ABSTRACT

Postmenopausal osteoporosis is a major concern to public health. Fractures are the major clinical consequence of osteoporosis and are associated with substantial morbidity, mortality and health care costs. Bone strength determinants such as bone mineral density and bone quality parameters are determined by life-long remodeling of skeletal tissue. Receptor activator of nuclear factor-kB ligand (RANKL) is a cytokine essential for osteoclast differentiation, activation and survival. Denosumab (Prolia®) is a fully human monoclonal antibody for RANKL, which selectively inhibits osteoclastogenesis, being recently approved for the treatment of postmenopausal osteoporosis in women at a high or increased risk of fracture by the FDA in the United States and by the European Medicines Agency in Europe since June 2010. FREEDOM, DECIDE and STAND are the phase 3 trials comparing denosumab with placebo and alendronate in postmenopausal osteoporosis. The authors aim to update denosumab role in postmenopausal osteoporosis with a physiopathological review.

Keywords: RANK; RANKL; Osteoprotegerin; Denosumab; Postmenopausal osteoporosis.

INTRODUCTION

Osteoporosis (OP) is a skeletal disease associated with an imbalance in bone resorption and formation, which turns to a loss of bone mass and deterioration of bone microarchitecture. This results in low bone mineral density (BMD) and poor bone quality, reduced bone strength and increased risk of fractures. Is a worldwide public health problem with serious consequences in personal suffering and economical costs. Clinical tools to diagnose OP and predict fracture risk are available but patients who are at risk for fracture or with a previous fracture are very often not identified or treated. The bone remodeling unit (BRU) includes a sequence of events, during which osteoclasts (OC) resorb bone over a period of 3 weeks, creating cavities that are termed as remodeling space. The resorption is followed by osteoblast (OB) activation and osteoid formation, filling the cavities over a period of 3 months. When the matrix synthesis is finished OB become embedded in the matrix as osteocytes (that will function as mechanoreceptors). Bone remodeling permits the repair of microdamage, maintains normal skeletal mass and participates in regulation of systemic calcium homeostasis. As bone resorption/formation is tightly coupled, inhibition of resorption eventually results in inhibition of formation. OP therapy may be classified as antiresorptive (estrogens, bisphosphonates, calcitonin and raloxifene) or anabolic [teriparatide (recombinant human parathyroid hormone PTH1-34) or PTH1-84]). Strontium ranelate appears to have both functions. Research is focusing on drugs that target the remodeling cycle acting in OC, OB and osteocytes or molecules that control the signaling pathway for cell functioning and gene transcription. Examples of studies on way include Glucagon-like peptide 2, cathepsin K inhibitors, PTH1-28, calcium-sensing receptors, Wingless (Wnt)-/catenin pathway, sclerostin and Dickkopf-1, activin (fusion protein ACE-011). However, the discovery of the receptor activator of nuclear factor KB ligand (RANK ligand, RANKL) osteoprotegerin (OPG) and RANK as a RANKL/OPG/RANK signaling pathway for the bone balance brought advances to the understanding of healthy bone turnover, and to osteolytic and destructive bone diseases like OP, rheumatoid arthritis (RA), Paget’s disease of bone and metastatic bone diseases. RANKL was identi-
fied as a potential target for therapeutic intervention in the treatment of these diseases. Possible strategies to down-regulate RANKL include inhibition of RANKL production, stimulation of endogenous OPG, and administration of exogenous OPG, soluble RANK or antibody to RANKL (Figure 2). Denosumab (Prolia®, AMG 162; Amgen Inc., Thousand Oaks, California, USA) is a fully human monoclonal antibody (IgG2 immunoglobulin isotype) with a high affinity and specificity for RANKL, currently approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) for clinical use in postmenopausal women OP with high risk of fracture and for the treatment of bone loss associated with hormone ablation in men with prostate cancer.

This manuscript reviews Denosumab pharmacological and clinical data in postmenopausal osteoporosis, with a previous physiopathology overview.

**ADULT SKELETON**

**PHYSIOLOGY**
The adult human skeleton is a metabolically organ with a coupled bone turnover that maintains the equilibrium in the trabecular and cortical bone. The total amount of bone loss is proportional to the number of BRUs activated at the bone surface at a given time. In the healthy adult, more than 1 million BRUs are active and up to 5-10% of existing bone is replenished annually. Full skeletal regeneration is accomplished once every decade. High bone turnover diminishes bone strength independently of BMD, because excessive number of resorption cavities act as areas of stress that may be a source of microcracks. This high turnover can eventually result in osteopenia or osteoporosis.

