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Molecular biology of bone remodeling: implications for new therapeutic targets for osteoporosis

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Abstract

Osteoporosis is a major public health problem for adults over age 55 years costing billions of Euros/Dollars. Over the last 20 years antiresorptive drugs were the treatment of choice for osteoporosis and most were derived from the Bisphosphonate molecule.

In the last 7 years remarkable advances in molecular biology and genetics have led to a detailed understanding of the bone remodeling cycle and as a result new therapeutic targets for treatment emerged.

These new compounds have different modes of action depending on their role in the bone remodeling cycle. A major discovery was the important role of RANKL (Receptor Activator for Nuclear Factor κ B Ligand) secreted by osteoblasts and responsible for stimulating osteoclastic bone resorption. This led to development of a potent monoclonal antibody that blocks its action. This drug should be available soon as a new treatment for osteoporosis. Other molecular targets in resorption have been identified and several specific antagonists are potential treatments. However, a significant limiting factor for a new anti resorptive drug is the cost of bringing it to the market because of the huge costs of a fracture trial.

Although anti resorptive agents have been the backbone of osteoporosis treatment they do not rebuild bone architecture and development of anabolic agents are needed. These are likely to evolve from an understanding of the LRP/Wnt signaling pathway. Already an antibody against sclerostin has shown promise in animal studies, and not to forget parathyroid hormone which was the first clinically useful anabolic treatment for osteoporosis.

Introduction

Osteoporosis is a major public health problem for healthy adults over age 55 years and one in two women will go on to develop an osteoporotic fracture compared to one in four men.

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In the USA around 10 million adults older than 50 years are estimated to have osteoporosis and another 34 million are at risk for osteoporosis (1). Without an intervention strategy it is likely that the number of people with osteoporosis will increase 3-fold over the next 25 years due to an increase in the aging population worldwide. Fracture incidence trends with age are similar in many countries although there is considerable geographical variation in incidence (~ 7-10 folds) in Europe (2). Survival after a spine or hip fracture is reduced by 20 percent (3,4).

Osteoporosis and particularly hip fractures have a large economic impact; the direct costs of osteoporotic fractures in the USA in 2005 were estimated to be \$ 19 billion (5). There are several effective treatments available for osteoporosis and treatment has reduced the incidence of osteoporotic fractures. However, research is continuing to investigate new and more potent therapies. Recent advances in bone biology have identified several molecules involved in the process of bone resorption and bone formation that will lead to new treatments for osteoporosis and this review will focus on the bone remodeling process and pending molecular targets along with a brief overview of existing therapies.

Because of the daily stress on the skeleton that leads to micro fractures it is essential that there is an efficient process that repairs bone and replaces the old bone with new bone. Bone is a very dynamic tissue and the process of repair occurs in bone remodeling units at the surface of cortical and trabecular bone. Bone remodeling follows a time sequence that lasts about 6 months. There are 4 stages, 1) Activation of osteoclast precursors that mature into multinuclear osteoclasts under the direction of cytokines and hormones, 2) Resorption of bone by osteoclasts causing a resorption cavity – a process that lasts about 3 weeks, 3) Reversal of the resorption signal 4) Formation of new bone that fills up the resorption cavity with new bone and lasts several months (Figure 1).

Osteoclast precursors and mature osteoclasts are derived from the monocyte/macrophage lineage of hematopoietic stem cells in the bone marrow. These cells need activation by two essential cytokines, M-CSF (Macrophage colony stimulating factor) and RANKL (Receptor Activator of NF- Kappa B Ligand) that are produced by marrow stromal cells and osteoblasts. M-CSF is responsible for proliferation, survival and differentiation of osteoclast precursors and RANKL is the most important cytokine that primes the precursor cells for osteoclast differentiation. It binds to the receptor RANK on the surface of osteoclast precursors and osteoclasts and is the key activator of osteoclast formation and action. T cells also secrete RANKL. This regulation of osteoclast formation and action is antagonized by a local inhibitor called Osteoprotegerin (OPG) that is secreted by local mesenchymal cells and binds to the RANKL.

In summary, the RANKL / OPG is the common central pathway regulating differentiation and activation of osteoclasts by osteoblasts (6-8) (Figure 1).

