Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/poamed

Review Article

A view at monoclonal antibodies in therapy of osteoporosis

Małgorzata Michałowska [a], *Beata Znorko* [b], *Ewa Oksztulska-Kolanek* [b], *Tomasz Kamiński* [a], *Dariusz Pawlak* [a,*]

[a] *Department of Pharmacodynamics, Medical University of Bialystok, Poland*
[b] *Department of Monitored Pharmacotherapy, Medical University of Bialystok, Poland*

ARTICLE INFO

Article history:
Received 19 June 2015
Accepted 31 July 2015

Keywords:
Osteoporosis
Antibody
Sclerostin
Dickkopf-1
Denosumab

ABSTRACT

Introduction: Osteoporosis is a bone disease, which leads to increased fracture risk and weakens bone strength. Drugs used in current therapies of this disease are far from perfect thus the search for new effective compounds is an ongoing process, and some researchers put great hopes in monoclonal antibodies in this field.
Aim: The purpose of this paper is to discuss monoclonal antibodies as potentially beneficial therapy of osteoporosis.
Material and methods: It was based upon the available literature and publications.
Results and discussion: Sclerostin is a glycoprotein that belongs to Wnt inhibitors. Wnt/β-catenin signaling pathway is essential for normal physiological cell functions such as differentiation or proliferation. Inhibition of sclerostin activity can result in increased bone mineral density, and can be achieved by using antibodies against this factor, i.e. romosozumab and blosozumab. Another compound that has an influence on Wnt/β-catenin signaling pathway is Dickkopf-1. Monoclonal antibodies against this factor have been tested in bone diseases and found to contribute to increased bone mineral density. Other anti-resorptive agent indicated for the treatment of osteoporosis is a receptor activator of nuclear factor-κB ligand (RANKL) inhibitor. Denosumab is a human antibody to RANKL, and it decreases osteoclastogenesis and osteoclast activity, leading to reduced bone resorption. It is currently used in treatment of postmenopausal osteoporosis.
Conclusions: The essential goal for the management of osteoporosis is to increase bone mass and reduce fracture risk by slowing or stopping bone loss. Monoclonal antibodies that have been recently developed are becoming an important option in the pharmacotherapy of osteoporosis.

* *Correspondence to*: Department of Pharmacodynamics, Medical University in Bialystok, Mickiewicza 2C, 15-222 Bialystok, Poland. Tel.: +48 857485601; fax: +48 857485601.
E-mail address: dariuszpawlak@poczta.onet.pl (D. Pawlak).

http://dx.doi.org/10.1016/j.poamed.2015.07.002

1. Introduction

Monoclonal antibodies are a group of biological agents that are hugely important in targeted therapies for many disorders. They specifically bind to exact targets, allowing improvement of clinical efficacy and safety. These developmental drugs, which in fact are immunoglobulins that modify immunological reaction, have become useful not only in cancer treatment, but also in treatment for other diseases including multiple sclerosis, psoriasis, age-related macular degeneration, rheumatoid arthritis and osteoporosis.[1]

Osteoporosis is a bone disease that affects both women and men. Its clear characteristics such as low bone mass and disturbed bone microarchitecture are caused by a lack of balance between the actions of osteoblasts and osteoclasts. All of these disturbances lead to increased fracture risk, and weaken bone strength.[2]

Nowadays, drugs with anti-resorptive or stimulating bone formation properties are used to prevent possible fractures and avoid any significant reduction in quality of life of osteoporotic patients. Despite all the benefits, drugs used in current therapies are far from perfect thus the search for new effective compounds is an ongoing process, and some researchers put great hopes in monoclonal antibodies in this field.[1]

2. Aim

This article discusses monoclonal antibodies that are currently being used or tested in the therapy of osteoporosis and other bone diseases, as promising therapeutic methods.

3. Material and methods

This article was based on the available literature and publications.

4. Results and discussion

4.1. *Anti-sclerostin antibodies*

The progress in discovery of mechanisms that are at the heart of the imbalance between bone formation and bone resorption contributes to development of new drugs, e.g. monoclonal antibodies against sclerostin.