**RANKL/OPG/RANK SIGNALING PATHWAY**
OB are mononuclear cells responsible for the deposition of bone matrix and for OC regulation. They originate from mesenchymal stem cells (MSC) by the ac-
tion of transcription factors like core binding factor α1 (Cbfa-1) also known as Runx2, osterix (Osx), activating transcription factor 4 (ATF4), and bone morphogenic proteins (BMP) as BMP4. OC are derived from mononuclear precursors in the myeloid lineage of hematopoietic cells that also originate macrophages. Macrophage-colony stimulating factor (M-CSF) expression by osteoblastic stromal cells is required for progenitor cells to differentiate into OC, but is unable to complete this process by its own. The principal final mediator of osteoclastic bone resorption is RANKL, highly expressed by OB and T cells (mainly T helper cells). RANK is located on the cell membrane of OC and pre-OC and is a receptor for RANKL. RANKL/RANK binding stimulates OC differentiation and survival, resulting in increased bone resorption. OPG is a “decoy receptor” for RANKL, preventing its interaction with RANK, OC differentiation, activation of mature OC and permits OC apoptosis. RANKL/OPG ratio represents an important determinant of bone resorption. This ratio is decreased by estrogens, interleukin-4 (IL-4), IL-13, interferon-γ (IFN-γ), transforming growth factor-β (TGF-β), and increased by glucocorticoids, PTH, PTH-related protein, prostaglandins, IL-1, IL-17, tumor necrosing factor-α (TNF-α), 1,25-dihydroxyvitamin D (1,25vitD) and BMP. The precise role of the RANKL/OPG/RANK signaling pathway in regulating bone remodeling and mass was validated by gene studies in mice, with genetic RANKL or its receptor deficiency and OPG overexpression resulting in osteopetrosis, and genetic OPG ablation resulting in osteoporosis. The Wnt signaling in OB is also a source of OPG regulation. This pathway integrated with RANKL/OPG/RANK enabled to understand the normal and diseased bone. Many familiar bone diseases are mediated by RANKL/OPG/RANK pathway (Table I). Evidence is accumulating that bone remodeling is modulated through the interaction of OC and OB with immune cells, cytokines and circulating hormones. It was demonstrated that activated T cells directly stimulate osteoclastogenesis through RANKL, and RANKL prolongs the survival of dendritic cells and thereby increases T cells activity. Infection and malignancy concerns have been raised by inhibiting RANKL, however it seems that RANKL/OPG/RANK system does not have an essential role in immune function of adults, who already have a fully developed immune system.

**Postmenopausal Osteoporosis**

Estrogen is a positive regulator of OPG expression and its decrease in postmenopausal women are associated with increased RANKL expression, which shifts the bone remodeling balance toward the bone resorption. The incidence of postmenopausal OP is growing due to changing demographics and increasing life expectancy, which will also increase its economic and social burden fracture-related. OP fractures occur more frequently in women, its frequency increases with age, and spine, hip and wrist are the skeletal sites typically associated to OP. Vertebral fractures may result from relatively mild trauma in osteoporosis and

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**TABLE I**

<table>
<thead>
<tr>
<th>RANKL/OPG/RANK-mediated diseases</th>
<th>Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal osteoporosis</td>
<td>↑ bone marrow stromal cell expression of RANKL</td>
</tr>
<tr>
<td>Primary hyperparathyroidism</td>
<td>↑ RANKL, ↑ OPG expression by osteoblasts</td>
</tr>
<tr>
<td>Bone Paget's disease</td>
<td>↑ stromal cell expression of RANKL</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>↑ RANKL expression by synoviocytes and T cells</td>
</tr>
<tr>
<td>Periodontal bone loss</td>
<td>↑ RANKL expression by activated T cells</td>
</tr>
<tr>
<td>Myeloma bone disease</td>
<td>↑ RANKL expression by myeloma cells</td>
</tr>
<tr>
<td>Osteolytic bone metastases</td>
<td>↑ RANKL expression by tumour cells</td>
</tr>
<tr>
<td>Humoral hypercalcaemia of malignancy</td>
<td>Parathyroid hormone related protein (PTHrP) mediated: ↑ RANKL, ↓ OPG by osteoblasts</td>
</tr>
<tr>
<td>Familiar expansile osteolysis</td>
<td>Activating mutations of the RANK gene</td>
</tr>
<tr>
<td>Idiopathic hyperphosphatasia (Juvenile Paget's disease)</td>
<td>Inactivating mutations of the OPG gene</td>
</tr>
</tbody>
</table>

