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REVIEW ARTICLE

Biologic Antiresorptive: Denosumab

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Abstract

Background: Osteoporosis is an age-related common bone disorder characterized by low bone mineral density and increased fragility fracture risk. Various Antiresorptive medications are being used to target osteoclast mediated bone resorption to prevent bone loss and reduce fracture risk.

About Denosumab: Denosumab is a novel biological antiresorptive drug that belongs to the class of monoclonal antibodies. It binds to and inhibits the cytokine receptor activator of nuclear factor kappa-B ligand (RANKL), which is requisite for osteoclast differentiation, function and survival.

Effectiveness: Denosumab has been shown to be a potent and effective therapy for osteoporosis, with clinical trial data demonstrating significant improvement in bone mineral density (BMD) and reductions in fracture risk at various skeletal sites for more than 10 years of treatment.

Safety Profile: Denosumab has a favourable benefit/risk profile, with low rates of complications such as infection, atypical femoral fracture and osteonecrosis of the jawbone.

Challenges: However, denosumab treatment requires continuous administration, as discontinuation leads to rapid bone mineral loss and increased risk of multiple vertebral fractures due to rebound of bone turnover. Therefore, modification to another anti-osteoporosis drug therapy after denosumab discontinuation is required to maintain bone health.

Conclusion: Denosumab is a promising biological antiresorptive therapy for osteoporosis that offers high efficacy and safety, but also poses challenges for long-term management.

Keywords Antiresorptive · Denosumab · Biologic · Osteoporosis treatment · Monoclonal antibody

Abbreviations

AFF	Atypical femur fracture
BMD	Bone mineral density
BRC	Bone remodeling compartment
BTMs	Bone turnover markers
CMP	Common myeloid progenitors
CTX	C-telopeptide
DAPS	Denosumab adherence preference satisfactions
DEFEND	Denosumab fortifies bone density
FREEDOM	Fracture reduction evaluation of denosumab in osteoporosis every 6 Months
GMP	Granulocyte/macrophage progenitors
GM-CSF	Granulocyte/macrophage colony stimulating factor
HALT	Hormone ablation bone loss trial
HSC	Hematopoietic Stem Cells
IGF-I	Insulin-like growth factor-I
IL	Interleukin and IL-6
MVF	Multiple vertebral fractures
ONJ	Osteonecrosis of jaw
OPG	Osteoprotegerin
OPGL	Osteoprotegerin ligand
P1NP	Procollagen type 1 N-terminal peptide
PTH	Human recombinant parathyroid hormone,
PTHrP	Synthetic PTH-related peptide
RANK	Receptor activator of nuclear factor kappa beta (NKfB)
RANKL	Receptor activator of nuclear factor kappa beta (NKfB) ligand
SCF	Stem cell factor
SERMs	Selective estrogen receptor modulators
SC	Subcutaneous
TGF-β	Transforming growth factor beta
TRANCE	TNF related activation induced cytokine

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Introduction

Osteoporosis is a metabolic disorder of bone metabolism characterized by more resorption than bone formation, translating to microarchitecture deterioration and a reduction in bone mineral density (BMD) with an enhanced risk of fractures.

Osteoporotic fragility fracture occurs in the elderly population when they are unable to withstand physiological stress. Commonly encountered sites of fractures are the dorso-lumbar spine, hip region, wrist: however, fragility fracture is common occurrence in the pelvis, humerus and ribs [1].

Fragility fractures have severe detrimental effects on patients and their families by affecting their physiology, psychology and financial condition, if not managed appropriately, they can be a cause of loss of life. That is why osteoporosis treatment should be started early, aiming to prevent of osteoporotic fractures.

For more than two decades, medications with different mechanisms of action have been used for the management of osteoporosis. They are have antiresorptive effects, osteoanabolic effects and drugs with dual modes of actions. Anabolic or bone-forming drugs like teriparatide and abaloparatide, used to create bone remodeling imbalance and stimulate the bone formation. Resultant effects are increase in BMD. While antiresorptive drugs focused on the inhibition of bone resorption by reducing the bone turnover through the alteration in osteoclast proliferation and maturation. These drugs classified into various categories like bisphosphonates, selective estrogen receptor modulators (SERMs), estrogen, monoclonal antibodies-denosumab and calcitonin [2].

At present, osteoporosis treatment revolves around the use of anti-resorptive drugs. Bisphosphonates are extensively used as the first treatment modality for osteoporosis due to the low cost and reliability of drugs. Drugs that are commonly used like Alendronate, Risedronate, Ibandronate and zoledronic acid have shown therapeutic effectiveness in moderation of fragility fracture at vertebral, non-vertebral sites and hip region. In all the above only Ibandronate was found to be in-effective in prevention of vertebral fracturs [3].