Sclerostin is a glycoprotein codified by the human SOST gene and is considered to be a member of the DAN family of bone morphogenetic protein antagonists. In the structure of this compound the C-terminal cysteine knot-like domain can be distinguished. Sclerostin is not only a monomeric protein, what Hernandez et al. showed in their work, but also occurs in other forms like dimers. Moreover, they described the sclerostin molecular weight to be detected more often as 47 kDa, 54 kDa or 70 kDa rather than the 27 kDa mentioned in former works.[3–5]

For a long time sclerostin was considered to be produced exclusively by osteocytes; however the SOST mRNA has been discovered in several parts of the organism such as kidneys, heart, lungs, liver, aorta, and chondrocytes. Nonetheless, osteocytes are still the main producers of glycoprotein, which in its 54 kDa dimeric form can be secreted from the bone and spread into various organs.[3–8]

Serum sclerostin levels depend on many factors, and increase with age or augmentation of fat mass; it can also be higher in men. Increased levels can also be observed in conditions such as type 2 diabetes, atherosclerosis or aortic calcifications.[6–10]

In contrast to those conditions, some disorders are associated with reduced serum sclerostin levels. Cases in point are sclerosteosis and van Buchem's disease – both are caused by mutations of the SOST gene and the decreased level of sclerostin that follows. In sclerosteosis two clinical phenotypes can be observed. As opposed to heterozygotes that are defined by reduced level of sclerostin, homozygotes' serum sclerostin level is undetectable. Furthermore, severe symptoms occur in homozygotes, including bone overgrowth, facial nerve paralysis or syndactyly. In heterozygotes none of these appear. They are phenotypically normal, and only aberrations that can be observable are higher than normal bone mass and rarely fracture. Contrary to sclerosteosis, van Buchem's disease is characterized by low levels of sclerostin in all patients, which results in increased bone mass and other similar but less severe symptoms. All skeletal disturbances in mentioned disorders are associated with sclerostin, and can be explained by physiological functions of this compound.[5,11]

Nowadays, it has become well-known that sclerostin belongs to Wnt inhibitors. Wnt/β-catenin signaling pathway is essential for normal physiological cell functions such as differentiation or proliferation. Sclerostin is a protein, which is able to bind to Wnt co-receptors low-density lipoprotein receptor-related protein (LRP) 5/6, causing receptor internalization through inhibition of β-catenin translocation into nucleus, and augmentation of β-catenin degradation. Regarding bones, Wnt pathway participates in balance maintenance between bone formation and resorption. The pathway stimulates osteoprotegerin (OPG), which results in counteracting excess bone resorption due to binding receptor activator of nuclear factor-κB ligand (RANKL). Besides LRP5 and LRP6, sclerostin can also bind to LRP4. Data from previous studies concerning LRP4 protein mutations showed occurrence of aberrations of skeletal phenotypes in the form of increased bone mass.[5,11–13]

Genetic modifications of sclerostin activity were observed both during researches, and in naturally occurred diseases such as sclerosteosis and van Buchem's disease. It was precisely its inhibition, resulting in increased bone mineral density (BMD) that opened up the possibility of therapeutic use of the observed effects. Monoclonal antibodies against sclerostin, through specific binding to this protein, can block its action and contribute to increased bone formation. Anti-sclerostin antibodies prevent binding of sclerostin to LRP6, as well as receptor internalization and degradation of β-catenin, all of which result in osteoanabolic effects. Amongst sclerostin antibodies there are blosozumab and romosozumab, which are the first humanized monoclonal antibodies against sclerostin.[5,9,13,14]

Phase I clinical trial in blosozumab in postmenopausal women was completed in 2014. Data indicated safety of a

single subcutaneous or multiple intravenous doses of blosozumab, and reflect anabolic effects on bone. In March 2015 another study was completed, in order to evaluate safety, tolerability, and pharmacokinetics of two formulation of blosozumab in postmenopausal women. Results from this study have not been published yet.[15,16]