Legend: OPG – osteoprotegerin; RANK – receptor activator of nuclear factor kappa B; RANKL – receptor activator of nuclear factor kappa B ligand.
are associated to an increased risk for subsequent fracture. Hip fractures are associated with worst outcomes and secondary complications, and their risk increases in women from the age of 70 years. Healthcare costs due to OP are difficult to calculate as they include the costs of acute hospital care, loss of working days for family carers, longterm care and medication. The International Osteoporosis Foundation (IOF) estimated that the number of osteoporotic fractures in 2000 was 3.79 million and the total direct costs resulting from these fractures was estimated at €31.7 billion, which is expected to increase to €76.7 billion by 2050. Prevention of osteoporosis-related fractures appears to be essential to the quality of life and independence of postmenopausal women and prevent the economic burden.

**OSTEOPOROSIS TREATMENT: DENOSUMAB AS A NEW OPTION**

The ideal anti-osteoporotic agent would have to meet the following favorable biological properties: a rapid onset action, long duration effect, patient’s compliance and persistence, documented cost-effectiveness and excellent safe profile. Denosumab is a fully human monoclonal immunoglobulin G2 antibody that binds with high affinity and targets the activity of human RANK ligand, preventing it to interact with RANK on the OC surface, which turns into the disruption of cellular signaling of bone resorption mediated by RANKL and OPG expression in human-derived OC cell lines. Denosumab inhibits numerous aspects of OC differentiation and function (fusion, differentiation, attachment to bone, activation and survival), by inhibiting the intracellular signal pathways that are activated by the RANKL/RANK binding. Denosumab does not bind to murine RANKL, and does not cross-react with other human proteins of similar structure to RANKL, such as TNF-α, TNF-, TNF-related apoptosis-inducing ligand (TRAIL) or CD40 ligand.

**PHARMACOKINETICS AND METABOLISM**

The pharmacokinetics of denosumab is nonlinear with dose. Although the absorption, bioavailability, distribution, and elimination are not well defined, studies with similar IgG antibodies showed that subcutaneous denosumab is absorbed by the lymphatic system with subsequent drainage into the vascular system. The bioavailability is probably 50-100% with a distribution that is about the same as the plasma volume, the clearance is probably by the reticuloendothelial system and no significant amount of denosumab seems to be filtered and excrated by the kidneys. Subcutaneous administration is characterized by 3 stages: a prolonged absorption phase with the maximum serum concentration (at 5-21 days post-dose) increasing disproportionately than the increase in dose; a long duration phase with half-life of a maximum of 32 days; a rapid terminal phase when serum concentration is lower than 1000ng/ml. The long duration of denosumab activity is probably due to a combination of a long half-life and a very potent antiresorptive effect at early stages of OC differentiation. A feature that distinguishes denosumab from bisphosphonates is the rapid reversibility of its antiresorptive effect once it has been eliminated and OC regeneration has occurred, since it is not incorporated in the bone matrix as the bisphosphonates. Its capacity to reduce bone resorption was measured by serum C-telopeptide (CTX-1), by 82% within 72 hours post-dose, with a sustained effect during the 6-month dosing interval.

