Summary
Denosumab, a fully human monoclonal antibody against the key osteoclastogenic factor RANK ligand, is currently approved for the treatment of postmenopausal osteoporosis. Denosumab differs from bisphosphonates in many aspects, such as that denosumab acts in the extracellular compartment and is likely to be distributed throughout the skeleton. In contrast, bisphosphonates have to be internalized by osteoclasts and are mainly located across bone surfaces. This could explain, why patients with osteoporosis who are already treated with bisphosphonates, might experience further benefit when switching to denosumab. Head-to-head studies revealed that transition to denosumab resulted in greater increase of bone mineral density (BMD) and greater reduction of bone turnover than did continued alendronate. Additional analyses of the phase 3 FREEDOM trial demonstrated that
fracture reduction was particularly high in cortical bone, such as the wrist. In addition, denosumab treatment for five and eight years showed sustained reduction in fracture risk, increase in BMD and continued to be well-tolerated. The seven year extension study of FREEDOM and a phase 3 trial evaluating denosumab for the treatment of male osteoporosis are still ongoing and will provide supportive data in the near future.

**Keywords:**
postmenopausal osteoporosis, denosumab, fractures, bone mineral density, review

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**Zusammenfassung**


**Schlüsselwörter**
Postmenopausale Osteoporose, Denosumab, Frakturen, Knochenmineraldichte, Review
**Introduction**

At menopause, the decrease in estrogen leads to increased bone resorption [Sambrook 2006]. At first this affects trabecular bone which is very sensitive to changes in bone resorption and formation [Zebaze 2010]. Turnover in cortical bone is much slower and less sensitive to changes in bone remodelling. Hence marked cortical bone loss occurs later after menopause from the age of 65 and above [Zebaze 2010].

The most devastating result of osteoporosis are osteoporotic fractures, especially those of the hip. Osteoporotic fractures occur throughout the skeleton and the incidence increases with age [Sambrook 2006]. Most common fracture sites are the hip, spine and wrist [Johnell 2006]. Vertebrae comprise of approximately 70% trabecular bone [Dempster 2008] and vertebral fracture incidence is high early on after menopause correlating with early trabecular bone loss. Fractures of the hip, a bone comprised of more than 75% cortical bone, increase later after menopause from the age of approximately 70 years and correlate with the accumulation of trabecular and cortical bone loss [Dempster 2008].

During the last 15 years key regulators of bone formation and resorption have been identified [Boyle 2003; Boyce 2007]. An essential mediator of osteoclast formation, function, and survival is RANK ligand (*receptor activator of nuclear factor kappaB* ligand). RANK ligand exerts its effects by binding to RANK, a receptor on osteoclast precursor cells and mature osteoclasts. This interaction results in activation, migration, differentiation, and fusion of hematopoietic cells of the osteoclast lineage finally giving rise to mature osteoclasts. Normally this process is regulated by an endogenous, soluble antagonist called osteoprotegerin (OPG). OPG is a decoy receptor which binds to RANK ligand preventing it from binding to RANK. Understanding of these basic processes enabled the development of new agents for the treatment of diseases associated with increased bone resorption.

**Denosumab and bisphosphonates – Different modes of action**

Denosumab is a fully human monoclonal antibody that specifically binds to human RANK Ligand [Tsourdi 2011; Moen 2011]. Binding of denosumab to RANK ligand inhibits the activation of RANK on the surface of osteoclasts and osteoclast precursors which results in decreased bone resorption and increased bone mass and strength in both cortical and trabecular bone. Denosumab is administered subcutaneously every 6 months [Denosumab SmPC]. After infusion, denosumab circulates continuously in the blood and extracellular fluid allowing to reach both trabecular and cortical bone [Baron 2011].

In contrast, bisphosphonates bind with high affinity to hydroxyapatite and inhibit bone resorption by inducing osteoclast apoptosis [Russell 2011]. Bisphosphonates localize to regions of bone where new mineral is being deposited beneath osteoblasts and to where bone is being resorbed by osteoclasts. They are embedded in bone and remain inactive until they are released by osteoclast-mediated bone resorption. They are then taken up by
osteoclasts by endocytosis. This internalization results in disruption of the cytoskeleton, loss of osteoclast orientation, and detachment of the osteoclast from the bone surface [Russell 2011; Weinstein 2009]. Bisphosphonates are primarily located across bone surfaces, mainly those with adjacent marrow, such as endocortical and trabecular surfaces. Only a minor amount reaches more lightly vascularized deep sites in cortical bone [Kimmel 2007]. Hence, bisphosphonates are likely to act mainly on trabecular bone.

One way in which denosumab differs from bisphosphonates is that bisphosphonates have to be internalized to act upon osteoclasts, whereas denosumab acts in the extracellular compartment [Baron 2011; Russell 2011]. In addition, denosumab varies from bisphosphonates in its likely distribution in bone. As denosumab is an antibody, this agent is expected to be capable of distributing throughout the skeleton, including intracortical sites [Baron 2011]. These differences become obvious when denosumab is used in patients pretreated with bisphosphonates.