Denosumab is an anti-receptor activator of nuclear factor kappa beta(NKfB) ligand.

(RANKL) monoclonal antibody acts specifically on RANKL, resulting in antiresorptive effect on bone and increases in BMD with effective and sustained risk reduction of fracture [3, 4].

Other group of medications are Selective estrogen receptor modulators (SERMs) which can either stimulate or block the effects of estrogen with a differential degree of expression on different tissues. SERMs have a suppression effect on osteoclastic activity and have fracture preventing effects [3].

Anabolic agents that stimulate bone formation include teriparatide- a human recombinant parathyroid hormone (PTH) and abaloparatide- a synthetic PTH-related peptide (PTHrP) analogue. Romosozumab is a new monoclonal antibody that targets sclerostin and inhibits its activity and helps to treat osteoporosis by promoting bone formation and inhibiting bone resorption. Anabolic drugs improve BMD in spine and hip with fracture risk reduction [5]. These drugs are reserved for individuals with high risk of fractures, due to limited duration of use.

Denosumab

Denosumab is a fully human monoclonal IgG2 antibody produced by genetically engineered techniques. Denosumab acts specifically and preferentially on the human receptor activator of the nuclear factor kappa-B ligand RANKL. RANKL is a major modulator of osteoclast activation and its precursors for bone resorption. Receptor activator of nuclear factor kappa beta (NKfB) (RANK) is a receptor for RANKL, present on the osteoclast surface, and osteoclast precursors and denosumab attaches to RANKL, restricting the activity of receptor RANK. This leads to inhibitory effect on osteoclast formation, activity, and vitality, resulting in a decline in bone resorption rate [6].

Mechanism of Action

Bone formation and bone resorption are balanced processes of the natural bone remodeling process, thus ensuring that the net bone mass remains the same at the end of each remodeling cycle. This bone remodeling process is influenced by many mechanisms, including:

(a) Systemic and local factors: all factors that contribute to the formation and activity of osteoclast cells and osteoblast cells [7, 8]. Systemic factors like hormones, hypoxia, acidosis, neurovascular signaling, and a large number of local factors that affect them include various cytokines, growth factors, cell adhesion molecules, proteases, and other matrix molecules.

(b) Various factors released by the bone matrix, like transforming growth factor beta.

(TGF-β) and insulin-like growth factor-I (IGF-I) during bone resorption, promote the coupled bone formation and bone resorption by activating osteoblast differentiation and formation [9, 10].

(c) While resorption occurs, osteoclast cells synthesize local factors having a stimulatory effect on osteoblast

differentiation and function, which is another local coupled mechanism that affects bone remodeling [11, 12].

All these coupling mechanisms of bone turnover occur in an enclosed compartment named the bone remodeling compartment (BRC), which plays a critical role. Moreover, the BRC concept explained how bone formation and resorption are coupled with osteoclast, osteoblast differentiation and vascular channels in the local microscopic area. In BRC area vascular channels, osteoblast, osteoclast, and cell lining play a differential role in different disease processes resulting in alteration in bone remodeling, which can be a targeted area for drug uses in different disease processes [13].

Osteoclasts originate from hematopoietic cells and are not related to the osteoblast lineage. This was confirmed in various experimental studies, which include, Gothlin et al.'s experiments which found that by joining the circulation of two rats, osteoclasts migrated from a normal rat to an irradiated rat [14, 15]. Other experiments by chimaeras of chicks and quail embryonic tissue demonstrated that hematopoietic tissues contain osteoclast precursors [16–18]. Scheven and co-workers, in an *in-vitro* experiment, reported that osteoclasts differentiated from stem cells in the co-culture technique of mouse bone marrow and fetal bone rudiments together [19]. The bone marrow contains hematopoietic stem cells (HSC) that can be activated by different factors, such as stem cell factor (SCF), interleukin-3 (IL-3), and IL-6. These factors trigger the HSC to produce common myeloid progenitors (CMP). The CMP then undergoes differentiation into granulocyte/macrophage progenitors (GMP) with the help of granulocyte/macrophage colony stimulating factor (GM-CSF). The GMP further develops into cells of the monocyte/macrophage lineage under the influence of M-CSF. These cells are the precursors of osteoclasts [20, 21]. The strongest evidence for the hematopoietic origin of osteoclasts comes from in vitro studies that demonstrated that monocytes can become osteoclasts when exposed to RANKL and M-CSF [22, 23].

