# CLINICAL
# INVESTIGATION

# Denosumab for treatment of osteoporosis in postmenopausal women

Osteoporosis leads to fragility fractures that are associated with adverse health outcomes, including increased mortality, disability, psychological deterioration and impaired health-related quality of life. Denosumab is a human monoclonal antibody that specifically blocks bone resorption. Denosumab has recently been approved for treatment of postmenopausal osteoporosis. Its efficacy in reducing the risk of fracture has been shown in a large prospective, randomized multicenter study of 7868 postmenopausal women with osteoporosis – the FREEDOM trial. Denosumab 60 mg injected subcutaneously every 6 months for 3 years significantly increased the bone mineral density of the lumbar spine, hip and radius and reduced the relative risk of new vertebral fractures by 68%, hip fractures by 40% and of nonvertebral fractures by 20%, compared with a placebo-treated group. Bone biopsies showed normal trabecular and cortical microarchitecture, normal mineralization and no adverse effects on the formation of lamellar bone. Denosumab is cleared by the reticuloendothelial system and may have advantages for the treatment of osteoporosis in patients with renal impairment. This article summarizes the receptor activator of nuclear factor κB ligand–osteoprotegerin mechanism and a brief description of clinically relevant aspects of denosumab for treatment of osteoporosis in postmenopausal women. The results of the 3-year FREEDOM study are compared with the pivotal fracture data from other antiresorptive therapies for treatment of postmenopausal osteoporotic women (alendronic acid, risedronic acid, raloxifene, ibandronic acid and zoledronic acid). The properties of denosumab for treatment of osteoporosis in patients with renal impairment are discussed.

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Current bone specific drugs for treatment of osteoporosis such as bisphosphonates, strontium ranelate and selective estrogen receptor modulators such as raloxifene (RAL) are based on comparatively small molecules, or on polypeptides such as teriparatide (PTH 1–34) or full length parathyroid hormone (PTH 1–84). While the principle of treatment with a recombinant monoclonal antibody is well established in rheumatology and hematology, it has recently been introduced to the bone field through the discovery and characterization of one of the most important and specific regulators of bone turnover, the receptor activator of nuclear factor κB (RANK) ligand (RANKL)–osteoprotegerin (OPG) system. The successive development of the first fully human monoclonal antibody, denosumab, which blocks the RANKL–RANK pathway, has made it possible to treat osteoporosis and skeletal-related events in cancer by this novel principle.

# FUTURE SCIENCE

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Denosumab is a potent antiresorptive agent and has been shown to increase bone mineral density (BMD) and to reduce the risk of new vertebral fractures, hip and nonvertebral fractures in postmenopausal women with osteoporosis [1].

After a short introduction to the RANKL–OPG mechanism and a brief description of denosumab, the results and lessons from the large FREEDOM study of treatment of postmenopausal osteoporotic women with denosumab are presented and discussed. As denosumab acts by inhibiting bone resorption, its efficacy of reducing the risk of fracture it is compared with other antiresorptive therapies in clinical use. In addition, the treatment of osteoporosis in patients with renal impairment is discussed.

### The RANKL–OPG system

For decades the molecular signal behind the coupling between bone resorption and bone formation was unknown. However, one of the many valuable results of the human genome project was the identification and cloning of OPG and RANK and its ligand RANKL, which is a member of the TNFα cytokine super family [2,3]. The RANK receptor is expressed on the surface of osteoclasts and osteoclast precursor cells while RANKL is located on the surface of osteoblasts and stromal cells in the bone marrow and to a limited extent as soluble RANKL [4,5]. RANKL controls the osteoclastogenesis by stimulating the differentiation of osteoclasts precursor cells and the formation, function and survival of osteoclasts, while OPG acts as a decoy receptor for RANKL and inhibits each of these effects [4,6–8]. The balance between the local concentration of RANKL and OPG in bone tissue is the key mechanism through which systemic hormones, local growth factors and cytokines regulate bone turnover and ultimately the bone mass [9] **(Figure 1)**. Inhibition of RANKL and increased levels of OPG leads to diminished bone resorption, while more RANKL and less OPG leads to increased bone resorption and a decrease in BMD as seen when the endogenous estrogen levels falls during the menopause phase.