**PRE-CLINICAL STUDIES**

The preclinical studies evaluating the RANKL/OPG/RANK role in bone showed: RANKL induces OC-like cells formation in cell cultures, recombinant OPG expression in human-derived OB cell lines, OPG knockout mice develop OP and fragility fractures, RANKL and RANK knockout mice have osteoporosis with the total absence of OC. Human monoclonal antibodies (mAbs) are the fastest-growing category of mAb therapeutics entering clinical studies. Murine antibodies are easier to produce, but are limited by safety issues and diminished efficacy owing to the immunogenicity of the mouse-derived protein sequences. One path started to develop mAbs containing a combination of rodent-derived and human-derived sequences, resulting in chimeric and humanized mAbs. During the 2000s, human mAbs stand for 45% of the mAb candidates in the clinic and 88 are now in clinical development. Denosumab is one of the 7 mAbs currently approved for marketing in the United States. Denosumab was generated by immunizing the XenoMouse with full-length human RANKL protein, producing a fully human IgG1 mAb known as AMG 161. Because human IgG1 can direct complement-dependent cytotoxicity or antibody-dependent cell cytotoxicity to target cells, appeared the
concern that AMG 161 could have a toxic profile caused by death of RANKL-producing cells. It was converted to noncytotoxic IgG2 mAb, AMG 162, with high affinity for human RANKL. The difficulty of AMG 162 to recognize rodent RANKL has complicated pre-clinical studies and the relevant data were obtained from cynomolgus monkeys. The majority of the preclinical studies in RANKL inhibition in mice and rats used other agents than denosumab. The most commonly used were fusion molecules of recombinant OPG or RANK and the Fc fragment of IgG, called OPG-Fc or RANK-Fc, respectively. These studies demonstrated that recombinant OPG prevents bone loss in ovariectomized (OVX) rats, recombinant OPG decreases OC differentiation in normal mice, resulting in non-lethal osteopetrosis, recombinant OPG prevents bone loss in mice with low BMD that over-express TNF-α, RANKL inhibition increases bone mineralization and improves mechanical strength in the femur of young male rats, in aged OVX rats a combination of rat OPG and PTH increased BMD more than either agent alone.

In the cynomolgus monkeys the preclinical studies showed: 5-year-old male monkeys treated with denosumab had an increased bone mass and improved bone strength in femur and lumbar vertebral bodies, skeletal benefits were demonstrated with the RANKL inhibition in RA models, ovariectomy models, multiple myeloma models and inflammatory bowel disease models. Knocking technology has been recently used to create a genetically engineered mouse expressing a chimeric form of RANKL (human/murine) that is bound and neutralized by denosumab, which has been studied for evaluating the skeletal effects of denosumab in different circumstances.

CLINICAL STUDIES ON POSTMENOPAUSAL OSTEOPOOROSIS

The first study of RANKL inhibition in humans was conducted with OPG-Fc, a phase 1 randomized, double-blind, placebo-controlled, sequential dose escalation study in healthy postmenopausal women. Each of the 52 subjects received a single subcutaneous dose of OPG-Fc 0.1, 0.3, 1.0, 3.0 mg/kg or placebo. It was shown that a single dose of OPG-Fc caused a rapid, reversible and dose-dependent suppression of bone resorption, with no adverse events (AEs) or neutralizing antibodies. This provided support for further clinical studies.

**Phase 1:** A phase 1 study was conducted in 49 healthy postmenopausal women who received a single subcutaneous dose of denosumab 0.01, 0.03, 0.1, 0.3 and 3.0 mg/kg or placebo. All the cohorts had a follow-up of 6 months, except the highest doses (0.1, 0.3 and 3.0 mg/kg) that were followed 9 months. Pharmacological effect was assessed by urinary N-telopeptide (NTX) and serum bone-specific alkaline phosphatase (BSAP) levels. Rapid and reversible NTX reduction was sustained for 6 months. Later BSAP reduction occurred with a lesser magnitude. Denosumab was well tolerated with no drug-related serious adverse events (SAEs) reported or study discontinuation due to AEs. Infectious events were similar in both groups (33% in placebo versus 38% in denosumab). Two hospitalizations: severe undetermined abdominal pain (in the 0.01mg/kg group) and a cholecystitis (in the 0.1 mg/kg group). Mild transient dose-dependent decrease in albumin-adjusted serum calcium and dose-dependent early increase in PTH levels (Table II).