Once the multinucleated osteoclasts have matured, they attach to bone surface through $\alpha_v\beta_3$ integrin receptor. This attachment seals off a resorption zone; osteoclasts secrete various enzymes and acid into this zone causing bone resorption. Cathepsin K is a protease that degrades bone matrix and other factors such as acid cause dissolution of bone mineral. A proton pump H^+-K^+ ATPase does acid secretion and the enzyme carbonic anhydrase (Type 2) is utilized in the process (Figure 2).

In the Reversal phase, mononuclear cells line the resorptive cavity and form a cement line (glycoprotein) that helps in attaching osteoblasts. Osteoblast precursors are derived from the stromal mesenchymal cells and converted into mature osteoblasts under the influence of many growth factors, hormones and cytokines. Osteoblasts synthesize collagenous bone matrix and

then complete its mineralization leading to the formation of bone matrix proteins like collagen type 1, osteopontin, osteocalcin, bone specific alkaline phosphatase and bone sialoprotein.

Recent research has shown that several proteins are essential for osteoblast proliferation, differentiation and survival. Of particular importance is the Wingless-type and integrase 1 (Wnt/ β -Catenin pathway) (9,10) (Figure 2). Mutations in the LRP5 protein can lead to either osteoporosis or high bone mass. The various factors in this pathway regulate the formation of osteoblasts, inhibit apoptosis of osteoblasts and increase their lifespan.

As bone formation continues, osteoblasts become embedded deeper in bone and become osteocytes that are inter- connected through dendritic processes within the canaliculi of bone. Actually osteocytes comprise about 95 percent of bone cells. They are sensitive to mechanical strain. Osteocytes can resorb bone adjacent to the cells, promote mineralization and also inhibit bone formation by secreting sclerostin that acts on the Wnt signaling pathway in osteoblasts. Besides these local effects osteocytes have systemic effects. It is now recognized that osteocytes are an important regulator of phosphate homeostasis through the secretion of FGF23 (fibroblast growth factor), DMP1 (dentin matrix protein) and PHEX. Mutations of DMP1 are the cause of recessive hypophosphatemic rickets. A high phosphate diet increases FGF23 which in turn increases phosphate excretion in the kidney, Although a low phosphate usually stimulates serum 1,25dihydroxyvitamin D, FGF23 inhibits its production. Thus, osteocytes play an important role in calcium and phosphorus homeostasis.

In a state of normal bone remodeling bone formation closely matches bone resorption, in other words each packet of bone that is removed is replaced by the same amount of bone. The greatest change in bone remodeling occurs at menopause when there is an increase in the number of resorption cavities but bone formation does not increase proportionately and resorption cavities are not completely filled in with new bone and this results in a permanent loss of bone mass. The difference is largest in the first 5 years after menopause and coincides with the surge in cytokines (11). The negative change in total bone calcium is about 100 mg/day during the first 3 years and this becomes less after about 5 years, averaging 30 mg/day (12). This postmenopausal loss can be attributed to estrogen deficiency and can be prevented with estrogen therapy. One possible mechanism for the effect of estrogen on bone is that it stimulates OPG that blocks RANKL activity and vice versa, estrogen deficiency leads to up-regulation of RANKL.

Treatment and prevention of osteoporosis

A. Established therapies

Anti-Resorptive Agents—Most of the anti resorptive agents available today also inhibit bone formation after several months and this limits the effect on increasing bone mass. Drugs that uncouple bone resorption from bone formation potentially have a greater effect in terms of increasing bone mass.

1. Estrogen therapy (ET) has long being shown to have a beneficial effect on bone mass, prevention of bone loss and fractures in postmenopausal women with or without established osteoporosis. These effects are exerted through estrogen receptors (ER) α and β , which are present on both the cells of monocyte lineage and osteoblasts. Estrogen leads to the direct suppression of osteoclasts both by reducing the expression of RANKL on marrow cells and by increasing OPG secretion by osteoblasts that binds to and inactivates RANKL (13). Other indirect effects include suppression of interleukin -1 (IL-1), IL-6, IL-7 and tumor necrosis factor α (TNF- α) and increased production of IGF-1 and TGF- β by osteoblasts. Concern about adverse effects of estrogen (ET) or estrogen and progestin therapy (HT) has limited the use of HT for

prevention and treatment of postmenopausal osteoporosis especially in older women, but ET /HT is still highly effective for osteoporosis management.