### Denosumab

Denosumab is a human IgG2 monoclonal antibody with high affinity and specificity for the human RANKL **(Figure 1)**. When denosumab binds to RANKL, the bone resorption is inhibited. In a prospective, randomized, double-blind Phase I trial, the highest dose caused serum concentrations of denosumab to rise rapidly during the 3 weeks after subcutaneous injection in healthy postmenopausal women, and these levels were maintained for up to 9 months [10]. The turnover markers for bone resorption fell within 12 h with a maximal suppression of approximately 84% and remained suppressed for up to 6 months [10]. Denosumab has no detectable binding to TNFα, TNFβ or TRAIL and no neutralizing antibodies have been seen in clinical trials. For treatment of postmenopausal osteoporosis denosumab is registered in the dose of denosumab 60 mg injected subcutaneously every 6 months.
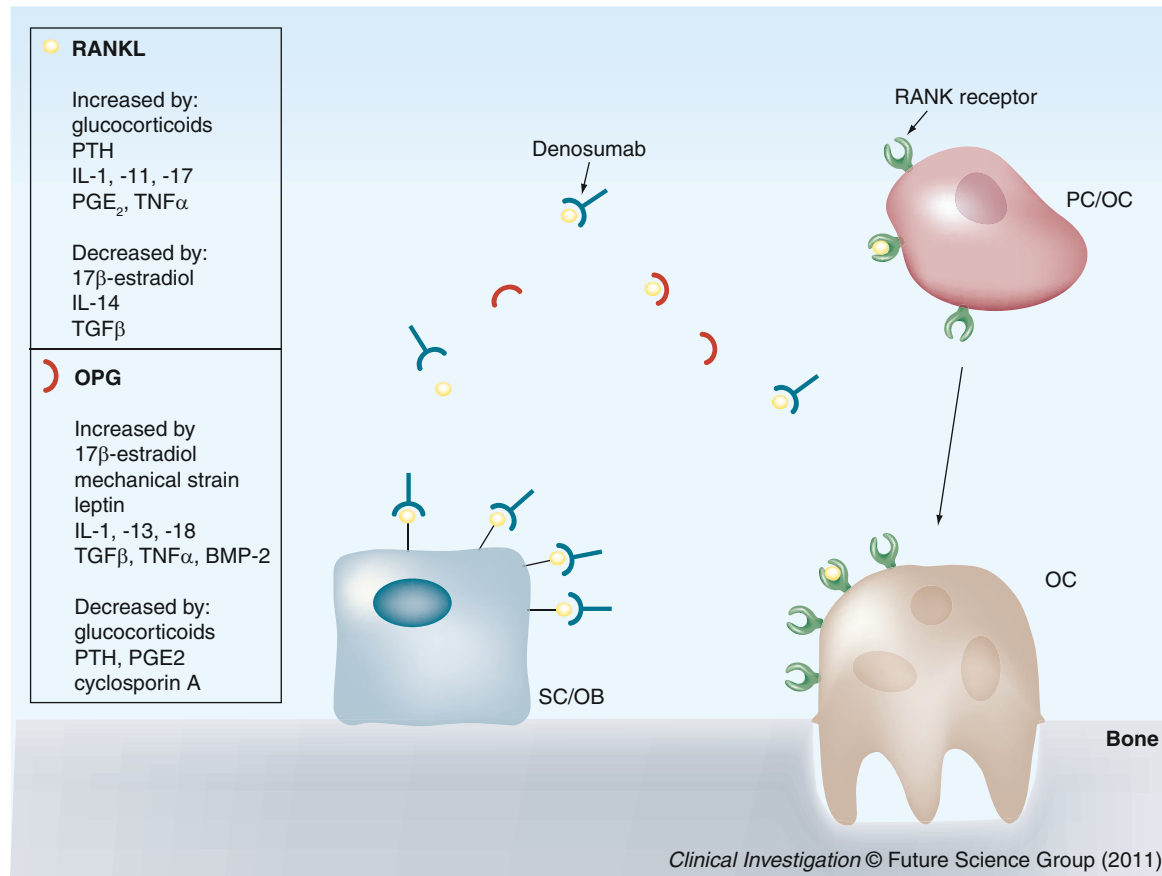
### FREEDOM study
#### ■ Study design

In the FREEDOM study 7868 postmenopausal women received either denosumab 60 mg or a placebo injection subcutaneously twice yearly for 36 months [1]. All women received a calcium supplement of at least 1000 mg and 400–800 IU vitamin D according to baseline concentration of serum 25-hydroxy vitamin D. The study was performed as an international, randomized, placebo-controlled trial in which 45% were recruited from Western and 35% from Eastern Europe, 12% were from Latin America, 7% from North America and 1% from Australia and New Zealand. Of the 7868 patients 31 subjects treated with denosumab and 29 with placebo at one center was excluded from all analyses due to inconsistency of data and adherence to the study protocol. Of the 7808 patients the denosumab-treated group consisted of 3902 women and the placebo group of 3906 women (mean age: 72.3 ± 5.2 [SD] years) for both groups. 82% completed 36 months of the study (3206 women in the placebo and 3272 in the denosumab group).

Included in the study were women between 60 and 90 years of age who had a BMD T-score of the lumbar spine or the total hip less than -2.5. Women who had used oral bisphosphonates less than 3 years before a 12-month period preceding the randomization were allowed to participate in the study.

Excluded for ethical reasons were women with severe osteoporosis if they had a T-score less than -4.0 SD at the lumbar spine or total hip or had any severe or more than two moderate prevalent vertebral fractures [11]. Also excluded were women who had taken oral bisphoshonates for more than 3 years or used intravenous bisphosphonates, fluoride or strontium for osteoporosis within 5 years. Women who had used PTH, selective estrogen receptor modulators, HRT, corticosteroids, tibolone, calcitonin or calcitriol within 6 weeks before the study start date or had conditions that influenced bone metabolism were also excluded. The primary end point was the incidence of new vertebral fractures at 36 months. Fracture efficacy was determined by annually lateral radiographs of the spine. Clinical fractures which occurred during the study were confirmed by diagnostic imaging or a radiologist's report. Prevalent and new vertebral fractures were assessed by a semi

**RANKL**

Increased by:
glucocorticoids
PTH
IL-1, -11, -17
PGE$_2$, TNF$\alpha$

Decreased by:
17$\beta$-estradiol
IL-14
TGF$\beta$

**OPG**

Increased by
17$\beta$-estradiol
mechanical strain
leptin
IL-1, -13, -18
TGF$\beta$, TNF$\alpha$, BMP-2

Decreased by:
glucocorticoids
PTH, PGE2
cyclosporin A

Denosumab

RANK receptor

PC/OC

OC

SC/OB

**Bone**

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**Figure 1. Systemic and local acting hormones, cytokines and growths factors regulate receptor activator of nuclear factor kB ligand and osteoprotegerin, and thereby the balance between bone resorption and formation.**
OB: Osteoblast; OC: Osteoclast; OPG: Osteoprotegerin; PC: Precursor cell; RANK: Receptor activator of nuclear factor $\kappa$B; RANKL: RANK ligand; SC: Stromal cell.

quantitative grading scale [11]. Secondary end points were the time to the first non vertebral fracture and the time to the first hip fracture.

