2011; Moustafa *et al.* 2012]. Therefore, any drugs that target the remodeling cycle by affecting osteoblasts, osteoclasts, and osteocytes, and/or

molecules that control the signaling pathways, will have a major impact on the targeted bone remodeling [Killock, 2011; Moriishi *et al.* 2012].

The cellular and molecular basis of osteoporosis

Osteoporosis is a systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue with a consequent compromised bone strength and increased susceptibility to fracture. According to the clinical definition of osteoporosis proposed by the World Health Organization, a patient is osteoporotic when the dual-energy X-ray absorptiometry measurement of bone-mineral density is 2.5 standard deviations below the typical peak bone mass of young healthy women [Blake and Fogelman, 2007]. Osteoporosis occurs in both genders, at all ages, and can be separated into three types: (a) primary osteoporosis in which no underlying cause can be clearly identified, but often follows menopause in women and occurs later in life in men; (b) secondary osteoporosis in which the underlying cause is known (e.g. hyperparathyroidism, hypophosphatasia, diabetes types 1 and 2, alcoholism, glucocorticosteroid use, etc.); (c) more rare forms of the disease, such as juvenile, pregnancy-related, and *postpartum* osteoporosis [Taxel and Kenny, 2000; Schnatz *et al.* 2010; Cook *et al.* 2013].

Osteoporosis is associated with typical fractures (e.g. lumbar spine, femoral neck or distal radius, vertebral fractures, and any fracture resulting from a low trauma in the elderly) that are associated with an increase in morbidity and mortality [Lindsay, 1996; Garnero, 2008; Hopkins *et al.* 2013]. Therefore, the goal of osteoporosis therapies is to prevent these fractures by inhibiting bone resorption and/or by stimulating bone formation [Sun *et al.* 2013]. While antiresorptive drugs lower bone turnover [Lewiecki, 2013], anabolic therapies increase bone modeling and/or remodeling osteoblastic activity [Khan and Khan, 2006].

Osteoporosis treatments

The most widely prescribed and first-line drugs for bone diseases are the bisphosphonates, such as alendronate, risedronate, ibandronate, or zoledronic acid [Fleisch, 2002]. These molecules are generally considered to be safe drugs, with the clinical benefits surpassing the risks associated with treatment. Due to their widespread usage in many patients suffering from different diseases, several adverse effects have been reported, including nausea, abdominal pain, ocular inflammation, difficulty in swallowing, and the risk of an inflamed esophagus or esophageal ulcers. However, the relationship between the drug and adverse events is often difficult to establish because clear correlations are often missing due to comedication or comorbidities. One of the most severe adverse events of bisphosphonate treatment is jawbone osteonecrosis, defined as an exposed area of bone in the maxillofacial region after a tooth extraction in which a section of jawbone persists for at least 8 weeks [Khosla *et al.* 2007; Rizzoli *et al.* 2008], dies, and deteriorates [Aspenberg, 2006]. Incidence of this rare side effect ranges from 1 per 10,000–110,000 patient years to approximately 10% in patients with myeloma [Khosla *et al.* 2007; Rizzoli *et al.* 2008]. The occurrence of jawbone osteonecrosis is often connected to underlying dental problems. In addition, bisphosphonate treatment has been linked to stress and atypical fractures of the femoral shaft, but data are not always significant [Schilcher *et al.* 2011]. These stress fractures seem to occur through inhibition of bone remodeling. When a patient is undergoing long-term bisphosphonate treatment, bone microdamages are not always repaired, and thus accumulate, and eventually lead to fractures occurring on compact bones at sites of high tensional stress.

Hormone-replacement therapies are also an established approach to the treatment and prevention of osteoporosis, with significant improvement in bone-mineral density, and reduction in hip and vertebral fracture. Estrogen started soon after menopause helps to maintain bone density. However, estrogen therapy in women may increase blood clots, and risk of endometrial cancer, breast cancer, and possibly heart disease [Maclean *et al.* 2008]. Treatment mimicking estrogen, such as raloxifene, has significant beneficial effects on bone density in postmenopausal women, without some of the side effects associated with estrogen, such as breast cancer. Osteoporosis in men may be related to a gradual age-related decrease in testosterone levels that may be treated by testosterone-replacement therapy, which has a lower impact on bone density than direct osteoporosis medications [Maclean *et al.* 2008].