M-CSF and Rankl/Rank/Osteoprotegerin (OPG)

M-CSF, also called CSF-1, is a hematopoietic growth factor that has a crucial role in stimulating osteoclast precursors. It plays a pivotal role and helps in the growth and differentiation of these cells as well [24, 25]. Another important factor that affects osteoclast formation is RANKL, which has other names such as OPGL, ODF, and TRANCE. RANKL belongs to the tumor necrosis factor (TNF) superfamily. RANKL works by binding to its receptor RANK, which is part of the TNFR family. Osteoprotegerin (OPG) is a protein that mimics RANK and competes with it for RANKL. By doing so, OPG prevents RANKL from activating osteoclasts and reduces bone resorption. [26, 27, 30, 31]. RANKL and RANK are essential for activating osteoclasts and maintaining their survival [28, 32, 33].

Pharmacodynamics

In clinical studies, subcutaneous (SC) denosumab at a dose of 60 mg was given, and the marker level of bone resorption C-telopeptide (CTX) was assessed and showed a reduction of up to 85% at three days. The level of detection was too low to assess in 39% and 68% of patients at 1 and 3 months, respectively.

After six months of the last dose, CTX levels were partially recovered from the lowest level of the assay suggesting some reversibility of bone remodeling suppression.

After stopping denosumab treatment, bone resorption markers increased by 40–60% more than the levels before treatment, but they returned to normal within a year, showing that the effects can be reversed, and bone formation markers (such as osteocalcin and P1NP) also decreased after one month [32, 33].

Pharmacokinetics and Metabolism

Denosumab's pharmacokinetics are non- linear in nature and dose-dependent at different dose ranges. It has a long absorption phase, a slow β phase, and a faster terminal phase [33]. After a single SC dose of 60 mg of denosumab, the average peak drug concentration (Cmax) was 6.75 mcg/ml. The peak concentration (T-max) was reached in a median of 10 days, with a range of 3 days to 3 weeks, indicating that denosumab is absorbed slowly through the SC route. After reaching the peak concentration, the drug levels in the serum decreased over a period of 4–5 months, with an average half-life of 25.4 days. Repeated doses of 60 mg SC every 6 months did not result in significant accumulation or change in the pharmacokinetics of denosumab over time [32].

A meta-analysis that included 11 studies of Phase I, Phase II, and Phase III studies found that the SC bioavailability of denosumab is 64%. The study concluded that drug dose adjustment is not required for changes in body weight, age, gender, or race, and a single fixed dose of 60 mg denosumab yields the same RANKL inhibition as a body weight adjusted dose [34]. Denosumab does not need dose adjustment for patients with kidney disease, from normal renal function to dialysis-dependent [35]. The effects and pharmacokinetics of denosumab have not been studied in patients with liver derangements.

Evidences

The role of denosumab in healthy women for osteoporosis treatment was evaluated in many randomized controlled trials for risk and benefits over a substantial period of time. In Phase I of the RCT, 49 healthy postmenopausal women were given a single SC dose of denosumab or placebo at 0.01, 0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg. They were assessed for the safety of the drug, tolerance, pharmacokinetics and level of bone turnover markers monitored (BTMs). There was suppression of urinary NTX, and serum calcium was lowered for a transient period. ALP levels at baseline did not change much after one month of the denosumab SC dose, and a transient increase in serum iPTH was observed, suggesting that the effect is primarily antiresorptive. The treatment was well tolerated, and no serious or drug-related adverse events were reported [33].

A larger Phase II study tested the effectiveness and safety of denosumab on 412 people. Only 262 (64%) of them were post-menopausal women with low BMD who finished the study for 48 months. The participants were randomly assigned to different groups that received SC Denosumab at different doses, placebo, or alendronate (open label) as a control. The low dose group (6, 14, and 30 mg) got Denosumab every 3 months, and the high dose group (14, 60, 100, or 210 mg) got it every six months for the first two years. After that, the groups were changed to continue, stop, or restart the treatment with Denosumab 60 mg every 6 months. The placebo group stayed on placebo, and the alendronate group stopped and was followed up [37]. Denosumab treatment received patients have shown 9.4–11.8% gain in BMD at the lumbar region and 4–6% at the hip region, with suppression of bone turnover markers [BTMs] observed until the study was completed. BMD was returned to baseline after discontinuation of therapy. The BMD dropped more than 6% after 12 months of discontinuation of denosumab, and BTM levels reached baseline values, while in the re-initiated group, BMD dropped after stopping treatment and improved after re-initiating treatment. The placebo group had a decrease in the BMD at the spine. Phase II study has demonstrated a strong correlation between BMD and BTM at baseline, two years of denosumab and 12 months of stopping of denosumab.

In the FREEDOM Phase III clinical trial, which lasted three years, 7868 postmenopausal women aged 60–90 with a lumbar spine or total hip T-score between − 2.5 and − 4.0 were enrolled. They were divided into two equal groups; one received a 6-monthly SC dose of 60 mg denosumab, and the other received a placebo. The study found that denosumab significantly reduced the risk of vertebral, nonvertebral, and hip fractures compared to placebo. This evidence supports the FDA's approval of denosumab for use in postmenopausal women at high risk of fractures due to osteoporosis [37].