More information concerning romosozumab as the first anti-sclerostin antibody is available. Romosozumab have been examined in a number of studies, and currently phase III studies are being conducted. Four of the phase III studies are related to postmenopausal osteoporosis, while there is also one study that focuses on osteoporosis in men. To get to this stage, as a general rule, promising results must have been achieved in earlier experiments with romosozumab. Both preclinical animal studies and phase I and phase II clinical studies had demonstrated that the antibody can augment bone formation as well as enhance BMD. Li et al. initially proved that romosozumab caused reversal of bone loss in aged ovariectomized rats, while it also increased their bone mass over normal level. An experiment on aged male rats followed, in which the influence of increased level of romosozumab on BMD and bone formation was confirmed through observation of increased level of bone formation parameters. The same effects of romosozumab in cynomolgus monkeys were proved by *Ovinsky et al.*, who also tested the usefulness of this antibody in fracture healing. The correlation between improvements at fracture site and BMD as well as bone formation at non-fractured site were observed. Moreover, Cui et al. showed in a mouse osteotomy model that in mice treated with romosozumab the maximum load of failure was enhanced, while also bone strength and BMD increased. Therefore, the authors suggested usability of romosozumab in the therapy of fracture healing, particularly at the early stage. The quality of bone matrix after sclerostin antibody treatment in rats and nonhuman primates was examined by Ross et al. Data from their study showed that parameters like crystallinity, mineral-to-matrix ratio and collagen cross-linking did not change, what could be construed as a suggestion that romosozumab treatment did not exert any negative effects on bone matrix quality. Phase I clinical trials concerning romosozumab were conducted by Padhi et al. with participation of postmenopausal women and healthy men with low BMD. Tested antibody was generally well tolerated, and only one adverse accident (hepatitis) was observed during treatment. Evaluation of BMD showed significant increases, in comparison to placebo. Moreover, bone formation markers (N-terminal propeptide of human procollagen type I, bone alkaline phosphatase, osteocalcin) were augmented, and level of serum carboxy-terminal collagen crosslinks – the marker of bone resorption – was decreased. Data from randomized, placebo-controlled, multi-dose phase II clinical trial of romosozumab in the treatment of postmenopausal women with low BMD was published at the beginning of 2014. The authors noticed greater increases in BMD in patients treated by romosozumab than in groups that were treated with alendronate or teriparatide. In the placebo group a decrease of BMD was observed. In addition, an observation of bone formation and resorption markers concentrations suggested that romosozumab chronically reduced bone resorption and promoted bone formation for a short term. Regarding safety and tolerability, romosozumab did not cause any serious side effects. One of the observed adverse events after the injection were mild site reactions.[5,9,14,17–20]

Obtained results indicated that monoclonal antibodies against sclerostin could become a promising new treatment of osteoporosis. Further examinations are necessary to evaluate activity and safety of blosozumab, and Phase III clinical studies data concerning romosozumab are awaited.

4.2. *Anti-Dickkopf-1 antibodies*

Another factor influencing Wnt/β-catenin signaling pathway is Dickkopf-1 (DKK-1). DKK-1 is a glycoprotein that binds to LRP5 and LRP6 and thereby can inhibit Wnt pathway and decrease bone formation. In contrast to sclerostin, DKK-1 can inhibit Wnt 3a class Wnt signaling (beyond this, DKK-1 inhibits Wnt 1 class Wnt signaling, which is also a common action for sclerostin), as well as bind to Kremen 1 and Kremen 2 receptors.[5]

Expression of DKK-1 is specific mainly to osteoblast and osteocytes, although it was also found in skin and in prostate. Moreover, the overexpression of DKK-1 was detected in the myeloma plasma cell. In patients with multiple myeloma, the level of DKK-1 was correlated with osteolytic bone destruction. Osteolytic lesions in murine model of this disease were suppressed by monoclonal antibodies to DKK-1.[2,5,13]

Due to DKK-1 effects on bone, monoclonal antibodies against this factor have been tested in bone diseases. In studies with ovariectomized mice and monkeys, anti-DKK-1 antibodies contributed to increased BMD. It is noteworthy that glucocorticoid-induced osteopenia was also attenuated by modulation of DKK-1 expression. Data from researches showed that DKK-1 blocking is an interesting area of study, which could potentially open further approaches to treatment of rheumatoid arthritis or low-bone mass diseases.[5,13,17,18,21]

4.3. *Anti-RANKL antibodies*

Other antiresorptive agent indicated for the treatment of osteoporosis is an inhibitor of RANKL, which is a cytokine essential to osteoclast differentiation, activation and survival.