**Phase 2:** A randomized phase 2 study was performed to evaluate the efficacy and safety of denosumab compared with alendronate or placebo, in postmenopausal women with low bone mass (lumbar vertebra T-score: -1.8 to -4 or total hip or femoral neck T-score: -1.8 to -3.5). The 412 patients enrolled in this study were randomized to receive subcutaneous denosumab 6, 14, or 30 mg every 3 months; 14, 60, 100, or 210 mg every 6 months; open-label alendronate 70 mg/week; or placebo for 24 months. Primary endpoint was the percent change in BMD at the lumbar spine at 12 months compared to baseline. At 12 months denosumab group achieved an increase of 3.0 to 6.7% in lumbar spine BMD compared to baseline, with a dose-dependent rapid and sustained decrease in the bone turnover markers (BTMs). At 24 months the patients continued the study with a 60mg subcutaneous denosumab dose each 6 months and were randomized to continue treatment for 24 months, discontinue, or switch to placebo for 12 months and resume denosumab treatment for 12 months. The 48 months denosumab group treatment showed an increase in BMD at the lumbar spine (9.4% to 11.8%) and total hip (4.0 to 6.1%) with the BTMs remaining suppressed, while the placebo group showed a loss of 2.4% and 3.5% at the spine and hip, respectively. Within the first 12 months of denosumab discontinuation BMD decreased 6.6% in the lumbar spine and 5.3% in total hip. Re-treatment with denosumab at month 36 increased...
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study phase</th>
<th>Number and mean age of patients/subjects</th>
<th>Study characteristics</th>
<th>Study duration</th>
<th>Primary endpoints</th>
<th>Key efficacy results</th>
<th>Key safety profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bekker et al</td>
<td>I</td>
<td>49 healthy PMW; 59.6 years</td>
<td>RT, DB, SD, DE</td>
<td>6 or 9 months</td>
<td>Bone antiresorptive depending on dosing</td>
<td>Dose-dependent, activity and safety rapid (within 12h), profound (up to 84%) and and sustained (up to 6 months) decrease in bone turnover</td>
<td>No related SAEs, no clinical significantly laboratory changes</td>
</tr>
<tr>
<td>McClung et al</td>
<td>II</td>
<td>412 PMW with low BMD; 63 years</td>
<td>RT, PC, dose-ranging, also included an open-label ALN group (70mg orally, once weekly)</td>
<td>12 months</td>
<td>Percent change from baseline in BMD at LS, changes in BTMs</td>
<td>Increased BMD (LS: 3.0 to 6.7% compared with a decrease of 0.8% in placebo) and decreased BTMs (within 72h) compared to placebo</td>
<td>No significant differences between the ALN, denosumab and placebo profiles</td>
</tr>
<tr>
<td>Lewiecki et al</td>
<td>II</td>
<td>412 PMW with low BMD; 63 years</td>
<td>Extension study of McClung et al; same design</td>
<td>24 months</td>
<td>Percent change from baseline in BMD at LS, changes in BTMs</td>
<td>Continuing increases in BMD (4.13% to 8.89% compared with – 1.18% with placebo) and maintained suppression of BTMs</td>
<td>6 cases of infections associated to hospitalization in the denosumab group; similar AEs</td>
</tr>
<tr>
<td>Miller and et al</td>
<td>II</td>
<td>412 PMW with low BMD; 63 years</td>
<td>Extension study of McClung et al; study design reviewed, modification of the denosumab regimens; in the ALN group no additional ALN for the 24 months period extension</td>
<td>48 months</td>
<td>Percent change from baseline in BMD at LS, changes in BTMs</td>
<td>Significant increases in BMD (LS: 9.4% to 11.8% and TH: 4.0% to 6.1%) and sustained suppression of BTMs; discontinuing therapy for 24 months decreased BMD to baseline levels; retreatment for 12 months increased LS BMD by 9% from original baseline values.</td>
<td>Similar AEs, more infections as SAEs in the denosumab group</td>
</tr>
<tr>
<td>Seeman et al</td>
<td>II</td>
<td>247 osteopenic, osteoporotic PMW; 60.6 years</td>
<td>DB; patients randomized to denosumab, ALN or placebo</td>
<td>12 months</td>
<td>Evaluation of the denosumab and ALN effects on cortical and trabecular microarchitecture with pQCT</td>
<td>Better effects with denosumab than ALN</td>
<td>Incidence of AEs similar in both groups</td>
</tr>
</tbody>
</table>

Legend: AEs – adverse events; ALN- alendronate; BMD – bone mineral density; BTMs – bone turnover markers; DB – double-blinded; DE – dose-escalation; LS – lumbar spine; PC- placebo-controlled; PMW – postmenopausal women; pQCT – peripheral quantitative computed tomography; RT – randomized trial; SAEs – serious adverse events; SD – single-dose
Denosumab: update

By month 48, BMD increased 9% at the lumbar spine and 3.9% at the total hip from baseline and the BTM values were similar to those of the continuous treatment group. In 3.2% of denosumab patients occurred infections, all were common community-acquired (no opportunistic infections), with required hospitalization solved with standard antibiotics. This original study was extended 4 additional years with all patients switched to denosumab 60 mg/6 months subcutaneous and a further analysis 2 years after (6 years of denosumab treatment) reported an BMD increase of 13.3% at the lumbar spine with sustained suppression of BTMs.\(^{37,60-62}\) (Table II).