Results of the FREEDOM extension studies for a total of 10 years of follow up including the three year initial FREEDOM trial, showed a continued rise in BMD up to 18.5% in the lumbar spine and 8.2% in the hip region with a rise in the BMD in the cross over group of 13.8% and 4.8% in lumbar spine and hip, respectively. The extension study has evaluated the rate of new fractures in the vertebral or non-vertebral regions. The rate of new fractures in the denosumab and crossover groups remained low in 8-year follow up [38]. The FREEDOM extension study also assessed anti-fracture efficacy results on discontinuation of denosumab therapy. They found that the percentage of new fractures was similar in both groups that stopped denosumab and those that stayed on placebo [39].

Not only fractures and bone turnover markers, but histology and structural strength were evaluated in the FREEDOM extension cohort. A total of 41 subjects (28 long-term and 13 cross-over) have undergone a trans-iliac crest bone biopsy. Both qualitative bone histology and structural indices like trabecular bone volume, number, and surface were assessed. They showed normal mineralized lamellar bone and similar structural indices in the long-term and crossover groups. In denosumab treated patients, dynamic remodeling indices were low, suggesting low bone turnover [40].

Denosumab is being used as an anti-resorptive drug in males as well, having severe osteoporosis and a high fracture risk. The ADAMO RCT enrolled 242 men aged 30–85 who were divided into denosumab or placebo groups to test the effectiveness and safety of denosumab. The main goal of the study was to measure the percentage change in BMD after one year. The results showed that denosumab increased BMD by 5.7% at the spine, 2.4% at the total hip, and 2.1% at the femoral neck after 12 months. Based on these findings, the FDA approved denosumab for treating men with osteoporosis who have a high risk of fractures [41].

Androgen deprivation therapy in males and adjuvant aromatase inhibitor therapy in females are known to have a decrease in bone mineral density as a side effect. Men who have non-metastatic, hormone sensitive prostate cancer and are on androgen-deprivation therapy may lose bone mineral density and have a higher risk of fractures. The HALT study tested how denosumab or placebo affected these men in a randomized controlled trial. The study had 734 men in each group who received the treatment for 2 years. The denosumab group had a significant increase in BMD of 5.6% and a lower rate of new spine fractures. The placebo group had a decrease in BMD of 1.0 percent [42].

The study by Ellis et al. divided women with non-metastatic breast cancer who were on adjuvant aromatase inhibitor therapy into two groups. One group received a

SC dose of 60 mg denosumab (127 women), and the other received a placebo (125 women). The study lasted for two years. At 12 and 24 months, the denosumab group had an increase in BMD at the spine of 5.5% and 7.6%, respectively. The study did not find any link between the change in BMD in the denosumab group and how long they were on aromatase inhibitor therapy [43].

The results of combination therapy with denosumab and teriparatide are also encouraging. They show marked improvement in BMD and a significant decrease in the incidence of new fractures. These results were in contrast to the effects of alendronate and teriparatide combination therapy on a daily dose basis. The purpose of the DATA (The Denosumab and Teriparatide Administration study) RCT was to evaluate the effectiveness and safety of using both drugs together in postmenopausal women with osteoporosis.

The study randomly assigned 94 participants into three equal groups. The first group received 20 mcg of teriparatide SC daily, the second group received 60 mg of denosumab SC every 6 months, and the third group received both drugs. The combination group had a higher increase in BMD at the lumbar spine/total hip (9.1%/4.9%) than the teriparatide (6.2%/0.7%) or denosumab (5.5%/2.5%) groups. Serum markers like OC and P1NP CTX levels showed elevated values in the teriparatide group only, and significant suppression was found in both denosumab and the combination groups well. This effect is likely due to the dissociated bone resorption and bone formation and possibly a reduction in teriparatide-induced bone resorption, with partial effect on teriparatide-induced bone formation [44].

In a recent metanalysis which included 11 RCTs of 12,013 postmenopausal women with osteoporosis or low BMD, the metanalysis showed denosumab treatment increased BMD percentage more than placebo at different skeletal sites: lumbar spine, total hip, radius, trochanteric, and total body. In addition, denosumab therapy significantly lowered the risk of fractures in the non-vertebral, vertebral and hip regions. They also concluded Denosumab did not pose excess risks of adverse events [45].