RANKL belongs to the tumor necrosis factor (TNF) family of cytokines, and is also known as TNF (ligand) superfamily member 11 (TNFSF11).[22] The cytokine is a type II membrane protein that has close homology to TNFSF members, TNF-related apoptosis-inducing ligand (TRAIL), FAS ligand (FasL) and tumor necrosis factor α (TNF-α).[23] Human RANKL is synthesized as a glycoprotein of 317 amino acids, and consists of cytoplasmic N-terminal domain, a transmembrane domain, and extracellular C terminal domain.[24] RANKL is produced in a membrane-bound and soluble form. The soluble form can be generated by proteolytic cleavage of the membrane-bound form or through alternative splicing. The proteolytic cleavage can be carried by proteinases, e.g. TNF-α converting enzyme (TACE),[25] a disintegrin and metalloproteinase (ADAM) 10 and matrix metalloproteinase (MMP) 14.[26]

Numerous cell types have been reported to express either RANKL mRNA or RANKL protein.[27] The list includes both T and B lymphocytes, mammary epithelial cells, keranocites, vascular endothelial cells, synovial fibroblasts and hypertrophic

chondrocytes as well as osteoblast precursors, mature osteoblasts, and osteocytes.[27]

Expression of RANKL is regulated by various osteoactive factors such as glucocorticoids, [1,25(OH)2D3] – the active form of vitamin D3, interleukin-1 (IL-1), TNF-α, transforming growth factor-β (TGF-β), Wnt ligands, and lipopolysaccharide (LPS).[24]

More than a decade ago, it was shown that some of the members of the TNF superfamily play crucial roles the regulation of bone metabolism. The molecular triad consisted of the OPG, RANK and RANKL members of that superfamily, and was defined as the key regulator of the differentiation and function of osteoclast biology, also playing a crucial role in regulating psychological and pathological bone turnover.[28] RANKL is a protein which exists as a cell surface molecule, and is able to bind to its cognate receptor activator of nuclear factor-κB (RANK) on the surface of osteoclast precursors.[29] This process is essential for generating new osteoclasts. Moreover, RANKL binding to RANK on mature osteoclasts promotes their adherence to bone and suppresses osteoclast apoptosis.[30] Such action between RANKL and RANK leads to activation of specific signaling pathways implicated in the formation and survival of osteoclasts.[28] OPG functions as an endogenous antagonist receptor that is able to bind to both membranous and soluble form of RANKL.[29] OPG, by interacting with RANKL, prevents RANK activation and subsequent osteoclastogenesis, and inhibits bone resorption as a result.[29] The central role of the RANKL/RANK/OPG signaling pathway in the regulation of bone remodeling has been proven in studies in genetically altered mice. Genetic ablations of both RANKL and RANK in mice exhibit severe osteopetrosis due to complete lack of osteoclasts.[27,31] Conversely, the ablation of OPG in mice results in osteoporosis, whereas overexpression of OPG causes osteopetrosis.[32,33]