**Phase 3:** Treatment of postmenopausal osteoporosis

“Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months” (FREEDOM)\(^{63}\), randomized 7668 postmenopausal women with osteoporosis into 2 groups: placebo or subcutaneous denosumab 60 mg/6 months. Primary endpoint was a reduction in the incidence of new radiographic vertebral fractures in a 3-years period. Secondary endpoints were reduction in hip and other nonvertebral fractures and changes in BMD and BTMs. Denosumab group presented a 68% reduction in new vertebral fracture risk compared to placebo (2.3% vs. 7.2%; \(p<0.0001\)), 40% reduction in the hip fracture (0.7% denosumab vs. 1.2% placebo; \(p=0.036\)), and 20% reduction in the nonvertebral fractures risk (6.5% denosumab vs. 8.0% placebo; \(p=0.011\)). Denosumab group showed a significantly increase in BMD at all skeleton, mainly in the lumbar spine.

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**Table III: Resume of the Phase III Trials of Denosumab in Postmenopausal Osteoporosis Treatment**

<table>
<thead>
<tr>
<th>Study</th>
<th>Number and mean age of patients</th>
<th>Baseline T-score</th>
<th>Treatment duration</th>
<th>Primary endpoints</th>
<th>Key efficacy results</th>
<th>Key safety profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREEDOM(^{63})</td>
<td>7868 osteoporotic PMW; 72.3 years</td>
<td>&lt; -2.5 at LS or TH and ≥ -4.0 at both sites</td>
<td>36 months</td>
<td>Changes in BMD at the TH, FN, L5 and DR from baseline</td>
<td>Greater increases in BMD at the lumbar spine and nonvertebral sites in the denosumab group</td>
<td>No fractures compared to placebo</td>
</tr>
<tr>
<td>DECIDE(^{65})</td>
<td>1189 PMW naïve with low BMD, 64.4 years</td>
<td>≤ -2.0 at LS or TH</td>
<td>12 months</td>
<td>Transition to denosumab increased BMD (1.9% denosumab vs. 1.05% ALN) and reduced BTMs to a greater extent than continued ALN</td>
<td>Similar overall rates of AEs and SAEs in both groups</td>
<td></td>
</tr>
<tr>
<td>STAND(^{66})</td>
<td>504 osteopenic POM osteoporotic PMW</td>
<td>≤ -4.0 at LS or TH</td>
<td>12 months</td>
<td>Changes in BMD at the lumbar spine, hip, and nonvertebral sites</td>
<td>Similar overall rates of AEs and SAEs in both groups</td>
<td></td>
</tr>
</tbody>
</table>

No differences of total incidence of AEs or SAEs were reported in both groups. The incidence of serious infections was similar in both groups. Infections resulting in death were 0.2% in both groups. Few denosumab patients were diagnosed endocarditis (n=3), pancreatitis (n=8), eczema was reported in 3% of denosumab group versus 1.7% in placebo group (p<0.001). Cellulitis as an SAE occurred in 0.3% (12/3886) in the denosumab group versus less than 0.1% (1/3876) in the placebo group (p=0.002). This study was extended for 2 years with all patients switched to open-label denosumab 60 mg/6 months (with a significantly CTX-1 reduction and BMD increase), and then it was extended 5 more years to complete a total of 10 years of denosumab exposition. Authors concluded that denosumab given subcutaneously twice yearly for 36 months reduced the risk of new vertebral, nonvertebral and hip fractures in women with OP.

**Head-to-head comparison with Alendronate**

“Determining Efficacy: Comparison of Initiating Denosumab Versus Alendronate” (DECIDE), a double-blind, double-dummy noninferiority 1-year study to compare the effects of denosumab and alendronate on BMD and BTM in naïve postmenopausal women (n=1189) with T-score of lumbar spine or total hip <-2. Patients were randomized to receive subcutaneous denosumab 60mg/6months plus oral placebo/week or oral alendronate 70mg/week plus subcutaneous placebo/6months. The primary endpoint was percent change from baseline in total hip BMD at month 12 and the secondary endpoints were percent change in femoral neck, trochanter, lumbar spine and one-third distal radius BMD, and changes in BTM. Denosumab group showed a greater BMD increase at the total hip versus placebo (3.5% vs. 2.6%; p<0.0001) and a greater reduction in BTMs compared to alendronate. The overall incidence of AEs, SAEs, infections or neoplasms was similar between both groups. Authors concluded that denosumab was associated with both significantly more reduction in bone resorption and greater gains in BMD at all measured skeletal sites compared to alendronate.