Adherence, compliance and persistence are paramount aspects for the success of any treatment. Compromised and poor results have been observed in non-compliance and non-adherence to treatment. The DAPS (Denosumab Adherence Preference Satisfactions) study showed the results of how well patients followed and liked the treatment in 221 women who had osteoporosis after menopause. In this study, patients were treated with either denosumab or oral alendronate every week, and crossover was done after a year. The result was suggestive of more adherence, compliance and persistence with denosumab treatment compared to oral alendronate [46].

Approved Indications for Treatment

1. Women with high risk for fracture: post-menopausal with low BMD or women receiving adjuvant aromatase inhibitor therapy for breast malignancy
2. Men with a high risk for fracture having osteoporosis with low BMD or receiving androgen deprivation therapy for treatment prostate malignancy.
3. In addition, it is used to prevent skeletal fracture events, irradiated bone or the treatment of bone tumors.

Denosumab is administered in a 60 mg dose by subcutaneous route, usually given in the abdomen, proximal thigh, and proximal arm. The same dose is repeated at every six month interval. As per available literature with data of more than 10 years, denosumab can be given for extended period of 10 years with limited side effects.

Side Effects and Complications

Common side effects may include muscle spasm, cramps, muscular pain, fatigue and flu like symptoms. Other side effects observed in various studies that are injection site related are cellulitis, eczema, and erysipelas. In the combined FREEDOM and extension trial, the skin-related complication rates were not statistically significant in the denosumab and placebo groups. No reports of increased malignancy risk were found [46–48].

Few patients reported atypical femoral fracture (AFF) and osteonecrosis of jaw (ONJ) but direct cause of denosumab treatment was not established in these patients because these patients were reported to have received treatment with alendronate, glucocorticoids or chemotherapy at some point in time and had associated invasive dental procedures or disease involvement [49, 50]. DEnosumab FortifiEs boNe Density (DEFEND) study also reflected that the overall rate of infections was similar to placebo but reported more serious side effects like lung infections, GI related infections such as diverticulitis, appendicitis, pyelonephritis, urinary tract infections, septicemia and skin-related infections in the denosumab treated patients [51].

Patients with chronic kidney disease develop seizure or tetany-like symptoms that could be a risk factor for hypocalcemia. In cases with kidney disease, surveillance of serum calcium, serum magnesium and phosphorus is recommended. Also, daily vitamin D 400 IU and 1000 mg calcium intake is advised. Anaphylaxis, although rare, was reported in five patients [52]. As RANKL is expressed by immune B cells and T cells, there is theoretical possibility of risk of altered immune response [53].

It's important to discuss the risk of multiple spine Fractures after stopping of denosumab. Phase 2 and phase 3 studies have observed the reversibility of denosumab effect after discontinuation of the drug after 12 months and a surge in the number of multiple levels of vertebral fractures (MVF) seen with the bone turnover marker also returning to the baseline [36, 39, 55]. Bone biopsies were taken 2 years after the denosumab discontinuation, confirming the reversibility of bone turnover markers [56]. This increases the risk of MVF is clinical repercussion of the rebound in bone turnover on withdrawal of denosumab [57–59]. In the majority of individuals, there were associated risk factors for fracturs like low BMD, treatment with a steroid, previous vertebral fracture, or aromatase inhibitor therapy. In such cases, discontinuation of denosumab therapy is not suggested [60]. The RCT of Freedom trial and its extension, 10-year study showed a decrease in fracture risk with denosumab and a subsequent increase in fracture risk in denosumab cessation group compared to the placebo group [61]. Contraindications of denosumab use include patients who have hypocalcemia, are pregnant or have had allergic reactions to the drug or its ingredients [32].

Conclusion

Denosumab is recognized as the first monoclonal antibody that inhibits RANKL, which has been approved for the treatment of osteoporosis and the prevention of fractures due to fragility. It has proven efficacy in increasing BMD significantly, and suppressing bone turnover markers, with effects reversible on discontinuation of therapy. Many studies, including more than 10 years of use of extended data, have proved sustained efficacy for increasing BMD and lowering fracture risk. Although beneficial effects are high with good tolerance and acceptable side effects compared to placebo and other antiresorptive drugs.

Data availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by the any of the authors.

Informed consent For this type of study informed consent is not required.