In addition to these treatments, other bone anabolic pathways can be targeted, such as the

powerful parathyroid hormone (PTH) analogous teriparatide that stimulates new bone growth and is more dependent on increasing the activation frequency, or the monoclonal antibody denosumab that binds to RANKL, a protein involved in the formation, function, and survival of bone-resorption osteoclasts. New treatments under development are aimed at targeting sclerostin and the canonical wingless-int (Wnt) signaling, which is more dependent on increasing bone modeling [Bringhurst, 2002; Deal, 2009; Lim and Clarke, 2012].

The osteocyte within bone tissue

Osteocytes, the most abundant cells in bone, represent 90–95% of all cells in the adult skeleton [Rochefort *et al.* 2010]. They are able to live for several decades within the bone matrix, whereas osteoblasts and osteoclasts have a lifespan of only a few days or weeks [Bonewald, 2011]. Osteocytes represent the terminal differentiation of osteoblasts [Klein-Nulend *et al.* 2003]. These cells send out long dendritic processes (the dendrites) through fine channels within the bone matrix (the canaliculi), thus forming a large dendritic network connecting these cells with each other and with osteoblasts, lining cells, and osteoclasts [Rosser and Bonewald, 2012]. Osteocytes and their dendritic processes are bathed in an interstitial fluid (the bone fluid flow) [Bivi *et al.* 2012]. The osteocytic dendrites are particularly important in the mechanical sensitivity of these cells [Burra *et al.* 2010], as well as the mechanical signals recorded by the cilia [Hoey *et al.* 2011; Uzbekov *et al.* 2012]. More sensitive than osteoblasts, osteocytes are able to respond to mechanical stimulation, particularly shear stress forces [Klein-Nulend *et al.* 2002], by secreting several molecules, including insulin-like growth factors, osteocalcin, sclerostin, c-fos, prostanoids, and nitric oxide [Uzbekov *et al.* 2012; Dallas *et al.* 2013]. Among all the functions assigned to this cell (see an overview in Figure 1) [Dallas *et al.* 2013]), osteocyte mechanoreception may stimulate the Wnt-signaling pathway as a negative regulator of sclerostin secretion, itself acting as a negative regulator of bone formation [Ozcivici *et al.* 2010; Post *et al.* 2013].

PTH-related therapies

The mechanism of action of the recombinant human PTH drug is still under investigation, but it probably affects multiple signaling pathways and alters the biological activity of several bone cells, including osteoblasts, lining cells, osteoclasts, and osteocytes [O'Brien *et al.* 2008: Bellido *et al.* 2013]. The PTH stimulates bone formation by increasing the number of osteoblasts [Wang *et al.* 2007]. The PTH effects are mediated by a G-protein-coupled receptor, the PTH receptor 1 [Maeda *et al.* 2013; Van Der Lee *et al.* 2013]. Different recombinant peptides, mimicking this PTH receptor 1, have different anabolic effects. Thus, the cyclic amino-terminal fragment may have a more anabolic profile than the $PTH_{1–34}$ or $PTH_{1–84}$ fragments [Fraher *et al.* 1999; Whitfield, 2006; Henriksen *et al.* 2013]. Selected amino-acid substitutions at various positions in the $PTH_{1–28}$ fragment have also revealed an increased activity of this recombinant hormone [Yang *et al.* 2007].

The release regulation of PTH, and the related regulation of calcium homeostasis, is under the influence of the calcium-sensing receptor, a G-protein-coupled, seven-pass transmembrane molecule present in the parathyroid gland, the kidney, and in osteoblasts and osteocytes [Brown, 2007; Fromigue *et al.* 2009; Xue *et al.* 2012]. Allosteric modulators of this calcium-sensing receptor can affect the secretion of PTH [Trivedi *et al.* 2008; Riccardi, 2012]. Positive calcium-sensing receptor agonists, called calcimimetics, such as cinacalcet, can reduce PTH secretion in patients with hyperparathyroidism and renal disease [Li *et al.* 2013; Tsuruta *et al.* 2013], whereas negative antagonists of this receptor, called calcilytics, can inhibit the receptor function thus inducing the release of a PTH pulse [Cabal *et al.* 2013]. Therefore, these molecules may represent new targets in the treatment of osteoporosis [Fraser *et al.* 2004; Nemeth, 2004; John *et al.* 2011]. However, to be useful as anabolic agents, calcilytic agents must induce the release of sufficient PTH to be anabolic, they must have a short half-life since sustained activation would result in prolonged PTH secretion and a catabolic state (hyperparathyroidism), and they should not deplete the parathyroid gland, and not result in hyperplasia [Avlani *et al.* 2013]. Recently, a calcilytic agent, called ronacaleret, has shown a strong PTH response, with a short half-life, and an increase in both cortical and trabecular bone formation in rodents [Balan *et al.* 2009; Atchison *et al.* 2011]. However, a recent clinical trial involving ronacaleret in humans showed a small, non-dose-dependent increase in bone-mineral density in the lumbar spine at 6 months [Fitzpatrick *et al.* 2012].