2. SERMs or selective estrogen receptor modulators probably exert their effect on bone in a similar way as estrogen. These compounds reduce the incidence of spine fractures but not non-vertebral fractures whereas estrogen reduces both types of fractures suggesting that the efficacy of SERMs is similar to that of low dose estrogen.
3. Calcitonin directly suppresses the osteoclast function by binding to a calcitonin receptor on osteoclasts and suppressing function. In a large study involving 1,255 postmenopausal women with established osteoporosis, calcitonin nasal spray significantly reduced the risk of new vertebral fractures by 33 percent (14). The effect on non-vertebral fractures was non significant. It caused mild to moderate rhinitis when used as a nasal spray. An oral formulation based on bio equivalence studies is presently being evaluated for osteoporosis. Due to the relatively low potency of calcitonin, it is generally reserved only for the treatment of osteoporosis in women who are > 5 yrs since menopause and are unable to take other medications or in men with mild bone loss.
4. Bisphosphonates are currently the drug of first choice for prevention and treatment of primary osteoporosis. They reduce fractures in the spine and non-vertebral sites by 50-60 percent. Bisphosphonates attach to the hydroxyapatite in bone and as bone is resorbed the drug is taken up by the osteoclasts. Bisphosphonates without a Nitrogen atom in the molecule (etidronate, clodronate, tiludronate) are incorporated by ATP and cause apoptosis of osteoclasts, whereas bisphosphonates with a Nitrogen atom in the molecule (pamidronate, alendronate, ibandronate, risedronate, zoledronate) alter the cytoskeleton of osteoclasts and decrease osteoclast activity and function by inhibiting an enzyme in the mevalonate pathway.

Anabolic Agents

Parathyroid Hormone (PTH): PTH plays a major role in calcium homeostasis and bone remodeling. An important difference in the effect of PTH on bone was noted many years ago when it was shown that chronic elevated levels of PTH caused bone resorption, it does so by binding to the PTH receptor on osteoblasts and increasing production of RANKL which activates bone resorption (Figure 1). However, when small intermittent pulses of PTH are given they have an anabolic action despite increasing bone resorption. This is demonstrated by changes in bone markers, initially there is a rapid increase in bone formation markers followed by an increase in bone resorption markers, this is often referred to as the 'anabolic window'. Bone biopsies show an increase in cortical bone width without an increase in cortical porosity.

These actions of PTH occur by two mechanisms – in cancellous bone where the rate of apoptosis of osteoblasts is high PTH leads to a decrease in osteoblast apoptosis, and in periosteal bone where the rate of apoptosis of osteoblasts is low PTH increases the survival of post-mitotic pre-osteoblasts (15). One potential mechanism for the anabolic action is that PTH reduces sclerostin production by osteocytes thus allowing osteoblasts to differentiate and prolong their survival.

In a trial of PTH1-34 treatment involving ~1600 osteoporotic women, fracture incidence was reduced by 65 percent. Other studies showed that PTH produced a larger increase in trabecular bone density compared to alendronate as measured by computerized tomography (20 percent vs. 5 percent respectively). Similar results in terms of improved fracture outcomes and improved bone micro architecture have been found in studies on hPTH (1-84). PTHrP (Parathyroid hormone related peptide) appears to have the same action and may well be developed as an osteoporosis drug in coming years.

Research is continuing on developing more potent PTH analogues that can permit optimization of signaling duration time, thus reducing the potential for resorptive actions of PTH on bone. Transdermal delivery of PTH is completing clinical trials and this form of treatment may soon be another option. Despite early concerns in animal studies, only 2 cases of osteosarcoma have been reported in teriparatide treated humans – both had complex medical histories and a history of pelvic radiation in the second case (16).

Strontium Ranelate: Strontium ranelate is an oral agent that decreases bone resorption and may increase bone formation. It is incorporated into the hydroxyapatite with a strontium atom replacing a calcium atom. Basic research shows that strontium inhibits osteoclast action through an effect on the calcium sensing receptor causing apoptosis (17). In cell models strontium enhances osteoblast proliferation, increases alkaline phosphatase and increases collagen synthesis, thus demonstrating an anabolic effect (18).

In two phase 3 clinical trials involving 1649 and 5092 postmenopausal women with osteoporosis, strontium ranelate 2 g/d reduced the risk of new vertebral fractures by about 50 percent and 39 percent respectively (19,20).