References

1. Rosen, H., Drezner, M. Clinical manifestations, diagnosis, and evaluation of osteoporosis in postmenopausal women-UpToDate [Internet]. 2018.
2. Compston, J., Bowring, C., Cooper, A., et al. (2013). Diagnosis and management of osteoporosis in postmenopausal women and older men in the UK: National osteoporosis guideline group (NOGG) update 2013. *Maturitas, 75*, 392–396.
3. Cosman, F., de Beur, S. J., LeBoff, M. S., et al. (2014). Clinician's guide to prevention and treatment of osteoporosis. *Osteoporosis International, 25*, 2359–2381.
4. Denosumab (Prolia): Treatment to increase bone mass in men with osteoporosis at high risk for fracture; or who have failed or are intolerant to other available osteoporosis therapy [Internet]. Ottawa (ON): Canadian agency for drugs and technologies in health; 2015 Oct.
5. Tsai, J., Burnett-Bowie, S., Lee, H., et al. (2017). Relationship between bone turnover and density with teriparatide, denosumab or both in women in the DATA study. *Bone, 95*, 20–25.
6. Zaheer, S., LeBoff, M., & Lewiecki, E. M. (2015). Denosumab for the treatment of osteoporosis. *Expert Opinion on Drug Metabolism & Toxicology, 11*, 461–470.
7. Raisz, L. G. (1988). Hormonal regulation of bone growth and remodelling. *Ciba Foundation symposium., 136*, 226–238.
8. Mohan, S., & Baylink, D. J. (1996). Insulin-like growth factor system components and the coupling of bone formation to resorption. *Hormone research., 45*(Suppl 1), 59–62.
9. Tang, Y., Wu, X., Lei, W., Pang, L., Wan, C., & Shi, Z. (2009). TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nature Medicine, 15*, 757–765.
10. Xian, L., Wu, X., Pang, L., Lou, M., Rosen, C. J., Qiu, T., Crane, J., Frassica, F., Zhang, L., Rodriguez, J. P., Xiaofeng, J., Shoshana, Y., Shouhong, X., Argiris, E., Mei, W., & Xu, C. (2012). Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nature Medicine, 18*, 1095–1101.
11. Falany, M. L., Thames, A. M., 3rd., McDonald, J. M., Blair, H. C., McKenna, M. A., Moore, R. E., Young, M. K., & Williams, J. P. (2001). Osteoclasts secrete the chemotactic cytokine mim-1. *Biochemical and Biophysical Research Communications, 281*(1), 180–185.
12. Martin, T., Gooi, J. H., & Sims, N. A. (2009). Molecular mechanisms in coupling of bone formation to resorption. *Critical Reviews in Eukaryotic Gene Expression, 19*, 73–88.
13. Andersen, T. L., Sondergaard, T. E., Skorzynska, K. E., Dagnaes-Hansen, F., Plesner, T. L., Hauge, E. M., Plesner, T., & Delaisse, J. M. (2009). A physical mechanism for coupling bone resorption and formation in adult human bone. *American Journal of Pathology, 174*, 239–247.
14. Gothlin, G., & Ericsson, J. L. (1976). The osteoclast: Review of ultrastructure, origin, and structure-function relationship. *Clinical orthopaedics and related research., 120*, 201–231.
15. Walker, D. G. (1973). Osteopetrosis cured by temporary parabiosis. *Science, 180*, 875.
16. Kahn, A. J., & Simmons, D. J. (1975). Investigation of cell lineage in bone using a chimaera of chick and quial embryonic tissue. *Nature, 258*, 325–327.
17. Walker, D. G. (1975). Bone resorption restored in osteopetrotic mice by transplants of normal bone marrow and spleen cells. *Science, 190*, 784–785.
18. Walker, D. G. (1975). Spleen cells transmit osteopetrosis in mice. *Science, 190*, 785–787.
19. Scheven, B. A., Visser, J. W., & Nijweide, P. J. (1986). *In vitro* osteoclast generation from different bone marrow fractions, including a highly enriched haematopoietic stem cell population. *Nature, 321*, 79–81.
20. Kondo, M., Wagers, A. J., Manz, M. G., Prohaska, S. S., Scherer, D. C., Beilhack, G. F., Shizuru, J. A., & Weissman, I. L. (2003). Biology of hematopoietic stem cells and progenitors: Implications