**Comparison of Denosumab versus Alendronate (Stand Study)**

A phase 3 multicenter, randomized, double-blind study investigated the effects of denosumab in bisphosphonate pretreated patients (STAND - Study of Transitioning from Alendronate to Denosumab)[Kendler 2010]. Post-menopausal women at the age of ≥55 years who had been receiving prior alendronate therapy for at least 6 months were either switched to denosumab (60 mg SC every 6 months, Q6M) or continued oral alendronate therapy (70 mg once weekly, QW). All patients received supplemental calcium and vitamin D. Eligible patients had a T-score ≤ –2.0 and ≥ –4.0 at the lumbar spine or femoral neck. After 12 months, significantly greater gains in bone mineral density (BMD) were observed at the lumbar spine (3.03% vs. 1.85%; p<0.0125), femoral neck (1.90% vs. 1.05%; p<0.0001), and one-third radius (0.87% vs. 0.15%; p<0.0125) with denosumab therapy compared to alendronate therapy. Increases in BMD at the lumbar spine, femoral neck and 1/3 distal radius were 64%, 81% and 480% greater with denosumab compared to alendronate. It is interesting to note that in patients already exposed to alendronate, treatment with denosumab leads to greater BMD increase than alendronate at all sites measured and that this difference tends to be greater at sites that are primarily cortical, such as 1/3 distal radius. This observation may be explained by the different mode of action of these drugs. Denosumab might reach out to parts of the bone that alendronate had not reached even when patients were treated for a long period (median 36 months).

Support of this hypothesis comes from a different comparative study evaluating PTH expression and cortical porosity of the distal radius with alendronate, denosumab or placebo therapy [Seeman 2011]. PTH-levels increased transitorily with denosumab and alendronate, but not during placebo treatment. This increase was significantly higher in the denosumab group (p<0.05). Associated with this, porosity at the distal radius decreased by 3.0% after 12 months of denosumab, whereas it increased in the alendronate- and placebo-group (+2.9% and 5.2%; figure 1). In conclusion, denosumab probably exerts it’s effects primarily by
directly inhibiting osteoclast activity resulting in reduced bone turnover. In addition, there might be an indirect effect of denosumab by increasing PTH. This could positively influence bone remodelling units leading to new bone formation.

**Reduction of Fracture Risk (Freedom Study)**

The FREEDOM (*Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months*) trial was a randomized, placebo-controlled phase 3 study for 36 months designed to evaluate the efficacy of denosumab in reducing the risk of fracture in 7868 post-menopausal women [Cummings 2009]. Subjects with a T-score of <-2.5 to ≥-4.0 were randomized to either 60 mg denosumab SC Q6M or placebo. In addition, all patients received calcium (500-1000 mg daily) and vitamin D (400-800 IU). Patient characteristics are summarized in table 1.

After 36 months the incidence of new vertebral fractures in the denosumab- and placebo-group was 2.3% and 7.2%, respectively. This corresponds to a reduction in relative risk of 68% (p<0.001). In comparison to placebo, denosumab reduced the relative risk of fractures in total hip and other sites by 40% (p=0.04) and 20% (p=0.01). Reduction of fracture risk was shown to be independent of age (interaction value 0.5) [Rizzoli 2010]. A post-hoc analysis of the study revealed, that in patients at risk for hip fractures, i.e. those older than 75 years, denosumab reduced significantly the number of hip fractures to the one of younger patients [Boonen 2011]. Relative risk reduction in patients older than 75 years was 62%, whereas it was 6% for younger subjects.

During the study, BMD improved by 9.2% (lumbal spine) and 6.0% (hip) in denosumab-treated patients versus placebo-treated subjects. This is reflected in a sustained reduction of bone turnover markers: after 1 month and 36 months denosumab therapy C-telopeptide levels decreased by 86% and 72%, respectively. Accordingly, P1NP levels at end of study were 76% lower in the denosumab group vs. the placebo group.

A recent subanalysis of the FREEDOM study evaluated fracture rate of the wrist as well as BMD at all radius regions, i.e. 1/3, ulradistal, and total radius [Simon 2011]. For the whole study population, the incidence of wrist fractures was 2.9% and 2.5% for placebo- and denosumab-treated subjects (hazard ratio 0.84; p=0.21). However, in high-risk patients (i.e. those with a T-score of ≤-2.5 at femoral neck) this difference was significant, with an absolute reduction of 1.6% (p=0.03). This corresponds to a relative risk reduction of 40% and was similar to the risk of low-risk patients treated with placebo (figure 2). In addition, denosumab improved significantly BMD and calculated bone strength compared to baseline levels and placebo. This effect on cortical bone might explain favourable data on non-vertebral fractures in patients at high risk.