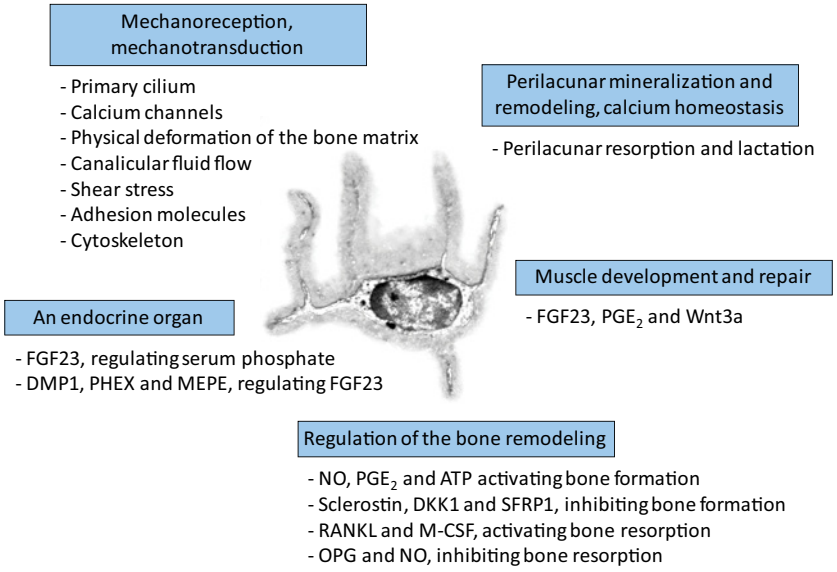

Figure 1. Functions assigned to osteocytes.
The osteocyte has several functions: (a) it senses and integrates mechanical signals (mechanoreception), and converts them into a biological message (mechanotransduction); (b) it directs the differentiation and activity of osteoblasts through the release of NO, PGE2, and ATP that activates bone formation, and sclerostin, DKK1, and SFRP1 that inhibits bone formation; (c) it directs the differentiation and activity of osteoclasts through the secretion of RANKL and M-CSF that activates bone resorption, and OPG and NO that inhibits bone resorption; (d) it controls the local mineralization of the surrounding bone matrix and calcium homeostasis that it can lyse locally to release calcium into the systemic bloodstream during periods of high demand (e.g. lactation); (e) it has an endocrine function by releasing into the bloodstream a specific endocrine factor, FGF23 and its related regulating factors DMP1, PHEX, and MEPE, to modulate phosphate homeostasis; (f) it may modulate the proliferation and tone of skeletal striated muscle cells through the expression of FGF23, PGE2,and Wnt3a. (Adapted with the publisher's permission from Dallas *et al*. [2013] and Rochefort and Benhamou [2013].) ATP, adenosine-triphosphate; DKK1, Dickkopf-related protein 1; DMP1, dentin matrix protein 1; FGF23, fibroblast growth factor 23; M-CSF, macrophage-colony stimulating factor; MEPE, matrix extracellular phosphoglycoprotein; NO, nitric oxide; OPG, osteoprotegerin; PGE2, prostaglandin E2; PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome; RANKL, receptor activator of nuclear factor kappa-B ligand; SFRP1, frizzled-related protein 1.

Recent studies demonstrated that some actions of PTH on the skeleton are mediated by direct effects on osteocytes [Bellido *et al.* 2013]. PTH thus down-regulates the expression of the *SOST* gene, encoding the potent inhibitor of bone formation sclerostin expressed in osteocytes (see below) [Bellido *et al.* 2005; Keller and Kneissel, 2005]. Furthermore, PTH increases the expression of fibroblast growth factor 23, a hormone expressed in osteocytes (and osteoblasts) that regulates phosphate reabsorption in kidney and contributes to mineral homeostasis [Lavi-Moshayoff *et al.* 2010; Bellido *et al.* 2013].