### Antifracture efficacy
#### ■ Vertebral fractures
The 3-year cumulative incidence of new vertebral fracture in the placebo group was substantially higher (7.2%) than in the denosumab group (2.3%). The risk ratio was significantly reduced with denosumab treatment 0.32 (0.26–0.41; $p < 0.001$) corresponding to a relative risk reduction of 68% for new vertebral fractures [1]. The risk ratio for subjects who received denosumab treatment compared with those who received placebo injections were consistent through the 3 years of study. Approximately a third of the morphometric new vertebral fractures were also clinical apparent fractures. The cumulative incidence of new clinical vertebral fracture was 2.6% in the placebo group compared with 0.8% in the denosumab group and a relative risk

ratio of 0.31 (0.20–0.47; $p < 0.001$). A similar reduction (61%) was also observed for more than two new vertebral fractures.

#### ■ Hip fractures
The cumulative incidence by denosumab treatment was significantly reduced compared with the placebo-treated group (0.7 vs 1.2%, respectively; hazard ratio 0.60 [0.37–0.97]; $p = 0.04$) **(Table 1)**.

#### ■ Nonvertebral fractures
The cumulative incidence of a nonvertebral fracture in the placebo group was 8.0 versus 6.5% in the denosumab group, respectively, and a hazard ratio 0.80 (0.67–0.95; $p < 0.01$).

#### ■ Denosumab versus other antiresorptive therapies
The various antiresorptive drugs for postmenopausal osteoporosis including RAL are effective with a 3-year relative risk reduction in the range of 30–70% for a

| Table 1. Hip fractures. | | | | | | | |
|---|---|---|---|---|---|---|---|
| Substance (study) | Baseline hip FN T-score (mean ± SD) | No. of patients (placebo/ treatment) | 3-year cumulative rate of hip fracture, % placebo group | 3-year cumulative rate of hip fracture, % treatment group | 3-year ARR% | 3-year RR (95% CI) | Ref. |
| ALN[†] (FIT 1) | N/A | 1005/1022 | 2.2 | 1.1 | 1.1 | 0.49 (0.23–0.99) | [16] |
| RIS[‡] (HIP) | -3.7 ± 0.6 | 1821/3624 | 3.2 | 1.9 | 1.3 | 0.6 (0.4–0.9) | [17] |
| RAL[§] (MORE) | N/A | 770/1534 | 0.7 | 0.8 | n.s. | 1.1 (0.6–1.9) | [13] |
| IBAN[¶] (BONE) | -2.0 ± 0.9 | 975/977 | N/A | N/A | N/A | N/A | [14] |
| ZOL[#] (HORIZON) | -2.8 ± 0.5 | 3875/3861 | 2.5 | 1.4 | 1.1 | 0.59 (0.42–0.83) | [15] |
| Denosumab[††] (FREEDOM) | -2.2 ± 0.7 | 3906/3902 | 1.2 | 0.7 | 0.5 | 0.60 (0.37–0.97) | [1] |

Pivotal randomized controlled Phase III trials over three years of treatment with the major antiresorptive drugs in clinical use for postmenopausal osteoporosis.
[†]Oral alendronic acid (ALN) 10 mg daily.
[‡]Oral risedronic acid (RIS): combined data for 2.5 mg (n = 1812) and 5 mg daily (n = 1812).
[§]Oral raloxifene (RAL) 60 mg daily, study group 1 and 2 combined.
[¶]Oral ibandronic acid (IBAN) 2.5 mg daily.
[#]Relative risk reduction (%).
[††]Infusion of zoledronic acid (ZOL) 5 mg intravenously every 12 months.
ARR: Absolute risk reduction; N/A: T-Score is not provided in the publication; n.s.: No significant risk reduction; RR: Risk reduction.

new vertebral fracture in the pivotal randomized clinical trials (Table 2) [12–16]. The FIT1 trial with alendronic acid (alendronate, ALN) included patients with severe osteoporosis and a low femoral neck bone mineral density ($0.53 ± 0.07$ g/cm²) [16]. All patients had more than one prevalent vertebral fracture and subjects with multiple or severe fractures were not excluded. As new drugs successively entered into Phase III studies, the clinical trials included less severely osteoporotic patients. This is illustrated by the decreasing prevalence of vertebral fractures at baseline for the antiresorptive drugs in Table 2. At the time the FREEDOM study was designed, effective pharmacologic treatments for osteoporosis were well established. It was no longer ethically acceptable to randomize patients with severe osteoporosis to receive calcium and vitamin D only. This is reflected by the low 3-year cumulative absolute risk for new morphometric vertebral fractures of 7.2% (Table 2) and 2.6% for new clinical vertebral fractures in the placebo group [1]. The relative risk reduction for new vertebral fractures was 68% and similar to the efficacy of zoledronic acid (zoledronate, ZOL) in the HORIZON study [15] (Table 2).

In the other three trials that showed a significant reduction for hip fractures, the risk in the placebo groups were substantially higher than in FREEDOM with a cumulative absolute risk ranging from 2.2–3.2% compared with 1.2% in FREEDOM (Table 1) [15–17].