New anti resorptive therapies for osteoporosis

Cathepsin K inhibitors—Cathepsin K is a cysteine protease that is selectively expressed in osteoclasts and leads to degradation of bone matrix proteins (Figure 2). Deficiency of Cathepsin K results in the human disease Pycnodysostosis characterized by osteosclerosis and decreased bone resorption (21) and has been seen in Cathepsin K knockout mice (22).

In a phase 2 placebo-controlled trial in postmenopausal women with osteopenia– an inhibitor balicatib was associated with a significant dose response increase in spine and hip BMD and a reduction in bone resorption markers and a minor effect on bone formation markers. There was an increased in serious skin reactions –rashes, and a morphea-like skin changes/skin scleroderma (a form of fibrosis), leading to discontinuation of the trial (23). In a trial evaluating another compound – odanacatib, a selective Cathepsin K inhibitor, given as a weekly oral dose for 2 years to ~ 400 postmenopausal women with osteopenia /osteoporosis, there was a dose dependent increase in spine (5.5 percent) and hip BMD (3.2percent) and decrease in bone resorption markers - urinary NTx/Cr and serum CTx, (24); in this study there was no increased incidence of skin reactions.

Glucagon like peptide 2 (GLP-2)—Bone remodeling follows the circadian rhythm with greater bone resorption at night that is inhibited by fasting (25). GLP-2 is an intestinal trophic hormone released in response to food. It probably acts by suppressing PTH secretion. The mechanism by which it does that is however not known; GLP-2 induced increased calcium absorption in gut or some other molecules released from small intestine (26). In two trials involving postmenopausal women, a significant dose-related reduction of serum CTx after injection of GLP-2 was seen showing anti-resorptive effects of GLP-2 with no significant effects on bone formation. Another 14 day RCT involving 60 postmenopausal women and 2 different doses of GLP-2 showed similar results (27). The most common adverse events included headache, abdominal pain and syncope. No serious adverse events were reported and there were no discontinuations. These authors then conducted another trial for 4 months enrolling 160 postmenopausal women with osteopenia (28). There was significant dose dependent increase in hip BMD of 1.1 percent from baseline for the highest dose (3.2mg/d) along with decrease in serum CTX. Adverse events included gastrointestinal symptoms, muscle pain and infections. The results should be carefully interpreted, as the studies conducted were too small and short. The benefit in terms of BMD was modest and long-term data on safety and efficacy is needed.

Denosumab (RANKL Antibody)—The vital importance of RANKL in controlling bone resorption has been discussed earlier. Denosumab is a human monoclonal antibody to RANKL that blocks the activation of osteoclasts thereby decreasing bone resorption (Figure 1, 2). It is a powerful inhibitor of bone resorption.

In a recently conducted large study involving 7868 osteoporotic women, Denosumab 60 mg, given subcutaneously every 6 months, reduced the risk of new radiographic vertebral fracture by 68 percent, hip fractures by 40 percent and non vertebral fractures by 20 percent (29). There were no significant differences in the incidence of total number of serious adverse events compared to placebo except for a small increase in skin infections including cellulitis.

Denosumab can also be used to decrease bone resorption in patients with arthritis and cancer since RANKL is the main cause of increased bone resorption in both diseases. Preliminary trial data in patients with prostate and breast cancer appear to be very promising.

Osteoprotegerin (OPG)—OPG binds to RANKL and prevents its binding to RANK and hence the activation of osteoclasts (Figure 1). It can be considered as a natural antibody to the RANKL. In a phase 1 clinical trial involving 52 healthy post menopausal women (age 40-70 years), OPG decreased the resorption marker urinary NTx by 80 percent by day 4 after a single dose and the effect gradually decreased to 17 percent after 6 weeks of follow up (30). However it was noted that antibodies to OPG appeared after several months and this therefore has limited its future use as a treatment for osteoporosis.

$\alpha_v\beta_3$ integrin antagonists (L-000845704)— $\alpha_v\beta_3$ integrin receptor or vitronectin receptor is present on the surface of osteoclasts and is required for the attachment of osteoclasts with bone matrix proteins (Figure 2). L-000845704 is an orally acting non-peptide antagonist of $\alpha_v\beta_3$ integrin receptor on osteoclasts and causes inhibition of bone resorption. In a phase 2 trial involving 227 women with postmenopausal osteoporosis, L-000845704 significantly decreased bone resorption markers by 40 percent and increased spine BMD by 3.5 percent at a dose of 200 mg bid (31). There were no serious adverse events. However, long-term trials have not been continued.