Wnt signaling, sclerostin, and DKK1

Wnt proteins are a large family of extracellular (secreted) cysteine-rich glycoproteins that regulate bone remodeling, but are also involved in several other physiopathological situations, including prostate adenocarcinoma [Yu *et al.* 2011], renal cancer [Banumathy and Cairns, 2010], most sporadic colorectal cancers [Scholer-Dahirel *et al.* 2011], melanoma [Lucero *et al.* 2010], breast cancer [Bu *et al.* 2008], as well as parathyroid carcinoma [Svedlund *et al.* 2010], and glioma [Liu *et al.* 2011].

The biological action of Wnt passes through canonical and noncanonical pathways. Canonical Wnt signaling employs extracellular Wnt ligands that bind frizzled and lipoprotein receptor-related protein (LRP) 5/6 coreceptors at the cell surface to transduce a signal that results in the intracellular activation of β-catenin (Figure 2). This canonical Wnt pathway regulates production of the β-catenin transcription factor by inhibiting its phosphorylation, ubiquitination, and degradation. Noncanonical Wnt signaling is defined as Wnt-initiated or frizzled-initiated signaling that is independent of β-catenin transcriptional function [Lee *et al.* 2010; Baron and Kneissel, 2013]. Noncanonical Wnt pathways are diverse and include Wnt-cGMP/Ca2+ signaling, Wnt-ROR2