**Transitioning from Alendronate to Denosumab**

“Study of Transitioning from Alendronate to Denosumab” (STAND), a randomized, double-blind, double-dummy, parallel-group, 1-year study in postmenopausal women (n=504) previously treated with alendronate at least 6 months, and with a lumbar spine or total hip T-score of -2.0 to -4.0. The primary endpoint was percent change in BMD at the total hip at 12 months for denosumab compared to alendronate. The study design allowed testing the primary endpoint for superiority if noninferiority was demonstrated. At 12 months denosumab group presented a significantly greater increase in BMD at the total hip (denosumab 1.9% vs. alendronate 1.05%; p<0.0001), lumbar spine, and distal one-third radius, compared with continuing of alendronate. AEs and SAEs were similar in both groups. Authors concluded that postmenopausal women with low BMD can be safely transitioned from weekly oral alendronate to 6-monthly subcutaneous denosumab to achieve an incremental increase in bone mass.

**Further Clinical Studies**

Histology and Histomorphometry with Denosumab

Reid et al, collected iliac crest biopsies from FREEDOM and STAND populations, after 12, 24 and 36 months of denosumab exposition. In the FREEDOM study median eroded surface was reduced by greater than 80% and OC were absent from greater than 50% of biopsies in the denosumab group. It was shown a reduction in fractures in the same cohort of patients in whom there was such a clear histological evidence of reduced turnover. In STAND study, the histomorphometry indicates that denosumab 60mg/6months produces greater inhibition of turnover than occurs with alendronate 70mg/week, but without evidence of any untoward interaction after the transition from alendronate to denosumab.

**Effects of Denosumab on Bone Microarchitecture**

Seeman et al, conducted a double-blind pilot study to compare the effect of denosumab and alendronate on cortical and trabecular microarchitecture at the radius and tibia in postmenopausal women using a quantitative computed tomography (QCT) and a high-resolution peripheral QCT (HR-pQCT). The women were randomized to receive subcutaneous denosumab 60mg/6months (n=83), oral alendronate 70mg/week (n=82) or placebo (n=82). Trabecular BMD and cortical thickness at the distal radius at month 12 decreased in the placebo group while was preserved in alendronate group. Denosumab prevented the decline or improved these variables. Similar results were achieved in the tibia.
DENOSUMAB IN PATIENTS WITH RENAL IMPAIRMENT

Block et al.\(^9\), conducted a phase 1 study to evaluate the pharmacokinetics, pharmacodynamics and safety of a single dose of 60mg subcutaneous denosumab in 55 patients with different degrees of renal function. Results showed that the renal impairment did not affect the denosumab pharmacokinetics and pharmacodynamics, and no dose adjustment is necessary in impairment renal function. About 30% of patients with severe renal function and hemodialysis showed symptomatic hypocalcaemia.

BESIDE POSTMENOPAUSAL OSTEOPOROSIS TREATMENT

PREVENTION OF POSTMENOPAUSAL OSTEOPOROSIS

“Denosumab Fortifies Bone Density” (DEFEND)\(^10\), was a randomized, placebo-controlled, trial to evaluate efficacy and safety of denosumab in postmenopausal women with osteopenia.

Denosumab is currently being studied for other indications as RA bone erosions, bone loss associated with androgen deprivation therapy, bone loss associated with aromatase inhibitor therapy and for the treatment of bone metastases. However these studies are beyond the scope of this paper.

SAFETY AND TOLERANCE

In the DEFEND, DECIDE, and STAND studies, AEs and SAEs, including infections and malignancies, were similar in both groups\(^11\). In DECIDE the most common types of infections were nasopharyngitis, influenza and upper respiratory tract infections. In STAND were nasopharyngitis and bronchitis\(^1\). In the FREEDOM study the only significant AE were cellulitis and eczema\(^7,2\). The longest exposure to denosumab reported to date is 6 years. No significant differences in infections, neoplasms were reported as SAEs for denosumab\(^11\). There were no denosumab-related cases of osteonecrosis of jaw, fracture repair problems, changes in white blood cells counts, T, B, or natural killer cell counts, immunoglobulins or antibodies to denosumab\(^1,11\).