Other Potential Anti-Resorptive Agents—Osteopetrosis is a group of human diseases characterized by defect in bone resorption by osteoclasts because of defects in either the production or functional capabilities. Mutations in the **TCIRG1** subunit of the vacuolar proton pump are responsible for about 50 percent of severe cases of autosomal recessive Osteopetrosis (32). This pump is required for the secretion of acid and digestion of bone mineral by osteoclasts (Figure 2). The osteoclasts are frequently normal in number but dysfunctional. Chloride channel **CIC7** mutations are responsible for severe recessive, dominant, and intermediate Osteopetrosis (33). This channel is required for secretion of acid from osteoclasts (Figure 2) and its deficiency has similar effects as TCIRG1 mutations. Chloride channels are highly expressed in osteoclasts, especially CIC-7 and ClC1, CIC-7 channels appear to be restricted to a few tissues whereas ClC1 channels are in many tissues. In an experimental model of rat osteoporosis a chloride channel inhibitor blocked osteoclastic acidification and bone resorption without affecting bone formation and bone loss was reduced by 50 percent. It may be that a more potent inhibitor is needed for human subjects.

Similarly, mutations in carbonic anhydrase (CA □□)(Figure 2) lead to decreased acid production by osteoclasts. Also recent studies have established a role of protein tyrosine phosphatases (PTP) in the regulation of osteoclast function and survival. Cyt-PTP-epsilon, PTP-PEST, and PTP-oc are positive regulators of osteoclast activity, while SHP-1 is a negative regulator (34). These molecules are essential for the osteoclast migration and attachment of $\alpha_v\beta_3$ integrin on osteoclast surface with bone matrix.

New anabolic therapies for osteoporosis

Sclerostin neutralizing antibodies—Sclerostin is a protein that is secreted by osteocytes after primary mineralization to limit further bone formation by osteoblasts (Figure 2). A deficiency of sclerostin due to a mutation in the SOST gene results in the human disease sclerosteosis which is characterized by increased bone density at all skeletal sites (35). Normally sclerostin which is produced by osteocytes acts on osteoblasts via Lrp5 and Lrp6 receptors to inhibit the Wnt pathway. Loss of function mutations in Lrp5 result in osteoporosis and pseudoglioma syndrome (36) and a gain of function mutation in the Lrp5 gene results in high bone mass. In rat and monkey models of osteoporosis, treatment with a monoclonal antibody to sclerostin (Scl-AbII) markedly increased bone formation on trabecular, periosteal, endocortical, and intracortical surfaces after 5 weeks of treatment (37). Sclerostin antibodies may soon start in clinical trials for osteoporosis.

Bone morphogenetic proteins (BMPs)—BMPs are growth factors belonging TGF- β superfamily. They are involved in a multitude of processes including embryonic and post natal development. They are potent bone inducers and are being used in clinical studies for local fracture healing. In murine models for osteopenia/osteoporosis, intra-peritoneal (i.p.) injections of rhBMP-2 increased the adult mesenchymal stem cell number, osteogenic activity, proliferation and bone formation activity (38). Rolipram is a specific inhibitor of phosphodiesterase-4, which increases the efficacy of rhBMP-2. However it also increases cAMP levels in brain and has strong anti-depressant properties. In a study analyzing effects of local release in mice, locally released rolipram enhanced the capacity of rhBMP-2 to induce bone formation as measured by bone mineral content of the formed bone (39). As described by authors, these findings have strong implications for clinical studies, since use of local rolipram decreases the systemic side effects.

Activin acting through soluble activin receptor IIA (ActRIIA) stimulates osteoclasts and inhibits osteoblasts. ACE-011 (ActRIIA-IgG1) is a human glycosylated dimeric fusion protein consisting of ActRIIA linked to the human IgG1 Fc domain. By binding activin, ACE-011 prevents activin from binding endogenous receptors and thus acts as a decoy. In a phase 1 clinical trial involving 48 healthy postmenopausal women, a single dose of ACE-011 caused a rapid and sustained dose-dependent increase in serum levels of Bone specific alkaline phosphatase (16.6 percent by 4 months with highest dose of 3 mg/kg iv) and a dose-dependent decrease in serum CTX and TRACP-5b levels (40). The most common adverse events were headache, infusion site reactions and toothache, which were mild and transient.