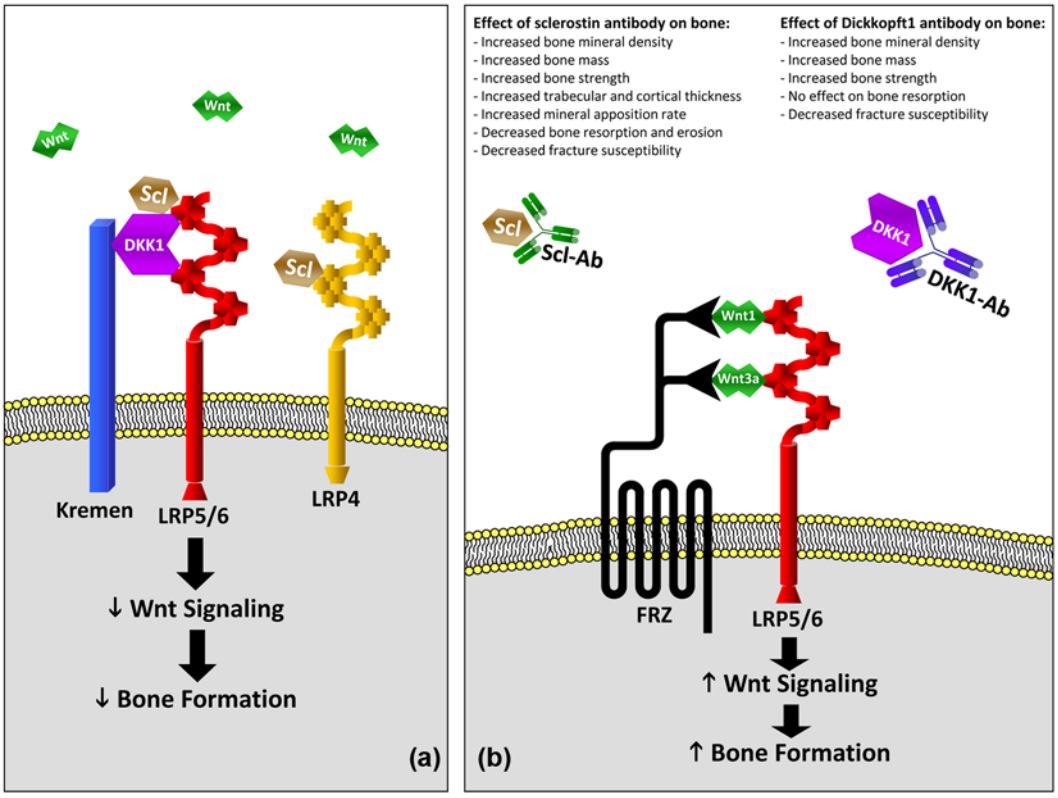

Figure 2. Wnt pathway, sclerostin, and DKK1; effects of monoclonal antibodies on bone.
(a) Scl and DKK1 bind Wnt coreceptors LRP5/6 to inhibit Wnt binding and signaling to decrease bone formation. Scl and DKK1 bind the first β-sheet of LRP5 and LRP6 to inhibit Wnt-1 signaling. DKK1 also binds the third β-sheet to inhibit Wnt-3a signaling. DKK1 and Scl can also utilize coreceptors, such as Kremen-1 or -2 receptors, to augment Wnt inhibitory activity resulting in the internalization of the complex. (b) Scl-Ab and DKK1-Ab prevent the interaction of these molecules with LRP5 and LRP6, thereby allowing Wnt-1 and Wnt-3a to bind the first and third β-sheet of LRP5/6, respectively. Actions of these antibodies on bone are summaries. Wnt proteins (either Wnt-1 or Wnt-3a) form a complex with FRZ receptors and LRP5/6 to transduce an intracellular signal leading to increased bone formation. (For an in-depth review see Ke *et al.* [2012]). DKK1, Dickkopf-related protein 1; DKK1-Ab, Dickkopf-1 antibody; FRZ, frizzled; LRP, lipoprotein receptor-related protein; Scl, sclerostin; Scl-Ab, sclerostin antibody.

signaling, Wnt-RYK signaling, Wnt-mTOR signaling, etc. [Lee *et al.* 2010; Sassi *et al.* 2013].

The Wnt protein also allows the activation of a protein complex consisting of axin, adenomatous polyposis coli, and glycogen synthase kinase 3 activating an intracellular signal. When the Wnt protein is absent, the membrane receptors frizzled and LRP5/6 are dissociated and glycogen synthase kinase 3 phosphorylates β-catenin, which is then degraded through the ubiquitin/proteasome pathway [Boudin *et al.* 2013]. When the Wnt protein is present, the frizzled membrane receptors are associated with LRP5/6, the protein complex is disrupted, and the phosphorylation of β-catenin does not occur. Therefore β-catenin accumulates, and is then translocated to the cell nucleus, and binds to transcription factors that can affect the transcription of genes related to bone formation [Lee *et al.* 2010; Baron and Kneissel, 2013; Boudin *et al.* 2013].

Elements of the Wnt-signaling pathway are well conserved in evolution and are found in primitive metazoans, such as cnidarians [Lengfeld *et al.* 2009]. The Wnt antagonist DKK1 is expressed by early invertebrates, such as the Hydra [Guder *et al.* 2006], whereas the expression of sclerostin is not found until the emergence of bony vertebrates, indicating a more specific role for sclerostin in the development and maintenance of the skeleton and a broader role for DKK1.

In addition to binding LRP5/6, both sclerostin and DKK1 can bind other transmembrane molecules, such as LRP4, also known as multiple epidermal growth factor-like domains 7 (Megf7) to increase their inhibitory activity on the

Wnt-signaling pathway (Figure 2) [Choi *et al.* 2009]. Indeed, Megf4 KO mice exhibit limb abnormalities with polysyndactyly [Simon-Chazottes *et al.* 2006], whereas loss-of-function mutations of this factor in humans, also known as Cenani–Lenz syndrome, cause syndactyly, kidney malformations, and bone overgrowth [Li *et al.* 2010b]. This indicates that LRP4 acts as a negative regulator of LRP5/6 signaling by increasing the inhibitory activity of sclerostin on the Wnt pathway, without affecting the activity of DKK1 [Leupin *et al.* 2011].

DKK1 may also bind to a two-member family of proteins referred to as Kremen-1 and Kremen-2, leading to the removal of LRP5/6 from the cell surface (Figure 2) [Mao *et al.* 2002]. Invalidation of both Kremen-1 and -2 in mice lead to subtle patterning defects in the forelimb with increased bone formation that were additionally enhanced by the deletion of a single DKK1 allele, indicating the importance of these factors in the regulation of bone mass through modulation of LRP5/6 [Ellwanger *et al.* 2008; Schulze *et al.* 2010]. However, the homozygous deletion of either Kremen-1 or -2 lead to normal bone formation and bone mass, suggesting a functional redundancy of Kremen-1 and -2 [Schulze *et al.* 2010].