COST-EFFECTIVENESS PROFILE

Affordability of a drug therapy is a concern for healthcare system and patients\(^7\). Hiligsmann et al\(^7\), assessed the potential cost effectiveness of denosumab in the treatment of postmenopausal osteoporotic women in an updated version of a validated Markov microsimulation model populated with cost and epidemiological data from Belgium and a base-case population defined from FREEDOM. It was concluded that denosumab is cost effective compared with no treatment\(^7\) and oral bisphosphonates\(^7\) for postmenopausal Belgian women similar to FREEDOM population. Additional published studies with data from England, Wales and Sweden concluded that denosumab has a higher probability of being cost-effective in some patients subgroups\(^7,2\). A cost-utility analysis of denosumab versus standard care (alendronate and colecalciferol) in the treatment of post-menopausal osteoporosis in Portugal concluded that denosumab is a cost-effective therapeutic strategy with an Incremental Cost-effectiveness Ratio (ICER) OF 14.887 € per Quality Adjusted Life Year (QALY) gained. The analysis was undertaken from a NHS perspective, efficacy data for denosumab was taken from FREEDOM and the comparator was taken from a meta-analysis conducted by National Institute for Health and Clinical Excellence (NICE). Epidemiological Portuguese data were complemented with Swedish data whenever the former were unavailable. The model pre-

### TABLE IV. REPORTED DIFFERENCES BETWEEN DENOSUMAB AND THE BIPHOSPHONATES

<table>
<thead>
<tr>
<th>Denosumab compared to biphosphonates</th>
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</thead>
<tbody>
<tr>
<td>• Denosumab blocks osteoclasts formation, function and survival while biphosphonates cause loss of resorptive function but “disabled” osteoclasts may persist(^7)</td>
</tr>
<tr>
<td>• Exerts its effect from within the extracellular fluid(^67)</td>
</tr>
<tr>
<td>• Rapid offset of action at about 6 months(^3,7,7)</td>
</tr>
<tr>
<td>• Induces more rapid and greater reduction in bone remodeling(^63)</td>
</tr>
<tr>
<td>• Superior pharmacokinetic properties (better distribution, no excretion of kidneys)(^64)</td>
</tr>
<tr>
<td>• Greater increase in bone mineral density(^64)</td>
</tr>
<tr>
<td>• Positive effect on both cancellous and cortical bone(^3,72)</td>
</tr>
<tr>
<td>• Better strength indexes and geometrical bone variables(^67,68)</td>
</tr>
<tr>
<td>• Completely reversible effect and no accumulation in bone(^3)</td>
</tr>
<tr>
<td>• Possible non-blunting effect on subsequent anabolic therapy(^10)</td>
</tr>
<tr>
<td>• Better patient's convenience and compliance(^1)</td>
</tr>
<tr>
<td>• Denosumab was the first anti-bone resorbing agent that showed to halt bone erosions in Rheumatoid Arthritis(^11)</td>
</tr>
</tbody>
</table>
dicts that relative to the comparator, denosumab would prevent 12 hip, 22 vertebral, 2 wrist and 1 other osteoporotic fractures, per 1000 patients, over a 10 years period, and the probability of cost-effectiveness was between 91% and 64%.

DIFFERENCES BETWEEN DENOSUMAB AND THE BIPHOSPHONATES
The effect of RANKL inhibition is quite unique among antiresorptive agents. Table IV shows how denosumab differs from the effects of byphosphonates on bone in several aspects.

CONCLUSION
In postmenopausal women with osteoporosis denosumab reduced the risk of vertebral and nonvertebral fractures versus placebo. In those with low BMD or OP it increased BMD and decreased BTMs more than alendronate, both in treatment-naive and who switched from alendronate. Denosumab was safety and well tolerated, being cost-effective.

CORRESPONDENCE TO
Inês Maria Crispim Gomes da Silva
Alta do Lumiar, Rua Helena Vaz da Silva n°4 3ºD
1750-429 Lisboa
E-mail: inescrispin@hotmail.com

REFERENCES
23. Boyle BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Archives of biochemistry and biophysics 2008;473:139-146.
30. Stolina M, Dwyer D, Morony S. Rats and mice overexpressing soluble OPG have high bone mass but no alteration in immunological parameters or lymphocyte function. Arthritis Rheum 2005;52:5708.