for clinical application. *Annual Review of Immunology, 21*, 759–806.
21. Metcalf, D. (2008). Hematopoietic cytokines. *Blood, 111*(2), 485–491.
22. Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N., & Suda, T. (1998). Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/ANKL. *Proc Natl Acad Sci U S A., 95*, 3597–3602.
23. Matsuzaki, K., Udagawa, N., Takahashi, N., Yamaguchi, K., Yasuda, H., Shima, N., Morinaga, T., Toyama, Y., Yabe, Y., Higashio, K., & Suda, T. (1998). Osteoclast differentiation factor (ODF) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. *Biochemical and Biophysical Research Communications, 246*, 199–204.
24. Metcalf, D. (1970). Studies on colony formation in vitro by mouse bone marrow cells. II. Action of colony stimulating factor. *Journal of Cellular Physiology, 76*, 89–99.
25. Xaus, J., Comalada, M., Valledor, A. F., Cardó, M., Herrero, C., Soler, C., Lloberas, J., & Celada, A. (2001). Molecular mechanisms involved in macrophage survival, proliferation, activation or apoptosis. *Immunobiology, 204*, 543–550.
26. Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., … Boyle, W. J. (1998). Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell, 93*, 165–176.
27. Anderson, D. M., Maraskovsky, E., Billingsley, W. L., Dougall, W. C., Tometsko, M. E., Roux, E. R., Teepe, M. C., DuBose, R. F., Cosman, D., & Galibert, L. (1997). A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature, 390*, 175–179.
28. Bucay, N., Sarosi, I., Dunstan, C. R., Morony, S., Tarpley, J., Capparelli, C., Scully, S., Tan, H. L., Xu, W., Lacey, D. L., Boyle, W. J., & Simonet, W. S. (1998). osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes & Development, 12*, 1260–1268.
29. Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Lüthy, R., Nguyen, H. Q., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colombero, A., Tan, H. L., Trail, G., Sullivan, J., Davy, E., Bucay, N., … Boyle, W. J. (1997). Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. *Cell, 89*, 309–319.
30. Lum, L., Wong, B. R., Josien, R., Becherer, J. D., Erdjument-Bromage, H., Schlöndorff, J., Tempst, P., Choi, Y., & Blobel, C. P. (1999). Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzymelike protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *Journal of Biological Chemistry, 274*, 13613–13618.
31. Wong, B. R., Besser, D., Kim, N., Arron, J. R., Vologodskaia, M., Hanafusa, H., & Choi, Y. (1999). TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. *Molecular Cell, 4*, 1041–1049.
32. Amgen, Inc. *Prolia (denosumab)* prescribing information. 2010 updated 2014.
33. Bekker, P., Holloway, D., Rasmussen, A., et al. (2004). A single-dose placebo-controlled study of AMG 162, a fully monoclonal antibody to RANKL, in postmenopausal women. *Journal of Bone and Mineral Research, 19*(7), 1059–1066.
34. Sutjandra, L., Rodriguez, R., Doshi, S., et al. (2011). Population pharmacokinetic metaanalysis of denosumab in healthy subjects and postmenopausal women with osteopenia or osteoporosis. *Clinical Pharmacokinetics, 50*(12), 793–807.
35. Block, G., Bone, H., Fang, L., et al. (2012). A single-dose study of denosumab in patients with various degrees of renal impairment. *Journal of Bone and Mineral Research, 27*(7), 1471–1479.
36. Miller, P., Bolognese, M., Lewiecki, E., et al. (2008). 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, 43*(2), 222–229.
37. Cummings, S., San Martin, J., Mcclung, M., et al. (2009). Denosumab for prevention of fractures in postmenpausal women with osteoporosis. *New England Journal of Medicine, 361*(8), 756–765.
38. Papapoulos, S., Lippuner, K., Roux, C., Lin, C. J., Kendler, D. L., Lewiecki, E. M., Brandi, M. L., Czerwiński, E., Franek, E., Lakatos, P., Mautalen, C., Minisola, S., Reginster, J. Y., Jensen, S., Daizadeh, N. S., Wang, A., Gavin, M., Libanati, C., Wagman, R. B., & Bone, H. G. (2015). The effect of 8 or 5 years of denosumab treatment in postmenopausal women with osteoporosis: results from the FREEDOM Extension study. *Osteoporosis International, 26*(12), 2773–2783.
39. Brown, J., Roux, C., Torring, O., et al. (2013). Discontinuation of denosumab and associated fracture incidence: analysis from the fracture reduction evaluation of denosumab in osteoporosis every 6 months (FREEDOM) trial. *Journal of Bone and Mineral Research, 28*(4), 746–752.
40. Brown, J., Reid, I., Wagon, R., et al. (2014). Effects of up to 5 years of denosumab treatment on bone histology and histomorphometry: the FREEDOM study extension. *Journal of Bone and Mineral Research, 29*(9), 2051–2056.
41. Orwoll, E., Teglbjærg, C., Langdahl, B., et al. (2012). A randomized, placebo-controlled study of the effects of denosumab for the treatment of men with low bone mineral density. *Journal of Clinical Endocrinology and Metabolism, 97*(9), 3161–3169.
42. Smith, M., Egerdie, B., Toriz, N., et al. (2009). Denosumab in men receiving androgen deprivation therapy for prostate cancer. *New England Journal of Medicine, 361*(8), 745–755.
43. Ellis, G. K., Bone, H. G., Chlebowski, R., et al. (2008). Randomized trial of denosumab in patients receiving adjuvant aromatase inhibitors for nonmetastatic breast cancer. *Journal of Clinical Oncology, 26*, 4875–4882.
44. Tsai, J., Uihlein, A., Lee, H., et al. (2013). Teriparatide and denosumab, alone or combined, in women with postmenopausal osteoporosis: The DATA study randomised trial. *Lancet, 382*(9886), 50–56.
45. Chen, Y., Zhu, J., Zhou, Y., Peng, J., & Wang, B. (2021). Efficacy and safety of denosumab in osteoporosis or low bone mineral density postmenopausal women. *Frontiers in Pharmacology, 14*(12), 588095.
46. Freemantle, N., Satram-Hoang, A., Tang, E., et al. (2012). Final results of the DAPS (Denosumab ADHERENCE PREFERENCE SATISFACTION) study: a 24-month, randomized, crossover comparison with alendronate in postmenopausal women. *Osteoporosis International, 23*(1), 317–326.
47. von Keyserlingk, C., Hopkins, R., Anastasilakis, A., et al. (2011). Clinical efficacy and safety of denosumab in postmenopausal women with low bone mineral density and osteoporosis: a meta-analysis. *Seminars in Arthritis and Rheumatism, 41*(2), 178–186.
48. Hageman, K., Patel, K., Mace, K., et al. (2013). The role of denosumab for prevention of skeletal-related complications in multiple myeloma. *Annals of Pharmacotherapy, 47*(7–8), 1069–1074.
49. Watts, N. B., Grbic, J. T., Binkley, N., et al. (2019). Invasive oral procedures and events in postmenopausal women with osteoporosis treated with denosumab for up to 10 years. *Journal of Clinical Endocrinology and Metabolism, 104*, 2443–2452.
50. Ferrari, S., Lewiecki, E. M., Butler, P. W., et al. (2020). Favourable skeletal benefit/risk of long-term denosumab therapy: a