Inhibitors of the Wnt pathway can target either frizzled (serum frizzled-related proteins), Wnt (Wnt inhibitory factors), or LRP5/6 (DKK1 and the osteocyte-released sclerostin) (Figure 2) [Rybchyn *et al.* 2011]. These agonist molecules can prevent Wnt from activating the frizzled LRP5/6 signaling pathway, inducing a decrease in Wnt signaling, and thus a significant bone decrease in bone formation [Glantschnig *et al.* 2011]. On the other hand, deficiencies in these inhibitors or antibodies targeting these inhibitors induce an increase of Wnt signaling and, therefore, induce an increase in bone formation [Glantschnig *et al.* 2010].

Complexity of Wnt signaling has increased since the recent description of distinct ligand-binding domains on LRP5/6 receptors that recognize different classes of Wnt proteins and inhibitors [Bourhis *et al.* 2010; Ettenberg *et al.* 2010; Gong *et al.* 2010]. The Wnt1 class, comprising Wnt 1, 2, 6, 7a, 7b, 9a, 9b, and 10b, was reported to bind the first β-sheet of LRP5/6, whereas the Wnt3 class, including Wnt3 and 3a, was shown to bind the third β-sheet of LRP5/6 (Figure 2). DKK1 was revealed to bind indifferently the first and third β-sheets of LRP5/6 [Bourhis *et al.* 2010], whereas sclerostin bound only the first β-sheet of LRP5/6 (Figure 2) [Ettenberg *et al.* 2010]. Therefore, DKK1 inhibited both the Wnt1 and Wnt3 classes, and sclerostin inhibited the Wnt1 class and enhanced the Wnt3 class [Ettenberg *et al.* 2010].

The sclerostin protein is encoded by the human *SOST* gene [Winkler *et al.* 2003; Robling *et al.* 2008; Cohen-Kfir *et al.* 2011]. Homozygous mutation of loss of expression of the *SOST* gene is responsible for two rare genetic disorders, sclerosteosis and van Buchem disease, which are associated with general progressive skeletal overgrowth and sclerosis of the axial and appendicular skeleton [Brunkow *et al.* 2001; Bhadada *et al.* 2013]. In sclerosteosis, bone formation is stimulated by the absence or decreased synthesis of sclerostin in humans, whereas bone resorption is not (or only mildly) affected [Van Lierop *et al.* 2011]. Van Buchem disease is a disorder resembling sclerosteosis but distinguished by its less severe phenotype and absence of hand malformations, such as syndactyly [Staehling-Hampton *et al.* 2002]. The difference between these two disorders might be explained by the fact that the deleted genomic region in van Buchem disease includes no regulatory elements required for sclerostin expression during the embryologic steps of digit formation [Brunkow *et al.* 2001; Uitterlinden *et al.* 2004]. Finally, heterozygous mutations in the *SOST* gene cause a mild increase in bone mass and fewer skeletal complications [Van Lierop *et al.* 2011].

SOST mRNA is expressed in many tissues during embryogenesis. However, the sclerostin protein is found only postnatally in terminally differentiated cells embedded within a mineralized matrix, including osteocytes, mineralized hypertrophic chondrocytes, and cementocytes [Ohyama *et al.* 2004]. In humans, SOST mRNA is detectable in tissues such as heart, aorta, liver, odontoblasts, and kidney, whereas sclerostin protein has never been detected in any organs other than bone [Balemans *et al.* 2001; Moester *et al.* 2010]. Since the sclerostin protein is almost exclusively produced by osteocytes in adult murine and human bone, antibodies targeting this offer a way to target specifically bone formation [Winkler *et al.* 2003; Van Bezooijen *et al.* 2005; Robling *et al.* 2008]. Sclerostin antibodies thus increase bone formation in osteopenic estrogen-deficient rats [Keller and Kneissel, 2005; Li *et al.* 2009]. A

single subcutaneous dose of a sclerostin antibody in postmenopausal women resulted in an increase in bone density and bone formation markers, without any modification of bone-resorption markers [Papapoulos, 2011; Lewiecki, 2013].