The absolute risk reduction (ARR) for a hip fracture in the denosumab group was 0.5%, which is lower than observed with ALN, risedronic acid (RIS) and ZOL (Table 1).

The ARR for antiresorptive therapies including denosumab is considerably higher (3.5–7.6%) for new vertebral fractures than for hip fractures (0.5–1.3%). The ARR for denosumab is similar to the potent bisphosphonates in preventing a new vertebral fracture (Table 2) but seem to be slightly less effective to prevent hip fracture judged only by ARR (Table 1). However, direct comparison of the antifracture efficacy of the various drugs as in Table 2 and Table 1 is difficult due to differences in inclusion criteria and study populations with respect to low or high risk of fracture.

To enable estimation of the efficacy of denosumab in higher risk cohorts post hoc subgroup analysis of the FREEDOM study has been performed [18]. In women with higher fracture risk due to multiple and/ or severe prevalent vertebral fractures, denosumab significantly reduced the risk of new vertebral fracture. The cumulative fracture risk was 7.5% in the denosumab group compared with 16.6% in the placebo group (p < 0.001). In the FREEDOM study high age also increased the risk of fracture. In subjects aged 75 years or older the risk of hip fractures was 0.9% in the denosumab-treated group compared with 2.3% in the placebo group (p < 0.01). The relative risk

reduction by denosumab treatment for hip fracture was 62% compared with 40% in the main FREEDOM cohort. In women at higher risk due to a T-score of less than 2.5 in femoral neck bone mineral density at baseline, the risk for a hip fracture was significantly reduced from 2.8% in the placebo group to 1.4% in denosumab group (p = 0.02) corresponding to a relative risk reduction by denosumab of 47%. The effects seen in the higher risk subgroup analysis were also seen in the lower risk subgroup [18]. They were consistent with and did not explain the results of the overall trial analysis of the FREEDOM study [18]. A prespecified subgroup analysis of the FREEDOM trial showed that new vertebral fractures in women with a T-score of less than 2.5 in femoral neck at baseline were reduced by denosumab treatment (3.1% denosumab vs 9.9% placebo; p < 0.001) [18].

### Bone mineral density

In a subgroup of 441 subjects in the FREEDOM trial BMD was measured by DXA of the hip and lumbar spine at 1, 6, 12, 24 and 36 months. After 3 years the placebo group had a stable, almost unchanged BMD (0.2%) in the lumbar spine but a significant decrease (-1.1%) in the total hip [1]. The relative increase in BMD in the denosumab group at the total hip was 6.0 and 9.2% in the lumbar spine as compared with the placebo. The increase in total hip BMD is similar to the gain seen at 36 months by 5.5% by infusion of ZOL 5 mg once yearly [20].

### ■ Denosumab versus bisphosphonates

Continued increase in lumbar spine and hip BMD has previously been seen (13.7 and 6.7%, respectively) after treatment with ALN for 10 years [21]. BMD at the total hip increased to 4% after 2 years and remained at that level despite 7 years of treatment with RIS [22]. Denosumab increases BMD in the femoral neck and distal third of the radius more after 12 months than did ALN 70 mg weekly in postmenopausal women (p < 0.13) [23]. In addition, in postmenopausal women, who were randomized to denosumab 60 mg twice yearly or weekly alendronate 70 mg in a Phase II study where high-resolution quantitative peripheral computed tomography (CT) was used, the changes in total and cortical BMD were significantly greater at 12 months in the denosumab group compared with the alendronate group (p ≤ 0.024) [24]. Denosumab increased volumetric BMD, BMC and thickness in cortical bone and of BMD in trabecular bone of the radius after 24 months treatment relative to placebo in women with low bone mass [25].

| Table 2. New vertebral fractures. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Substance (study) | Age (mean ± SD) | Prevalent vertebral fracture at baseline % | No. of patients (placebo/ treatment) | 3-year cumulative rate of new vertebral fracture, % placebo group | 3-year cumulative rate of new vertebral fracture, % treatment group | ARR% | RR (95% CI) | Ref. |
| ALN[†] (FIT 1) | 71 ± 6 | 100 | 1005/1022 | 15.0 | 8.0 | 7.0 | 0·53 (0.41–0.68) | [16] |
| RIS[‡] (VERT-NA) | 69 ± 8 | 80 | 820/821 | 16.3 | 11.3 | 5.0 | 0.59 (0.43–0.82) | [12] |
| RAL[§] (MORE) | 68 ± 7 | 37 | 2292/2259 | 10.1 | 6.6 | 3.5 | 0.7 (0.5–0.8) | [13] |
| IBAN[¶] (BONE) | 69 ± 6 | 94 | 975/977 | 9.6 | 4.7 | 4.9 | 52[#] (28–68) | [14] |
| ZOL[††] (HORIZON) | 73 ± 5 | 62 | 3039/3045 | 10.9 | 3.3 | 7.6 | 0.30 (0.24–0.38) | [15] |
| Denosumab[‡‡] (FREEDOM) | 72 ± 5 | 23 | 3906/3902 | 7.2 | 2.3 | 4.9 | 0.32 (0.26–0.41) | [1] |

Pivotal randomized controlled Phase III trials over 3 years of treatment with the major antiresorptive drugs in clinical use for postmenopausal osteoporosis.
[†]Oral alendronic acid (ALN) 10 mg daily.
[‡]Oral risedronic acid (RIS) 5 mg daily.
[§]Oral raloxifene (RAL) 60 mg daily, study group 1 and 2 combined.
[¶]Oral ibandronic acid (IBAN) 2.5 mg daily.
[#]Relative RR (%).
[††]Infusion of zoledronic acid (ZOL) 5 mg intravenously every 12 months.
[‡‡]Injecton of denosumab 60 mg subcutaneously every 6 months.
ARR: Absolute risk reduction; RR: Risk reduction.