The first fully human antibody to RANKL – denosumab decreases osteoclastogenesis and osteoclast activity, in consequence of which bone resorption reduces. Denosumab is a fully human IgG2 monoclonal antibody that binds to human RANKL with high affinity. By binding to RANKL the drug prevents the maturation and differentiation of preosteoclasts and promotes apoptosis of osteoclasts, and thereby bone resorption is slowed.[34] Denosumab, formerly known as AMG 162, was approved in June 2010 by the US Food and Drug Administration (FDA) as well as by the European Medicines Agency in Europe for treatment of postmenopausal osteoporosis in women at a high or increased risk of fracture.[35] Moreover, in Europe denosumab has also been accepted for treatment of bone loss associated with hormone ablation in men with prostate cancer, while in the USA it is used (as Xgeva) for prevention of skeletal-related events in patients with bone metastases from solid tumors.[36] Denosumab is administered subcutaneously as a single 60 mg dose once every 6 month, and may be injected in the upper arm, upper thigh or the abdomen.[37] The pharmacokinetics of this drug is nonlinear. Despite that neither absorption, bioavailability, distribution, nor elimination are determined, studies with similar IgG antibodies have shown that subcutaneous denosumab is absorbed by the lymphatic system with subsequent drainage into vascular system.[38] The bioavailability ranges from 50% to 100%. The clearance is probably by the reticuloendothelial system, and no significant amount of the drug seems to be filtered and excreted by kidneys.[38]

The phase I trial showed the ability of denosumab to reduce levels of bone turnover markers in healthy postmenopausal women.[39] Some patients received a single subcutaneous dose of denosumab of 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg or 3.0 mg/kg, while others were given placebo. The patients were then observed for 6 months, and those receiving the highest dose were monitored for the period of 9 months. Pharmacological effects were assessed by rapid and reversible reduction in the level of urinary N-telopeptide (NTX) that sustained for 6 months, and by later reduction of bone-specific alkaline phosphatase (BSAP).

Phase II was performed to evaluate the efficacy and safety of denosumab. It was established in a 4-year dose-ranging study in postmenopausal women (n = 412) with low bone mass or osteoporosis,[30,40] and continued in the extension studies.[41,42] Those studies demonstrated the significant increase in BMD in the treatment group (compared to the placebo group) after 12 months[43] and 48 months[30,40] with doses of denosumab of 30 mg once every 3 months or 60 mg once every 6 months. The extension studies confirmed the progressive improvement in BMD observed over 6 years[42] and 8 years[41] of treatment.

Therapeutic efficacy of denosumab was demonstrated in three experiments of phase III trials consisting of a comparison with placebo – 'Fracture Evaluation of Denosumab in Osteoporosis Every Six Month' (FREEDOM) trial,[44] and a head-to-head comparison with alendronate – 'Determining Efficacy: Comparison of Initiating Denosumab versus Alendronate' (DECIDE),[45] and the 'Study of Transitioning from Alendronate to Denosumab' trials (STAND).[46]

The FREEDOM trial enrolled 7808 postmenopausal women with osteoporosis, who were randomly assigned to receive either denosumab or placebo subcutaneously twice a year for 36 months.[44] At the end of the 36th month, compared to placebo, denosumab significantly reduced the risk of radiographic vertebral fractures by 68%, hip fracture by 40% and nonvertebral fracture by percent. Moreover, in comparison with placebo, denosumab improved lumbar spine BMD (0.2% vs. 0%) and total hip (4% vs. −2%) after 3 years of therapy. On top of that, biochemical markers of bone turnover were also significantly reduced in treated patients.

The DECIDE trial[45] was a double-blind, double-dummy noninferiority 1-year study to compare the effect of denosumab and alendronate on BMD and bone turnover markers (BTMs) in 1189 postmenopausal women with T-score of lumbar spine or total hip less than −2. Patients were randomly divided into groups, and either received a 60 mg dose of denosumab subcutaneously once every 6 months, accompanied by a weekly oral dose of placebo, or took an oral 70 mg dose of alendronate once every week together with a subcutaneous dose of placebo. The study revealed that the denosumab group had greater BMD increase in total hip in comparison to placebo, and greater reduction in BTMs than the alendronate group. Data also showed that denosumab was associated with significant reduction of bone resorption and greater gains in BMD at all measured sites in comparison to alendronate.

The STAND trial[46] was a 1-year randomized double-blind, double-dummy, parallel-group study in 504 postmenopausal women previously treated with alendronate for at least 6 months and with lumbar spine or total hip T-score of −2.0 to −4.0. At 12 months denosumab showed significant increase in BMD at the total hip, lumbar spine and distal one-third radius compared to the group continuing with alendronate. Those results revealed that postmenopausal women with low BMD could be safely transitioned from receiving weekly oral alendronate doses to 6-monthly subcutaneous denosumab doses in order to achieve incremental increase in bone mass.