Next to sclerostin, mutations in the gene encoding LRP5/6 inducing a gain of function cause an increase in bone mass [Kim *et al.* 2007; Hoeppner *et al.* 2009]. These mutations induce impairment of the binding of DKK1 to frizzled LRP5/6, thus allowing an increase in the Wnt-signaling pathway, and an increase in bone formation [Choi *et al.* 2009]. Antibodies targeting DKK1 and/or LRP5/6 induce the increase in bone mass, volume, and formation in rodents [Van Dinther *et al.* 2013]. Therefore, antibodies targeting DKK1 could also be used as anabolic or antiresorptive agents for the treatment of patients with low bone mass [Ahn *et al.* 2011].

Effects of monoclonal antibodies on bone

The pharmacologic inhibition of the sclerostin protein, using monoclonal antibody, has confirmed efficacy in animal models of bone diseases, including estrogen deficiency-induced bone loss [Li *et al.* 2009], age-related, or androgen deficiency-induced bone loss [Li *et al.* 2010a], disuse/immobilization-induced bone loss [Tian *et al.* 2011], glucocorticoid-induced bone loss [Marenzana *et al.* 2011], chronic inflammation-induced bone loss [Eddleston *et al.* 2009], bone loss associated with type 2 diabetes mellitus [Gaudio *et al.* 2012], as well as in a rodent model of osteogenesis imperfecta [Sinder *et al.* 2013], and fracture healing [Gamie *et al.* 2012; Cui *et al.* 2013]. Effects of sclerostin monoclonal antibody have also been reported in human preclinical models of bone loss, including osteogenesis imperfecta, fracture healing, implant diseases. and other bone disorders (Figure 2) [Eddleston *et al.* 2009; Li *et al.* 2009, 2010a]. The use of sclerostin antibody in these conditions has demonstrated a consistent ability to increase bone formation, bone mass, and bone strength [Ke *et al.* 2012].

A human antisclerostin antibody, known as AMG 785 or romosozumab, is being developed by Amgen (Thousand Oaks, CA, USA) and UCB Inc. (Smyrna, GA, USA), and a phase 1 randomized, double-blinded, placebo-controlled study using this antibody has been conducted on men and postmenopausal women [Padhi *et al.* 2011]. In this trial, antibody injection was associated with substantial increases in bone-formation markers and reductions in bone-resorption markers, as well as a dose-dependent increase in bone-mineral density at the lumbar spine and total hip after 3 months. Results from the phase ll trial on postmenopausal osteoporosis have not been published at this time, but a recent press release by Amgen and UCB Inc. reported positive results with the cohort using the monoclonal antisclerostin antibody, including a significant increase in bone-mineral density of the lumbar spine at 12 months compared with placebo. Furthermore, this antisclerostin antibody was positively compared with teriparatide and alendronate. Positive phase ll results of this antibody in patients with postmenopausal osteoporosis have been announced by Amgen and UCB Inc. (NCT00896532, May 2009), whereas phase lll programs on fracture healing, and in patients with postmenopausal osteoporosis are currently ongoing (NCT01631214, May 2012; NCT01796301, February 2013).

In the same manner, the pharmacologic inhibition of DKK1, using monoclonal antibody, has also demonstrated efficacy in animal models of bone diseases, including gonad-intact rodents [Li *et al.* 2006], rodent models of fracture healing [Li *et al.* 2011], and implant fixation [Agholme *et al.* 2011]. However, unlike Scl-Ab, DKK1 demonstrated no efficacy in adult ovariectomized rats [Li *et al.* 2006], and a modest improvement in ovariectomized rhesus monkeys [Glantschnig *et al.* 2011; Li *et al.* 2011]. DKK1 antibody demonstrated efficacy in rodent models of rheumatoid arthritis [Diarra *et al.* 2007], ankylosing spondylitis [Uderhardt *et al.* 2010], and multiple myeloma [Fulciniti *et al.* 2009].

Conclusion

Treatments of low bone mass and osteoporosis have advanced significantly beyond hormone therapy administered at menopause. These advances are mainly the result of increased understanding of the mechanisms underlying osteoblast, osteoclast, and osteocyte biology. Novel anabolic and antiresorptive agents, affecting osteocyte-associated sclerostin, the calcium-sensing receptor, or Wnt signaling, offer promise for the treatment of bone disorders. Additional therapies treating patients with established fractures are needed next to reduce the burden of this other disease.

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Conflict of interest statement

The authors declare no conflict of interest in preparing this article.

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