DENOSUMAB-A NEW THERAPEUTIC OPTION FOR OSTEOPOROSIS

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INTRODUCTION

Osteoporosis is a multifactorial progressive skeletal disorder characterized by reduced bone mass and deterioration of bone microarchitecture, predisposing it to increased fracture risk.[1] The capacity of bone to resist mechanical forces and fractures depends not only on the quantity of bone tissue but also on its quality.[2] Osteoporosis is called a "silent disease" because it progresses without symptoms until a fracture occurs. Because of larger skeletons and no period of rapid hormonal change osteoporosis progresses more slowly in men than in women.[3] The fractures caused by osteoporosis have a great impact on public health, as they are often associated with increased morbidity, mortality and high economic cost. Thus, in the last two decades pharmacological and non-pharmacological treatment (usually based on physical exercise) options have been largely developed to reduce the risk of fractures in osteoporotic patients.[1]

At present there are many therapies available for the treatment of osteoporosis, but the existing therapies have certain issues including efficacy and long term safety issues. Estrogen role for the maintenance of bone integrity was recognized early on, but estrogen therapy has several non-skeletal adverse consequences including vascular events and breast carcinoma.[4] Hormone replacement therapy was recommended to prevent osteoporosis. However, widespread use of parathyroid hormone (PTH) is limited because of its cost, the need for daily injections and prolonged use.[5] Selective estrogen receptor modulators (SERMs) have many potential uses and are currently approved for postmenopausal women with or at risk for osteoporosis. The safety profile of these agents is thus of great interest. Both tamoxifen and
raloxifene are associated with a two to three fold increase in venous thromboembolic events. Lesser adverse events that are causally related to these drugs include leg cramps and an increase in reports of hot flushes. Problems with side effects and in demonstrating efficacy to reduce fractures have prevented other SERMs, such as arzoxifene, from continuing toward approval. From longtime, osteoporosis therapeutics have been dominated by anti-resorptive agents like bisphosphonates but their anti-fracture efficacy is found to be very less than desired. According to the evidences available it is found that they can reduce fracture risk by only 50%. Also, all of these anti-resorptive agents have to be given on a long term basis. Hence there are issues of safety and compliance. Osteonecrosis of the jaw (ONJ) is an uncommon adverse reaction seen with high dose intravenous bisphosphonate therapy. Consequently, there is a definite need to improve upon the existing therapies and to develop newer agents which will be useful in the prevention as well as treatment of osteoporosis in the future.

**Osteoporosis in India**

Osteoporosis is becoming a serious problem for the public economy and health, because of the increase in elderly population in the near future. Conservative estimates in a study suggest that 20% of women and about 10-15% of men are osteoporotic in India. In developing countries like India, hip fractures are a major health problem. Hip fractures cause mainly physical impairment and mortality in the elderly patients. A survey carried out by the Indian Society for Bone and Mineral Research (ISBMR) among orthopedic surgeons across the country, revealed that in Government hospitals, about 80%-85% hip fractures are surgically treated whereas in private hospitals, almost 100% receive surgical treatment.

**Nutritional factors**

Despite abundant sunshine, vitamin D deficiency is widespread in India. A recent International Osteoporosis Foundation (IOF) report on the global status of Vitamin D nutrition highlights South Asia, especially India, as one of the most deficient regions. This is due to factors such as skin pigmentation, clothing habits and absence of vitamin D fortification. Also in India calcium intakes are also far below western recommendations. Thus, low vitamin D level and low calcium intake seem to be major contributing factors to poor bone health and osteoporosis in India. Confusion about the amount of vitamin D necessary to avoid deficiency has arisen in recent years, as tests that measure levels in patient's blood have become widely used. Poor sunlight exposure, skin pigmentation and
vitamin D-deficient diet are some obvious causes for this finding. Atmospheric pollution has also been suggested as a contributor to vitamin D deficiency in both urban and semi-urban Indians, postmenopausal women, pregnant women, specifically school children and newborns.