Further clinical study was conducted by Reid et al.,[47] who collected iliac crest biopsies from the FREEDOM and STAND populations after 12, 24 and 36 months of denosumab exposition. The data revealed that in the FREEDOM study, median eroded surface was reduced by more than 80%, and osteoclasts were absent from more than 50% of biopsies in the denosumab group, whereas in STAND the histomorphometry demonstrated that denosumab in a dose of 60 mg every 6 months showed greater inhibition of turnover than through a weekly 70 mg dose of alendronate.

In conclusion, denosumab is an effective and safe drug used in the treatment of postmenopausal osteoporosis and osteoporosis in men as well as bone loss in men with prostate cancer and in women with breast cancer. It has been proven that this human monoclonal antibody to RANKL is associated with reduction in bone turnover markers, increase in BMD and decrease in the risk of vertebral fractures, hip fractures and nonvertebral fractures.

5. Conclusions

In summary, osteoporosis is the most common disorder affecting humans. Low bone density, loss of bone strength, increased bone fragility and increased fracture risk are the major clinical consequences of osteoporosis. The essential goal for the management of osteoporosis is to increase bone mass and reduce fracture risk by slowing or stopping bone loss. Progress in the understanding of the molecular regulators of bone remodeling has opened the possibility of creating new drugs in the therapy of osteoporosis. Recently developed monoclonal antibodies, including anti-RANKL anti-sclerostin and anti-DKK-1, represent a new class of compounds for the therapy of osteoporosis. By causing reduction of bone resorption and increase of bone mineral content, bone mass and bone strength, these antibodies are becoming an important option in the pharmacotherapy of this disease

Conflict of interest

None declared.