The transient 70–90% increase in PTH after each new denosumab injection may be of importance for the long-term increase in BMD, as has been described in an earlier study [26]. Whether the PTH elevation causes an anabolic effect on bone is unknown, but a very early transient increase in the bone formation marker procollagen type I N-terminal propeptide (PINP) was seen in the FREEDOM study (but may have been missed in the Phase II study by Lewiecki *et al.*), which could strongly support an initial anabolic process [1,24,27].

### ■ Effects on bone histology & microstructure

In the FREEDOM trial bone biopsies from the iliac crest were obtained in the placebo (n = 51) and denosumab group (n = 52). 37 and 25 women in the placebo group and 31 and 22 in the denosumab group had biopsies taken at 24 months and at 36 months respectively. At both time points 23 women had biopsies taken. A total of 66 and 43% of the biopsies had no tetracycline labeling in trabecular and cortical bone, respectively, but the absence of label was not related to the level of bone turnover markers. Qualitative assessment of the bone biopsies showed normal trabecular and cortical microarchitecture and normal mineralization with an absence of osteoid accumulation [28]. The authors conclude that denosumab markedly reduces bone turnover [28]. Longer follow-up than 3 years is necessary to enlighten the consequences of long term inhibition of bone turnover by denosumab.

Micro CT at 24 months showed significantly greater cortical volumetric BMD in the denosumab group (866 g/cm$^2$) compared with the placebo group (851 g/cm$^2$; p < 0.02) [28]. The placebo group also had a significantly higher percentage of cortical porosity (4.58%) compared with the denosumab group (3.64%; p = 0.01). Cortical thickness was numerically, but not significantly higher, in the denosumab group (0.89 mm) than in the placebo group (0.72 mm). At 36 months there was no difference between the two groups. Nor were there any differences in trabecular structural indices at 24 and 36 months. The authors conclude that denosumab maintained normal microarchitecture and found no evidence of adverse effects on mineralization or formation of lamellar bone [28].

### ■ Effects on bone strength

In a study of denosumab treatment of women with low bone mass, using quantitative CT and calculations of polar moment of inertia, the strength of the radius increased significantly after 6 months compared with the placebo group and had improved further at 24 months, especially in the ultradistal region of radius [29].

### ■ Effects on bone turnover markers

In a subgroup of 160 subjects in the FREEDOM trial, serum carboxy-terminal collagen crosslinks (CTX) and tartrate-resistant acid phosphatase, both are biochemical markers for bone resorption, and two markers for bone formation, serum PINP and BALP, were followed [30]. The markers were measured at baseline, one month after the first injection and just before the next injection at 6, 12, 24 and 36 months. Denosumab caused a rapid and profound inhibition of bone resorption seen in all patients at one month with a decrease in CTX by 85–90% and of TRAC P5b by 55–60%. All patients had CTX concentrations below the marker level for the placebo group and below the lower limit of the premenopausal reference interval for the method at one month. In total, 79% of the women in the denosumab group had CTX concentrations at 6 months which were below the lower limit of the reference range at baseline. A decrease of PINP to 70% and of BALP to 40% at 6 months was also seen [30]. Patients with higher baseline concentrations of CTX had higher levels of bone turnover markers before the 6-month injection both in the denosumab and the placebo-treated group. A slight increase in the concentration of CTX was also seen before each 6 month injection in the FREEDOM study and which also has been observed in other studies with denosumab [19,23,24,27,30].

The antiresorptive profile of denosumab thereby seem to differ from the continuous suppression of bone resorption by the bisphosphonates such as ZOL with respect to the intermittent, short and low grade reactivation of bone remodeling before the next injection [15]. Bisphosphonates have high affinity to hydroxyapatite and binds to bone matrix [31]. They are retained in bone tissue even after discontinuation of treatment and cause apoptosis of the osteoclasts during resorption of bone which contains a bisphosphonate. Denosumab, on the other side acts as a circulating soluble protein with no apparent distribution on bone surfaces and is localized in medullary blood vessels and blood vessels penetrating cortical bone [32]. A different mode of action may also be important. During treatment with ZOL, ALN and RIS the bone turnover markers for resorption are reduced and remain rather constant during the treatment in contrast to denosumab. In the FREEDOM trial, the increase in BMD was significantly correlated to the CTX reduction in the denosumab group [30]. Whether the slight decrease in bone resorption (CTX) in the pre-injection period followed by a transient PTH release after the denosumab injection is beneficial for the bone formation is unknown. It could perhaps be an important factor for the continued long-term increase in BMD by denosumab.

## Discontinuation of denosumab

What happens to bone turnover when the treatment with denosumab is stopped? In a randomized Phase III trial of postmenopausal 256 women with low bone mass treated with the registered therapeutic dose for osteoporosis, denosumab 60 mg twice a year, for 24 months were followed for an additional 24 months [19]. After denosumab was discontinued at 24 months the median for the marker for bone resorption CTX increased above baseline within 3 months to reach a maximum at 6 months of approximately 60% and then declined to baseline within 48 months. The formation marker PINP increased above baseline within 6 months to approximately 40% and declined somewhat slower to baseline at 48 months [19]. The increased bone resorption is associated with a decrease in BMD of the lumbar spine to just above baseline after 12 months. BMD of the total hip declined in the distal radius, both declined to 0.5–1% below baseline at 12 months after discontinuation of denosumab [19].