**Pathophysiology of bone loss**

Bone resorption takes place through the action of osteoclast cells, which resorbs the bone matrix by secreting hydrochloric acid, which dissolves calcium phosphate and enzymes such as collagenase and other proteases.

After the action of osteoclast cells at the bone resorption site, osteoblast cells synthesize new bone.\(^\text{[15,16]}\) The organic component of the bone matrix, which is mainly composed of Type-I collagen fibers, is produced by Osteoblast cells. Osteonectin, sialoproteins and osteocalcin are the three important proteins secreted by osteoblast cells that are incorporated into the bone matrix. Two stages of mineralization mediated by osteoblasts then follow, firstly between the collagen fibrils, hydroxyapatite crystals get deposited. In this process of mineralization, alkaline phosphatase located on the membrane of osteoblast plays very important role. In the second stage deposition of additional minerals occurs on the bone resorption site.\(^\text{[17,18,19]}\)

Estrogen is another systemic hormone with direct effects on bone and playing an important role in osteoporosis. In postmenopausal women, deficiency of estrogen leads to an up regulation of RANK L (Receptor, Activator of Nuclear Factor – κB Ligand) on bone marrow cells, which is an important determinant of increased bone resorption,\(^\text{[20]}\) whereas estrogen itself stimulates OPG production in osteoblasts and thus exerts anti-resorptive effects on bone.\(^\text{[21]}\)

Extra skeletal effects of estrogen deficiency are mainly based upon increased renal calcium excretion and decreased intestinal calcium absorption. Estrogen deficiency also cause a continuous increase in serum parathyroid hormone (PTH) levels.\(^\text{[20]}\)

The production of many different cytokines and other inflammatory mediators, such as interleukin (IL)-1, IL-6, TNF-α and prostaglandin E\(_2\), are involved in the pathogenesis of osteoporosis. Nevertheless, it is also known that estrogen treatment increases the production of insulin-like growth factor-1 (IGF-1) and transforming growth factor (TGF)-β by
More recent studies deal with the effects of estrogen deficiency on T cell function. It was demonstrated that estrogen withdrawal results in increased production of IL-7, leading to T cell activation accompanied by an increased production of interferon (IFN)-γ and TNF-α by T cells. One major action of IFN-γ is the up regulation of major histocompatibility complex (MHC) class II molecules on antigen presenting cells. This leads to a further activation of T cells, which now produce more RANKL and TNF-α. As already mentioned, these two cytokines have a pronounced osteoclastogenic activity. The effects of IFN-γ on bone metabolism are scientifically challenging, as IFN-γ acts as pro-osteoclastogenic cytokine in the context of ovariectomy, whereas it is regarded as antiosteoclastogenic in general (Figure 1).

Recent advances have identified the Receptor Activator for Nuclear Factor _B Ligand (RANKL) as a critical mediator of bone remodeling. RANKL is essential for the formation, function and survival of the osteoclasts. It binds to its cognate receptor RANK on the surface of precursors and mature osteoclasts and stimulates these cells to mature and resorb bone. The physiological inhibitor of RANKL is osteoprotegerin (OPG), a soluble receptor that competes with RANK for binding to RANKL, thus neutralizing RANKL effects. Multiple preclinical models were used to study the effects of inhibiting RANKL showing that it leads to improved bone geometry and increased bone density and strength. Denosumab is a novel antiresorptive agent that also inhibits osteoclast-mediated bone resorption but works through a different pathway than bisphosphonates. Denosumab is a fully human monoclonal antibody (IgG2) that inhibits RANKL with high specificity, mimicking the effects of OPG on RANK L. In recent years, a number of clinical trials evaluating the efficacy and safety of denosumab for the treatment of osteoporosis in humans have been published.

**Identification of the osteoclast differentiation factor, RANKL**

Bone-resorbing osteoclasts originate from hematopoietic cells, which are hypothesized to be members of the colony forming unit-megakaryocyte-derived monocyte–macrophage family. Takahashi et al and Udagawa et al developed a mouse coculture system of hematopoietic cells and primary osteoblasts to investigate osteoclast formation in vitro. In this coculture system, several systemic and local factors were found to induce the formation of tartrate resistant acid phosphatase-positive multinucleated cells and these cells exhibited a number of osteoclast characteristics. In addition,
Figure 1: The mechanism of action of denosumab on bone metabolism.