Summary

Due to recent advances in molecular biology and a comprehensive understanding of the bone remodeling process many molecules have been discovered that play important roles in bone biology. Our understanding of the current mechanisms underlying the pathophysiology of osteoporosis has expanded and more compounds are moving from translational research into clinical trials. However, with these new biological agents also comes the potential for adverse events – possibly because of unknown systemic non-target tissue actions of these new compounds, as illustrated above in the studies of the Cathepsin K inhibitor balicatib. In moving from translational to clinical research one has to be aware that cytokines and proteins involved in bone remodeling are also present in other tissues throughout the body and the use of antibodies may cause unexpected adverse events. However, an increased role for compounds like Denosumab, odanacatib, sclerostin antibodies and PTH analogues is emerging in the treatment of osteoporosis.

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Figure 1.

Bone Remodeling - showing the various stages and the factors involved. Also shown is the development of osteoblasts and osteoclasts from precursors.

The factors in bold red are currently being used and/or under active investigation in clinical studies, others in green are potential targets based on animal and in vitro studies.

RANK (Receptor Activator of Nuclear Factor-Kappa B), RANKL (Receptor Activator of Nuclear Factor-Kappa B Ligand), OPG (Osteoprotegerin), TNF α (Tumor Necrosis Factor alpha), IL (Interleukin), PGE 2 (Prostaglandin E 2), PTHrP (Parathyroid Hormone related peptide), PTH (Parathyroid Hormone), 1,25(OH)₂ D3 (1,25 – dihydroxyvitamin D 3), CBF A1 (Core Binding factor alpha 1) BMP (Bone Morphogenic protein), TGF β (Transforming Growth Factor beta), IGF (Insulin like growth factor), m-CSF (Monocyte colony stimulating factor), NF- κ B (Nuclear factor kappaB), NFAT (Nuclear Factor of Activated T-cells)

*The function of Vitamin D3 in bone is complex and is dependent on serum calcium. If serum calcium is low, vitamin D increases bone resorption and if its high/normal, vitamin D promotes bone formation.

Figure 2.

Osteoclasts, osteoblasts and osteocytes: various molecules involved in bone remodeling. Those shown in bold red are currently being used and/or under active investigation in clinical studies, others in green are potential targets based on animal and in vitro studies.

CA □□ (Carbonic anhydrase iso enzyme 2), Cl channel (Chloride channel), TRAP (Tartarate resistant acid phosphatase), Trph1 (Tryptophan hydroxylase 1), CREB (cAMP (cyclic adenosine mono phosphate) response element binding protein), Wnt (Wingless type and integrase 1), Dkk (Dickkopf-1), WIF (Wnt inhibitory factor), sFRP (Secreted frizzled related protein), Lrp5/6 (Low density lipoprotein receptor related protein 5/6)

Table 1**Osteoporosis Treatment – Established therapies**

Agents	Clinical Trials	Type
Bisphosphonates	FDA approved for both prevention and treatment	Anti-resorptive
Estrogen	FDA approved only for the prevention of post menopausal osteoporosis	Anti-resorptive
Selective estrogen receptor modulators (SERM) - Raloxifene	FDA approved for the prevention and treatment of post menopausal osteoporosis	Anti-resorptive
Calcitonin	FDA approved for the treatment of post menopausal osteoporosis in women > 5 years menopause	Anti-resorptive
PTH	FDA approved for treatment of osteoporosis	Anabolic
Strontium	Approved in Europe but not US	Both anabolic and anti-resorptive
RANKL Antibody - Denosumab	Approved in Europe but not US	Anti-resorptive

Table 2**Osteoporosis Treatment – Novel Therapies**

Agents	Clinical trials	Type
Cathepsin K inhibitors (Balicatib and odanacatib)	Phase 1 studies with balicatib terminated due to serious adverse events like morphea, Phase 2 studies of Odanacatib ongoing	Anti-resorptive
Glucagon like peptide (GLP-2)	Phase 1	Anti-resorptive
Osteoprotegerin (OPG)	Phase 1	Anti-resorptive
$\alpha_v\beta_3$ integrin antagonists (L-000845704)	Phase 1, 2	Anti-resorptive
Sclerostin neutralizing antibodies (AMG 167)	Phase 1	Anabolic
rhBMP	Phase 2	Anabolic
Activin antibody (ACE-011)	Phase 1	Anabolic