REFERENCES

1. Powroznik B, Kubowicz P, Pekala E. Monoclonal antibodies in targeted therapy. *Postepy Hig Med Dosw (Online)*. 2012;66:663–673 [in Polish].
2. Cairoli E, Zhukouskaya VV, Eller-Vainicher C, Chiodini I. Perspectives on osteoporosis therapies. *J Endocrinol Invest*. 2015. http://dx.doi.org/10.1007/s40618-014-0236-9 [in press].
3. Costa AG, Bilezikian JP. Sclerostin: therapeutic horizons based upon its actions. *Curr Osteoporos Rep*. 2012;10(1):64–72. http://dx.doi.org/10.1007/s11914-011-0089-5.
4. Hernandez P, Whitty C, John Wardale R, Henson FM. New insights into the location and form of sclerostin. *Biochem Biophys Res Commun*. 2014;446(4):1108–1113.
5. Ke HZ, Richards WG, Li X, Ominsky MS. Sclerostin and Dickkopf-1 as therapeutic targets in bone diseases. *Endocr Rev*. 2012;33(5):747–783. http://dx.doi.org/10.1210/er.2011-1060.
6. Compton JT, Lee FY. A review of osteocyte function and the emerging importance of sclerostin. *J Bone Joint Surg Am*. 2014;96(19):1659–1668.
7. Ott SM. Bone cells, sclerostin, and FGF23: what's bred in the bone will come out in the flesh. *Kidney Int*. 2015;87(3):499–501. http://dx.doi.org/10.1038/ki.2014.360.
8. Weivoda MM, Oursler MJ. Developments in sclerostin biology: regulation of gene expression, mechanisms of action, and physiological functions. *Curr Osteoporos Rep*. 2014;12(1):107–114. http://dx.doi.org/10.1007/s11914-014-0188-1.
9. Clarke BL. Anti-sclerostin antibodies: utility in treatment of osteoporosis. *Maturitas*. 2014;78(3):199–204. http://dx.doi.org/10.1016/j.maturitas.2014.04.016.
10. Garnero P. New developments in biological markers of bone metabolism in osteoporosis. *Bone*. 2014;66:46–55. http://dx.doi.org/10.1016/j.bone.2014.05.016.
11. Yavropoulou MP, Xygonakis C, Lolou M, Karadimou F, Yovos JG. The sclerostin story: from human genetics to the development of novel anabolic treatment for osteoporosis. *Hormones (Athens)*. 2014;13(4):323–337. http://dx.doi.org/10.14310/horm.2002.1552.
12. Brandenburg VM, D'Haese P, Deck A, et al. From skeletal to cardiovascular disease in 12 steps—the evolution of sclerostin as a major player in CKD-MBD. *Pediatr Nephrol*. 2015. http://dx.doi.org/10.1007/s00467-015-3069-7 [in press].
13. Rossini M, Gatti D, Adami S. Involvement of WNT/b-catenin signaling in the treatment of osteoporosis. *Calcif Tissue Int*. 2013;93(2):121–132. http://dx.doi.org/10.1007/s00223-013-9749-z.
14. Reichert JM. Antibodies to watch in 2015. *MAbs*. 2015;7(1):1–8. http://dx.doi.org/10.4161/19420862.2015.988944.
15. Jean G, Chazot C. Sclerostin in CKD-MBD: one more paradoxical bone protein? *Nephrol Dial Transplant*. 2013;28(12):2932–2935. http://dx.doi.org/10.1093/ndt/gft222.
16. Recker RR, Benson CT, Matsumoto T, et al. A randomized, double-blind phase 2 clinical trial of blosozumab, a sclerostin antibody, in postmenopausal women with low bone mineral density. *J Bone Miner Res*. 2015;30(2):216–224. http://dx.doi.org/10.1002/jbmr.2351.
17. Bhutani G, Gupta MC. Emerging therapies for the treatment of osteoporosis. *J Midlife Health*. 2013;4(3):147–152. http://dx.doi.org/10.4103/0976-7800.118991.
18. Ng KW, Martin TJ. New therapeutics for osteoporosis. *Curr Opin Pharmacol*. 2014;16:58–63. http://dx.doi.org/10.1016/j.coph.2014.03.004.
19. McClung MR, Grauer A, Boonen S, et al. Romosozumab in postmenopausal women with low bone mineral density. *N Engl J Med*. 2014;370(5):412–420.
20. Padhi D, Allison M, Kivitz AJ, et al. Multiple doses of sclerostin antibody romosozumab in healthy men and postmenopausal women with low bone mass: a randomized, double-blind, placebo-controlled study. *J Clin Pharmacol*. 2014;54(2):168–178.
21. Lim V, Clarke BL. New therapeutic targets for osteoporosis: beyond denosumab. *Maturitas*. 2012;73(3):269–272. http://dx.doi.org/10.1016/j.maturitas.2012.08.002.