The study corroborates similar findings of bone turnover markers and BMD using different doses and timing schedules of denosumab [33]. The reversibility of the changes in BMD and bone turnover markers obtained during denosumab treatment illustrates that bone resorption and formation seems to remain coupled after discontinuation. During the follow-up period between 24 and 48 months 3% of the patients sustained a new fracture in both groups [19]. Whether treatment with a bisphosphonate after discontinuation of denosumab will preserve the gain in BMD and the reduced fracture risk obtained during denosumab treatment needs to be investigated. The denosumab-treated patients, however, had higher BMD than the placebo-treated group at 48 months despite the 2 year period of treatment.

## Health-related quality of life & mortality

Fragility fractures are associated with adverse health outcomes including increased mortality, disability, psychological deterioration and impaired health-related quality of life (HRQoL) [34]. In a subset of the MORE-study with RAL, women with a prevalent vertebral fracture especially in the region of L1–L4 had significantly lower scores on physical function, emotional status, clinical symptoms, and overall HRQOL compared with women without a prevalent fracture [35]. With each subsequent fracture the patients acquired, the HRQOL-scores were reduced. Similar observations were made in a sub-study of the teriparatide registration study [36,37]. Women who fractured reported a significant decline in physical functioning, emotional status, and symptoms. However, in neither the MORE nor the teriparatide trials was there a difference between the treatment and the placebo groups regarding HRQoL [35,37].

Preliminary results from the FREEDOM trial showed that HRQoL was not different in the denosumab-treated group compared with the placebo-treated group after 3 years [38]. Incident clinical fractures, however, were associated with significant decreases in HRQoL.

### ■ Mortality

A significant decreased mortality in postmenopausal women after 3 years was observed in the HORIZON trial with ZOL 5 mg intravenously every 12 months [20]. A recent meta-analysis of eight clinical trials showed an 11% reduction in mortality of four antiresorptive drugs (risedronate, strontium ranelate, ZOL and denosumab). The reduction was greatest in trials conducted in populations with higher risk of fractures such as older, frailer individuals [39].

The patients in the FREEDOM study had less severe osteoporosis, which may be a contributing explanation as to why a significant reduction in the death rate in the denosumab-treated group (70 patients of 3886, 1,8%) compared with the placebo group (90 of 3876 patients, 2.3%; $p = 0.08$) was not seen.

### ■ Adverse effects

During the 36 months of the FREEDOM study there was no difference in the number of total adverse events between the denosumab group and the placebo-treated group (92.8 vs 93.1%, respectively) [1]. Neither was there any difference in the occurrence of serious events (25.8 vs 25.1%) or fatal events (1.8 vs 2.3%). Nor of events leading to discontinuation of the participation of the subjects in the study (2.4 vs 2.1%) or of denosumab (4.9 vs 5.2%, respectively). Infections were the most frequent reported adverse events in both groups 53% for denosumab and 54% for the placebo group. Cancer occurred similarly in the two groups (4.8 vs 4.3%, respectively). Presumably, owing to the calcium and vitamin D supplement given during the study, hypocalcemia was not seen in denosumab-treated patients and rarely in the placebo group (0.1%). Osteonecrosis of the jaw or fracture of the femur shaft was not observed during the 3 year study period [1].

Serious adverse events leading to hospitalization such as infections, cancer, cardiovascular event, stroke, coronary heart or peripheral vascular disease or atrial fibrillation all occurred with similar frequencies in the two groups. Eczema was seen in 3.0 and 1.7% ($p < 0.001$) of the denosumab and placebo group respectively. Cellulitis, including erysipelas, as a serious adverse event with low frequency occurred significant more with denosumab (0.3%) compared with placebo-treated patients (<0.1%; $p = 0.002$) [1]. There were significantly fewer falls (4.5 vs 5.7%) and less concussion (<0.1 vs 0.3% ) in the denosumab arm versus placebo.

No patient developed hypersensitivity or neutralizing antibodies to denosumab. The rates of adverse events and serious adverse events did not differ between the denosumab or placebo-treated women when the groups were stratified by kidney function [40].

The increased risk of cellulitis including erysipelas and eczema in the FREEDOM study is, however, to be noted and requires further studies of a possible causal association with denosumab treatment. RANKL is also expressed by T cells and acts on synoviocytes in rheumatoid arthritis, dendritic cells, monocytes, macrophages and other cells expressing RANK. A theoretical possibility of an impaired immune function with increased number of opportunistic infections by denosumab treatment has been raised [41,42]. The number of opportunistic infections and the occurrence of cancer, however, were similar and not significantly different between the two treatment groups in the FREEDOM study [1]. Moreover, denosumab treatment in rheumatoid arthritis does not significantly alter the inflammatory processes [43].

### Denosumab in renal impairment

Impaired renal function is common above 70 years of age and implies a considerably increased risk of fracture [42]. Only approximately 25% have normal renal function, 49% have mildly and 25% moderately impairment according to NHANES III [44]. Osteoporosis also is common above 70 years of age. It is therefore necessary to take the patients renal function into account when a drug for treatment of osteoporosis is chosen.