Notes
Denosumab (a fully human monoclonal anti-RANKL antibody) binds to osteoblast-produced RANKL, thereby preventing RANKL from binding to the osteoclast receptor, RANK. By preventing RANKL from binding to RANK, there is less osteoclastogenesis and bone-resorbing activity so that bone resorption is markedly suppressed.

Abbreviations
- c-Fms, colony stimulating factor-1 receptor; M-CSF, macrophage colony-stimulating factor; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand.

Cell-to-cell contact between osteoblastic cells and osteoclast progenitors was shown to be essential for the induction of osteoclastogenesis. Based on these findings, Suda et al proposed that osteoblastic cells induce the membrane-associated osteoclast differentiation factor in response to various osteotrophic factors. In 1997, it was first reported that RANKL and its receptor, receptor activator of nuclear factor-κB (RANK), regulate interactions between
dendritic cells and T-cells.\(^{[27]}\) Furthermore, when osteoclast differentiation factor was cloned from a complimentary DNA library of mouse stromal ST2 cells treated with bone-resorbing factors, it was found it was found to be identical to RANKL, to tumor necrosis factor (TNF)-related activation-induced cytokine, and to the osteoprotegerin (OPG) ligand. These results were independently validated by other research groups. In these studies, RANKL was also shown to induce osteoclast differentiation from mouse hematopoietic cells and human peripheral blood mononuclear cells in the presence of macrophage colony-stimulating factor.\(^{[28]}\) To date, RANK is the only signaling receptor that has been identified for RANKL for the induction of osteoclastogenesis and the activation of mature osteoclasts. OPG, which lacks transmembrane and cytoplasmic domains and is released in a soluble form by a variety of cells including osteoblasts, also serves as a decoy receptor for RANKL. As such, OPG competes with RANK to inhibit the differentiation and activity of osteoclasts.

**A crucial role for RANKL in bone metabolism**

RANKL is a membrane-anchored molecule that is released from the cell surface following proteolytic cleavage by matrix metalloproteinases such as matrix metalloproteinases.\(^{[29,30]}\)

Both the soluble and membrane-bound forms of RANKL function as agonistic ligands for RANK, with the membrane-bound form functioning more efficiently.\(^{[31]}\) Using a knockout mouse model of OPG, a natural inhibitor of RANKL, a link between RANKL and the development of osteoporosis was demonstrated. Furthermore, overexpression of OPG in mice results in lower numbers of osteoclasts and greater bone mass. Correspondingly, for patients experiencing estrogen deficiency, hyperparathyroidism, or other disorders that stimulate bone resorption, perturbations in the ratio of OPG to RANKL have been detected.\(^{[32]}\) More recently, Nakashima et al reported that purified osteocytes express higher levels of RANKL and undergo enhanced osteoclastogenesis in vitro, while osteocyte-specific RANKL knockout mice exhibit a severe osteopetrotic phenotype. Taken together, these results indicate that osteocytes represent a major source of RANKL for bone remodeling in vivo. Mutations in *RANKL*, *RANK* and *OPG* genes have also been identified in patients with bone disorders such as autosomal recessive osteopetrosis, familial expansile osteolysis and juvenile Paget’s disease, Respectively Moreover, when RANKL expression is upregulated in response to factors such as vitamin D3, prostaglandin E\(_2\), parathyroid hormone, interleukin (IL)-1, IL-6, IL-11, IL-17 and TNF-\(\alpha\), pathological osteoclastogenesis has been observed.\(^{[33]}\) Therefore,
regulation of the RANKL/RANK/OPG axis represents a potential therapeutic target for the treatment of osteoporosis, rheumatoid arthritis, and cancer bone metastasis.