virtual-twin analysis of fractures prevented relative to skeletal safety events observed. *Bone, 134*, 115287.
51. Bone, H. G., Bolognese, M. A., Yuen, C. K., Kendler, D. L., Wang, H., Liu, Y., et al. (2008). Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism, 93*, 2149–2157.
52. Geller, M., Wagman, R., Ho, P., et al. (2014). SAT0479 early findings from Prolia® post-marketing safety surveillance for atypical femoral fracture, osteonecrosis of the jaw, severe symptomatic hypocalcemia, and anaphylaxis. *Annals of the Rheumatic Diseases, 73*, 766–767.
53. Ferrari-Lacraz, S., & Ferrari, S. (2011). Do RANKL inhibitors (denosumab) affect inflammation and immunity? *Osteoporosis International, 22*, 435–446.
54. Miller, P., Bolognese, M., Lewiecki, E., et al. (2008). 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, 43*(2), 222–229.
55. Bone, H. G., Bolognese, M. A., Yuen, C. K., Kendler, D. L., Miller, P. D., Yang, Y. C., Grazette, L., San Martin, J., & Gallagher, J. C. (2011). Effects of denosumab treatment and discontinuation on bone mineral density and bone turnover markers in postmenopausal women with low bone mass. *Journal of Clinical Endocrinology and Metabolism, 96*(4), 972–980.
56. Brown, J. P., Dempster, D. W., Ding, B., et al. (2011). Bone remodeling in postmenopausal women who discontinued denosumab treatment: Off-treatment biopsy study. *Journal of Bone and Mineral Research, 26*, 2737–2744.
57. Aubry-Rozier, B., Gonzalez-Rodriguez, E., Stoll, D., & Lamy, O. (2016). Severe spontaneous vertebral fractures after denosumab discontinuation: three case reports. *Osteoporosis International, 27*, 1923–1925.
58. Lamy, O., Gonzalez-Rodriguez, E., Stoll, D., Hans, D., & Aubry-Rozier, B. (2017). Severe rebound-associated vertebral fractures after denosumab discontinuation: 9 clinical cases report. *Journal of Clinical Endocrinology and Metabolism, 102*, 354–358.
59. Kendler, D., Chines, A., Clark, P., Ebeling, P. R., McClung, M., Rhee, Y., Huang, S., & Stad, R. K. (2020). Bone mineral density after transitioning from denosumab to alendronate. *Journal of Clinical Endocrinology and Metabolism, 105*(3), e255–e264.
60. Cummings, S. R., Ferrari, S., Eastell, R., et al. (2018). Vertebral fractures after discontinuation of denosumab: A post hoc analysis of the randomized placebo-controlled FREEDOM trial and its extension. *Journal of Bone and Mineral Research, 33*, 190–198.
61. Kendler, D. L., Chines, A., Brandi, M. L., et al. (2019). The risk of subsequent osteoporotic fractures is decreased in subjects experiencing fracture while on denosumab: Results from the FREEDOM and FREEDOM extension studies. *Osteoporosis International, 30*, 71–78.