For chronic kidney disease (CKD) stage 1 (estimated glomerular filtration rate [eGFR] >90 ml/min) and stage 2 (eGFR: 60–89 ml/min) according to the National Kidney Foundation's (KDOQI) staging of 2002, osteoporosis can be managed as in the general population and all skeletal specific drugs for treatment of osteoporosis can be used including calcium and vitamin D [45]. In CKD stage 3 (30–59 ml/min) determination of serum calcium, phosphorus, PTH, (bone-specific) alkaline phosphatase and 25-hydroxy-cholecalciferol and, if available serum FGF-23 is helpful to decide whether the patient also has a CKD-related mineral and bone disorder (CKD–MBD) [45]. In moderate renal impairment vitamin D insufficiency and secondary hyperparathyroidism should be corrected before antiresorptive drug therapy is started. *Post hoc* analyses of the major registration trials of osteoporosis drugs have documented efficacy in reducing the risk of vertebral fractures down to eGFR of 30 ml/min for RIS and ALN and 30–35 ml/min for ZOL [46–48]. For strontium ranelate and RAL there is no fracture data in patients with CKD, but RAL increases BMD in the femoral neck and lumbar spine in patients with eGFR above 30 ml/min [49]. For teriparatide decreased vertebral and non vertebral fracture risk also has been shown as well as an increase in BMD of the lumbar spine and hip in patients with eGFR above 30 ml/min [50].

For CKD stage 4 (15–29 ml/min) and 5 (<15 ml/min) the risk of CKD–MBD and renal osteodystrophy increases and a bone biopsy should be considered if serum PTH or bone-specific alkaline phosphatase is very abnormal and before bisphosphonates therapy is started [45]. The diagnosis of osteoporosis in these patients is complex and a measurement of BMD is at best of limited use [45]. If the patient has had fractures previously and prevention of new fractures is required, the choice of drug should be made with great caution. Bisphosphonates are cleared by the kidney and their use in this situation is not without risk [51]. An extended period of low bone turnover with adynamic bone disease has been described with the use of ALN [52]. *Post hoc* analysis of the use of RIS in CKD stage 4 has shown decreased risk of vertebral fracture in a comparatively small number of patients from the VERT study [47]. The skeletal retention of ALN can last up to several years even in women with normal kidney function [31]. Although the retention of RIS may be shorter there is still a high risk for prolonged antiresorptive action of bisphosphonates in CKD stage 4 and 5 and bisphosphonates should be used only with great caution or not at all [51].

Denosumab is eliminated from the body through the reticuloendothelial system and not by renal clearance. Thereby denosumab could be feasible for the treatment of osteoporosis with renal impairment. In a *post hoc* subgroup analysis of the FREEDOM trial the antifracture efficacy of denosumab was analyzed based on the CKD staging [40]. 842 women had normal renal function (stage 1), 4069 had mild impairment (stage 2), 2817 had moderate (stage 3) and 73 women had severe renal impairment, CKD stage 4. Denosumab reduced the incidence of new vertebral fractures significantly independent of the level of the patients kidney function. The odds ratio for the incidence of new vertebral fractures was 0.30 (95% CI: 0.23–0.39) and for non vertebral fractures 0.78 (95% CI: 0.66–0.93) [40]. For denosumab-treated women with severe renal impairment, CKD stage 4, the reduction in incidence for new vertebral was of the same magnitude (odds ratio = 0.31 [0.02–5.08]) but not significantly reduced [40]. The BMD increased 8.8% in the lumbar spine, 5.2% in the femoral neck and 6.4% in the total hip after 36 months and was independent of the level of kidney function [40].

In mild and moderate renal impairment denosumab therefore has an advantage over the bisphosphonates due to lack of skeleton retention and ease of administration of the drug. Based on the FREEDOM *post hoc* analysis, denosumab may also be considered as a treatment option to prevent new vertebral fractures in patients with severely

impaired kidney function, CKD stage 4 without CKD–MBD. Since patients with severe renal impairment and CKD–MBD were excluded from the FREEDOM trial through the exclusion criteria, fracture data on the effect of denosumab in patients with CKD stage 4 and CKD–MBD and in CKD stage 5 in hemodialysis are lacking. Denosumab treatment of these patients should only be given after great cautiousness or possibly be avoided if

## Executive summary

- While the principle of treatment with a recombinant monoclonal antibody is well established in rheumatology and hematology, it only recently has been introduced to the bone field through the discovery and characterization of the receptor activator of nuclear factor κB (RANK) ligand (RANKL)–osteoprotegerin (OPG) system.
- The first fully human monoclonal antibody, denosumab, blocks the RANKL–OPG pathway and made it possible for clinicians to treat osteoporosis and skeletal related events in cancer by this new principle.
- Denosumab is a potent antiresorptive agent and has been shown to increase bone mineral density (BMD) and to reduce the risk of new vertebral fractures, hip and nonvertebral fractures in postmenopausal women with osteoporosis.

### The RANKL–OPG system
- One of the many valuable results of the human genome project was the identification and cloning of OPG and RANK and its ligand RANKL. RANKL controls the osteoclastogenesis by stimulating the differentiation of osteoclasts precursor cells and the formation, function and survival of osteoclasts, while OPG acts as a decoy receptor for RANKL and inhibits each of these effects. The balance between the local concentration of RANKL and OPG in bone tissue is a key mechanism controlling the bone metabolism and bone mass.

### Denosumab
- Denosumab is a human IgG2 monoclonal antibody with high affinity and specificity for the human RANK ligand. By its binding to RANKL the bone resorption is inhibited. After one subcutaneous injection the turnover markers for bone resorption are suppressed for 6 months. For treatment of postmenopausal osteoporosis, denosumab is registered at the dose of 60 mg denosumab injected subcutaneously every 6 months.