**Immunological function of RANKL**

Prior to their identification in bone cells, RANKL and RANK were found to effect T-cell activation and dendritic cell survival. Moreover, during early development, RANKL signaling regulates the microenvironment of the thymus, thereby facilitating the deletion of self-reactive T-cells to provide self-tolerance and prevent autoimmunity. These results imply that inhibition of RANKL by denosumab may alter immune function, or increase susceptibility to infections. In studies of mice deficient in RANKL or RANK, an absence of lymph nodes and significantly smaller Payer’s patches were observed. These findings demonstrate the critical role that RANK activation has in the early stages of lymphoid tissue inducer cell development in peripheral lymphoid organs. A pathological model of inflammatory bowel disease has also demonstrated a role for RANKL in the stimulation of dendritic cells, suggesting that RANKL may mediate the activation of dendritic cells under certain autoimmune conditions. On the other hand, inhibition of RANKL by OPG has not been found to alter cellular or humoral immunity, nor does it render mice susceptible to bacterial challenge. Thus, although dendritic cells and T lymphocytes express RANK and RANKL, it would appear that they play a minor or redundant role in the mammalian immune response. However, these results do not guarantee the safety of denosumab treatments.

**In vitro studies of denosumab**

Direct binding assays have demonstrated that denosumab is able to bind human RANKL, yet does not bind murine RANKL, human TNF-related apoptosis-inducing ligand and other human TNF family members. Denosumab also does not suppress bone resorption in normal mice or rats, although it prevented a resorptive response in mice challenged with human RANKL (huRANKL). huRANKL knock-in mice have been generated and these mice exclusively express chimeric (human/murine) RANKL and are responsive to denosumab. In studies of young huRANKL mice treated with denosumab, trabecular osteoclast surfaces were reduced by 95% and bone density and volume increased. In contrast, adult huRANKL mice treated with denosumab exhibited reduced bone resorption, increased cortical and cancellous bone mass and improved trabecular microarchitecture. The same group also reported that subcutaneous administration of denosumab (25 or 50 mg/kg/month) for up to 16
months prevented the loss of cancellous bone and preserved indices of bone strength for adult ovariectomized cynomolgus monkeys.\textsuperscript{[37]}

**Clinical development of denosumab as a prophylactic and/or therapeutic agent for osteoporosis**

To evaluate whether inhibition of RANKL has clinical utility, 52 healthy postmenopausal women were given single doses of an osteoprotegerin-immunoglobulin Fc segment complex (Fc:OPG) (0.1, 0.3, 1.0, or 3.0 mg/kg) in a Phase I randomized placebo-controlled study.\textsuperscript{49} Urinary levels of the cross-linked N-telopeptide of type I collagen (NTX), a specific marker of bone resorption and bone-specific alkaline phosphatase (BSAP), an index of bone formation, were subsequently monitored for 84 days. Within 12 hours of receiving Fc: OPG, a dose-dependent decrease in NTX/creatinine ratios was observed. Furthermore, this ratio decreased by 70\% to 80\% within 5 days for the highest doses of Fc: OPG. After several weeks, levels of NTX/creatinine returned to baseline. A significant decrease in levels of BSAP were also observed for the 1.0 mg/kg and 3.0 mg/kg doses. For the latter group, inhibition of BSAP occurred more slowly, with levels 30\% below baseline observed after 60 days. There were also no serious adverse events reported in this study. In one patient, a transient neutralizing antibody to OPG was detected, although this did not have any obvious clinical effect. These data provide evidence that inhibition of RANKL by its natural inhibitor, OPG, can result in clinically measurable effects. However, the development of OPG as a therapy for osteoporosis was not further pursued due to its potential immunogenicity, and because immunologic resistance to OPG could have negative effects on the skeleton.\textsuperscript{[38]}