22. O'Brien CA. Control of RANKL gene expression. *Bone*. 2010;46 (4):911–919. http://dx.doi.org/10.1016/j.bone.2009.08.050.
23. Wong BR, Josien R, Lee SY, et al. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J Exp Med*. 1997;186 (12):2075–2080. http://dx.doi.org/10.1084/jem.186.12.2075.
24. Walsh MC, Choi Y. Biology of the RANKL-RANK-OPG system in immunity, bone, and beyond. *Front Immunol*. 2014;5:511. http://dx.doi.org/10.3389/fimmu.2014.00511.
25. Lum L, Wong BR, Josien R, et al. Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *J Biol Chem*. 1999;274(19):13613–21368. http://dx.doi.org/10.1074/jbc.274.19.13613.
26. Hikita A, Yana I, Wakeyama H, et al. Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. *J Biol Chem*. 2006;281 (48):36846–43655. http://dx.doi.org/10.1074/jbc.M606656200.
27. Li J, Sarosi I, Yan XQ, et al. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. *Proc Natl Acad Sci U S A*. 2000;97(4):1566–1571. http://dx.doi.org/10.1073/pnas.97.4.1566.
28. Tat SK, Pelletier JP, Velasco CR, Padrines M, Martel-Pelletier J. New perspective in osteoarthritis: the OPG and RANKL system as a potential therapeutic target? *Keio J Med*. 2009;58 (1):29–40. http://dx.doi.org/10.2302/kjm.58.29.
29. Romas E. Clinical application of RANK-ligand. *Intern Med J*. 2009;39(2):110–116. http://dx.doi.org/10.1111/j.1445-5994.2008.01732.x.
30. Miller PD, Bolognese MA, Lewiecki EM, et al. Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: a randomized blinded phase 2 clinical trial. *Bone*. 2008;43 (2):222–229. http://dx.doi.org/10.1016/j.bone.2008.04.007.
31. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. 1999;397(6717):315–323. http://dx.doi.org/10.1038/16852.
32. Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early inset osteoporosis and arterial calcification. *Genes Dev*. 1998;12(9):1260–1268. http://dx.doi.org/10.1101/gad.12.9.1260.
33. Mizuno A, Amizuka N, Irie K, et al. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Commun*. 1998;247 (3):610–615. http://dx.doi.org/10.1006/bbrc.1998.8697.
34. Suresh E, Abrahamsen B. Denosumab: a novel antiresorptive drug for osteoporosis. *Cleve Clin J Med*. 2015;82(2):105–114. http://dx.doi.org/10.3949/ccjm.82a.13173.
35. Diab DL, Watts NB. Denosumab in osteoporosis. *Expert Opin Drug Saf*. 2014;13(2):247–253. http://dx.doi.org/10.1517/14740338.2014.860133.
36. Tella SH, Gallagher JC. Biological agents in management of osteoporosis. *Eur J Clin Pharmacol*. 2014;70(11):1291–1301. http://dx.doi.org/10.1007/s00228-014-1735-5.
37. Amgen. *Prolia® (denosumab)*. Thousand Oaks, CA: Amgen Inc.; 2010. http://pi.amgen.com/united_states/prolia/prolia_pi.pdf. Accessed 16.2.2015.
38. Choi HJ. New antiresorptive therapies for postmenopausal osteoporosis. *J Menopausal Med*. 2015;21(1):1–11. http://dx.doi.org/10.6118/jmm.2015.21.1.1.
39. Bekker PJ, Holloway DL, Rasmussen AS, et al. A single-dose placebo controlled study of AMG 162, a fully human monoclonal antibody to RANKL in postmenopausal women. *J Bone Miner Res*. 2004;19(7):1059–1066. http://dx.doi.org/10.1359/JBMR.040305.
40. Lewiecki EM, Miller PD, McClung MR, et al. Two-year treatment with denosumab (AMG 162) in a randomized phase 2 study of postmenopausal women with low BMD. *J Bone Miner Res*. 2007;22(12):1832–1841. http://dx.doi.org/10.1359/jbmr.070809.
41. McClung MR, Lewiecki EM, Geller ML, et al. Effect of denosumab on bone mineral density and biochemical markers of bone turnover: 8-year results of phase 2 clinical trials. *Osteoporos Int*. 2013;24(1):227–235. http://dx.doi.org/10.1007/s00198-012-2052.
42. Miller PD, Wagman RB, Peacock M, et al. Effect of denosumab on bone mineral density and biochemical markers of bone turnover: six-year results of a phase 2 clinical trial. *J Clin Endocrinol Metab*. 2011;96(2):394–402. http://dx.doi.org/10.1210/jc.2010-1805.
43. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med*. 2006;354(8):821–831. http://dx.doi.org/10.1056/NEJMoa044459.
44. Cummings SR, San Martin J, McClung MR, et al. FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med*. 2009;361(8):756–765. http://dx.doi.org/10.1056/NEJMoa0809493.
45. Brown JP, Prince RL, Deal C, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. *J Bone Miner Res*. 2009;24(1):153–161. http://dx.doi.org/10.1359/jbmr.080901.
46. Kendler DL, Roux C, Benhamou CL, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy. *J Bone Miner Res*. 2010;25(1):72–81. http://dx.doi.org/10.1359/jbmr.090716.
47. Reid IR, Miller PD, Brown JP, et al. Effects of denosumab on bone histomorphometry: the FREEDOM and STAND studies. *J Bone Miner Res*. 2010;25(10):2256–2265. http://dx.doi.org/10.1002/jbmr.149.