### FREEDOM study
- The FREEDOM study is a multinational, randomized, placebo-controlled trial in which 7868 postmenopausal women between 60 and 90 years with a BMD T-score of the lumbar spine or the total hip less than -2.5 received either denosumab 60 mg or a placebo injection subcutaneously twice yearly for 36 months.

### Effects on fracture risk, BMD, bone turnover markers & histology
- Denosumab treatment significantly reduced the relative risk for new vertebral fractures by 68%, for hip fractures by 40% and nonvertebral fractures by 20%.
- The relative increase in BMD in the denosumab group at the total hip was 6.0% and in the lumbar spine 9.2% compared with placebo after 36 months.
- After denosumab injection, all patients had concentrations of the resorption marker carboxy-terminal collagen crosslinks below the level for the placebo group and the lower limit of the premenopausal reference interval for the method at 1 month. A total of 79% of the women in the denosumab group had carboxy-terminal collagen crosslink concentrations at 6 months that were below the lower limit of the reference range at baseline. The rest had a clear reduction of bone resorption compared with baseline. Bone biopsies showed normal trabecular and cortical microarchitecture and normal mineralization with absence of osteoid accumulation.

### Adverse effects of denosumab
- There was no difference in the number of total adverse events between the denosumab group and the placebo-treated group. Neither was there any difference in the occurrence of serious events or fatal events. Infections were the most frequent reported adverse events in both groups, 53% for denosumab and 54% for the placebo group. Cancer occurred similarly in the two groups (4.8 vs 4.3%, respectively). Eczema was seen in 3.0 and 1.7% (p < 0.001) of the denosumab and placebo group, respectively. Cellulitis, including erysipelas, as a serious adverse event with low frequency, occurred significantly more with denosumab (0.3%) compared with placebo-treated patients (<0.1%; p = 0.002). No patient developed hypersensitivity or neutralizing antibodies to denosumab.

### Denosumab in renal impairment
- Denosumab is eliminated from the body through the reticuloendothelial system and not by renal clearance. Denosumab significantly reduced the incidence of new vertebral fractures, independent of the level of the patients kidney function in mild and moderate renal impairment (chronic kidney disease (CKD) stage 1–3; glomerular filtration rate ≥30 ml/min). In these patients denosumab seemed to have an advantage over the bisphosphonates due to lack of skeleton retention and ease of administration of the drug.
- The effects of denosumab treatment on fracture risk and of side effects in patients with severe renal impairment (CKD stage 4 with CKD-related mineral and bone disorder and in CKD stage 5 in hemodialysis) requires further study.

serum PTH is below twice the upper normal reference range for the PTH method, as it may signify the presence of adynamic bone disease with low turnover [45].

When antiresorptive drugs are used in renal impairment it is important to pay attention to possible development of hypocalcemia. Therefore, sufficient vitamin D and calcium supplements are essential to avoid hypocalcemia due to the very potent antiresorptive actions of denosumab.

## Summary

Denosumab is a human monoclonal antibody that specifically blocks RANKL and is a potent antiresorptive drug. Denosumab has recently been approved for treatment of postmenopausal osteoporosis. Its antifracture efficacy has been proven in a large prospective, randomized multicenter study of 7808 postmenopausal women with osteoporosis (FREEDOM trial). Denosumab 60 mg injected subcutaneously every 6 months for 3 years significantly increased BMD in the lumbar spine, hip and radius and reduced the relative risk of new vertebral fractures by 68%, hip fractures by 40% and of nonvertebral fractures by 20% compared with the placebo-treated group. Bone biopsies showed a marked reduction of bone turnover but normal trabecular and cortical microarchitecture, normal mineralization and no sign of osteoid accumulation. Future studies are needed to establish whether extended inhibition of bone turnover for more than 3 years influences the fracture risk. Denosumab is cleared by the reticuloendothelial system and has been shown to significantly reduce the risk of new vertebral fracture in osteoporotic postmenopausal women with mild-to-moderate renal impairment.

## Future perspective

Despite the increased attention during the last three decades from healthcare providers, patient organizations, public healthcare systems and the pharmaceutical industries, osteoporosis is still a burden. Improved diagnostic tools for measurements of bone mineral density, increased knowledge of risk factors behind a fracture gained from large cohorts and the successive development of a fracture risk assessment tool (FRAX) together with the development of effective drugs for treatment of osteoporosis has put the clinician and patient in a much better situation today than ever before. We now have a number of effective drugs, such as the bisphosphonates, RAL and denosumab with antiresorptive properties, intermittent teriparatide and PTH 1–84 for stimulating bone formation, and strontium ranelate, which seems to act through both processes.

The newest of the drugs, denosumab, is an antibody that blocks bone resorption through a specific mechanism, the RANKL–RANK system. The clinical data for treatment of postmenopausal osteoporosis with denosumab in the large FREEDOM study show a risk reduction for new vertebral fractures, hip and nonvertebral fractures. It is likely that the obtained fracture risk reductions are close to what is maximal possible through blocking of the bone resorption. Anabolic agents such teriparatide and PTH 1–84 lead to profound increases in BMD and reduced fracture risks, but their use is hampered by the need for daily injections, high costs and they are limited to 24 months of treatment. Therefore, it seems there is a need for new anabolic drugs that are safe and convenient for the patient, reduce the fracture risk at least as well as the antiresorptive drugs, and that have a feasible cost:benefit ratio. Other possible steps towards better treatment of osteoporosis are the development of regiments where anabolic drugs are followed by, or combined with, an antiresorptive.

## Financial & competing interests disclosure

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