Since denosumab specifically binds RANKL, it is less likely to affect the immune system or other regulatory systems. Moreover, denosumab does not have the potential for autoimmunization against a vital regulatory protein and is characterized by a longer half-life, which permits less frequent dosing.\textsuperscript{[39]} Each of these attributes makes denosumab a more attractive therapeutic agent than forms of OPG. To evaluate the safety, pharmacokinetics (PK) and possible bone resorption effects of denosumab, a Phase I study was conducted. Subcutaneous administration of various concentrations of denosumab (0.01 mg/kg to 3.0 mg/kg) were administered to 49 healthy postmenopausal women. The PK of denosumab were found to be nonlinear with dose. A prolonged absorption phase also occurred, with maximum serum concentrations reached between 5 days and 21 days after the women received the initial dose. Conversely, the disappearance of denosumab from the serum occurred in two
phases: a slow phase and a fast phase. The initial slow phase was associated with half-lives of approximately 20 days for the lower doses of denosumab, and approximately 32 days for the higher doses. When circulating levels of denosumab were ∼1,000 ng/mL, clearance occurred more rapidly. Urinary NTX levels were also found to decrease within 12 hours of dosing. Overall, the magnitude of the initial response was similar among the doses, although the duration of the effect was dose-dependent. These results are consistent with the pharmacokinetic data. By the end of the 9-month follow-up period, NTX levels had returned to baseline for all of the doses. Alternatively, serum levels of BSAP remained stable for the first two weeks following dosing, then decreased in a dose-dependent manner. Taken together, these results suggest that the effect of denosumab on bone formation is indirect.\[^{39}\]

**Optimizing the dose of denosumab for osteoporosis**

To evaluate the safety, tolerability, PK and pharmacodynamics (PD) of denosumab, a randomized double-blind dose-escalation study was conducted. For a group of healthy postmenopausal Japanese women, denosumab was administered subcutaneously at doses of 0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg and was compared with a placebo.\[^{40}\] Suppression of bone turnover markers (BTM) was rapidly detected (within 2 days of dosing) and the duration of suppression was dose-dependent. Moreover, there was no marked differences in the PK and PD profiles between Japanese and non-Japanese and denosumab was well tolerated. In another study, the efficacy and safety of three doses of denosumab (14, 60 and 100 mg) were compared with a placebo over 12 months for a group of postmenopausal Japanese women with osteoporosis. The results associated with the 60 mg dose of denosumab were consistent with the results of a similar Phase II study of osteoporosis in a Caucasian population that was conducted in the United States.\[^{41}\]

**Safety**

Although denosumab has been shown to be safe in the collective data from Phase II and III clinical trials, the clinical concern was the potential risk for infections or neoplasms due to the ubiquitous presence of RANKL throughout many tissues. In a meta-analysis of randomized placebo-controlled trials involving denosumab, including the large FREEDOM registration trial,\[^{42}\] a borderline increased risk of serious infection was observed (risk ratio =1.25, 95% confidence interval: 1.00–1.54) for women with postmenopausal osteoporosis when intention-to-treat analysis was used. However, a nonsignificant risk ratio of 2.1 was
observed when a per-protocol analysis was employed. Thus, the incidence of infection and neoplasms in ongoing larger Phase III trials will be of interest.

While accumulating evidence indicates that denosumab is a safe treatment, there remains the potential for side effects from this treatment. For bisphosphonate therapy, ONJ (Osteo Necrosis of Jaw) has recently emerged as an adverse side effect,[43] although the nature and cause of ONJ remains controversial. Given the capacity for denosumab to strongly inhibit osteoclastic bone resorption similar to bisphosphonates, it will be important for future studies of denosumab to monitor the incidence and clinicopathologic characteristics of ONJ. Another potential side effect to consider is the so-called frozen bone process, whereby complete inhibition of remodeling leads to an accumulation of microfractures and an increased risk for atypical femoral fractures. This complication was considered in an analysis of postmenopausal women receiving bisphosphonate therapy based on the findings of animal studies. In trials that have continuously administered denosumab for up to 5 years, there have been no reports of atypical femoral fractures. However, two cases of atypical femoral fracture have been confirmed in patients receiving denosumab 60 mg for 2.5 years or more participating in the ongoing open-label extension study of the pivotal Phase III fracture trial in postmenopausal osteoporosis (FREEDOM). It is possible that differences in shorter half-life of denosumab compared with bisphosphonates (5 years or longer) can account for less incidence of atypical femoral fractures. Tsai et al also recently reported that a combination treatment of teriparatide and denosumab increased BMD to a greater extent than either agent alone.[44]

Since RANK and RANKL are also expressed by endothelial cells and lymphocytes, additional studies are needed to evaluate the potential effects of denosumab therapy on the cardiovascular and immune systems of the body. Continued documentation and quantification of the efficacy of denosumab for large numbers of patients will also be important. Recently, RANKL signaling was implicated in the pathogenesis of hepatic insulin resistance and type 2 diabetes mellitus. This may provide a link between inflammation and disrupted glucose homeostasis, and may also contribute to pharmacological strategies being developed for the treatment of RANKL-related diseases.[45]

Finally, Freemantle et al reported that postmenopausal women with osteoporosis were more adherent, compliant and persistent with subcutaneous injections of denosumab every 6 months than with once-weekly alendronate tablets in a 2-year randomized crossover study. In
addition, the women expressed greater satisfaction with injectable denosumab and preferred it over oral alendronate. Thus, preferences in the administration of denosumab may influence patient persistence and adherence to therapy, and this represents an important consideration for the treatment of chronic conditions that require long-term therapy.\[46\]

The most common adverse effects identified in initial studies of postmenopausal women include arthralgia (25%), nasopharyngitis, back pain, headache, extremity pain, upper respiratory infection, constipation, urinary tract infection, and shoulder pain. Sore throat, rash, and asymptomatic hypocalcemia have also been reported. Malignancy has also been a concern with denosumab; however, current studies have not demonstrated a statistically significant increase in these events. There are no reported drug interactions at this time. Denosumab is contraindicated in patients with severe hypocalcemia. Caution should be used in patients with impaired renal function as they are at an increased risk of hypocalcemia. There is an increased risk of serious infections, including skin infections, with denosumab.\[47\] Patients with impaired immune systems or those on concomitant immunosuppressant agents may be at an increased risk and the benefits and risks of starting denosumab should be evaluated. Dermatologic reactions, such as rash, eczema, and dermatitis have been reported with denosumab. Osteonecrosis of the jaw has been observed in patients receiving denosumab, and all patients should receive an oral exam prior to therapy initiation and maintain good oral hygiene during therapy. Bone turnover is significantly suppressed with denosumab and all patients should be monitored for the consequences of bone suppression, such as ONJ, atypical fractures, and delayed fracture healing.

FOR THE TREATMENT OF OSTEOPOROSIS, DENOSUMAB 60 MG EVERY 6 MONTHS IS ADMINISTERED AS A SUBCUTANEOUS INJECTION IN THE UPPER ARM, UPPER THIGH, OR ABDOMEN. ALL PATIENTS SHOULD TAKE 1000 MG OF CALCIUM AND AT LEAST 400 IU OF VITAMIN D DAILY IN CONJUNCTION WITH DENOSUMAB. IF A DOSE OF DENOSUMAB IS MISSED, ADMINISTER THE INJECTION AS SOON AS CONVENIENT AND THEN SCHEDULE INJECTIONS EVERY 6 MONTHS FROM THE DATE OF THE LAST INJECTION. PRIOR TO ADMINISTRATION, DENOSUMAB SHOULD BE REMOVED FROM THE REFRIGERATOR AND BROUGHT TO ROOM TEMPERATURE. THE GREY NEEDLE CAP ON THE SINGLE-USE PREFILLED SYRINGE CONTAINS DRY NATURAL RUBBER (A DERIVATIVE OF LATEX).\[48\]

CONCLUSION

Osteoporosis is a widespread condition with significant morbidity and mortality affecting millions of people worldwide. Incidence is expected to increase as the population ages. Guidelines are available from many organizations throughout the world. Pharmacists,
Pharmacologists and clinicians can contribute to the overall care of patients with osteoporosis by familiarizing themselves with these guidelines and the available treatment options. All therapies require an analysis of their risk-benefit profile.

REFERENCES