There was no significant difference between both study groups with regard to the incidence of adverse events, serious adverse events, and discontinuation of the study due to adverse
The incidence of serious skin infections (cellulitis) was significantly higher in the denosumab group (0.3% vs. <0.1%; p=0.002). However, the overall incidence of cellulitis was not significantly different (1.2% vs. 0.9%; p).

**Long term data**

All patients completing the FREEDOM study were offered to participate in an ongoing, open-label extension study for additional 7 years [Papapoulos 2011]. Patients of the placebo group were switched to denosumab and patients, who were initially randomized to denosumab, continued on this drug. Hence, in the future there will be long-term efficacy and safety data available of patients treated with denosumab for 10 years.

In total 4550 patients (70.2%) of the FREEDOM study were included in the long-term extension trial - 2207 patients previously received placebo (de novo group) and 2343 patients were treated with denosumab (long-term group). A planned interim analysis after two years of the extension trial demonstrated that denosumab treatment for 5 years in the long-term group continued to significantly increase BMD without reaching a plateau. During the 4th and 5th years, BMD at lumbar spine further increased by 1.9% and 1.7% and total hip BMD by 0.7% and 0.6% (all p<0.0001 compared to extension baseline). Total BMD increases with 5-year denosumab treatment were 13.7% and 7.0%, at lumbar spine and total hip, respectively. In the de novo group BMD improved comparably to the first two years of treatment in the long-term group, by 7.9% at the lumbar spine and 4.1% at total hip. Incidence of new vertebral and non-vertebral fractures continued to be low during the 4th and 5th treatment year, with 1.4% of new vertebral fractures and 1.2% and 1.1% of non-vertebral fractures. Fracture rates during the first three years in the placebo group were between 2.2% and 3.1%.

The safety profile and incidence of adverse events in the extension trial was comparable to that of the FREEDOM study [Bone 2011]. Yearly incidences of serious infections and malignancies did not increase over 5 years of denosumab treatment. In addition, the imbalance in serious skin infections seen during first three years in the FREEDOM trial was not observed with denosumab in the extension study.

Further long-term data on denosumab treatment were assessed during a phase 2 study and it’s extension [Miller 2008; McClung 2011]. In this study, postmenopausal women with a T-Score of -1.8 to -4.0 at the lumbar spine and/or -1.8 to -3.5 at the hip or femoral neck were randomized to placebo, alendronate or different dosing of denosumab [Miller 2008]. After four years patients could participate in an extension study for (additional) 4 years of denosumab, so that patients initially randomized to denosumab received this drug for a total of eight years, and patients randomized to the other two groups received denosumab for 4 years [McClung 2011]. During the extension all patients were given denosumab at a dose of 60 mg Q6M. In patients receiving denosumab for a total of 8 years (n=80), BMD at lumbar
spine and hip increased by 16.8% and 6.9% compared to baseline (figure 3). In comparison to the extension baseline increases at lumbar spine and hip were 5.8% and 2.0%, respectively. Patients who switched to denosumab in the extension trial (n=11) showed comparable improvements in BMD after 4 years as patients initially randomized to denosumab during the first 4 years.

**Additional data – the ADAMO study**

Besides to broad efficacy and safety data in women with postmenopausal osteoporosis and cancer patients receiving hormone ablation therapy, additional data in male patients with osteoporosis will be provided by the ADAMO study (*A multicenter, randomized, double-blind, placebo-controlled study to compare the efficacy and safety of DenosumAb 60 mg every 6 months versus placebo in Males with Osteoporosis*) [Orwoll 2011]. This trial is still ongoing. Men were eligible for inclusion if they had a T-score ≤ −2.0 and ≥ −3.5 (lumbar spine or femoral neck) or had experienced a prior major osteoporotic fracture and had a T-score ≤ −1.0 and ≥ −3.5. Up to now, 242 subjects were enrolled with a mean age of 65.0 years and 37.2% having a previous fracture. The study results are not available since the trial is ongoing and remains blinded.

**Acknowledgments**

**Conflict of interest**
References


Table 1:
Selected baseline characteristics of subjects in the phase 3 FREEDOM study [Cummings].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Denosumab (N = 3902)</th>
<th>Placebo (N = 3906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, yrs</td>
<td>72.3 ± 5.2</td>
<td>72.3 ± 5.2</td>
</tr>
<tr>
<td>Age groups, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70 yr</td>
<td>1030 (26.4)</td>
<td>1028 (26.3)</td>
</tr>
<tr>
<td>70–74 yr</td>
<td>1637 (42.0)</td>
<td>1642 (42.0)</td>
</tr>
<tr>
<td>≥75 yr</td>
<td>1235 (31.7)</td>
<td>1236 (31.6)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.0 ± 4.1</td>
<td>26.0 ± 4.2</td>
</tr>
<tr>
<td>T-score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>−2.82 ± 0.70</td>
<td>−2.84 ± 0.69</td>
</tr>
<tr>
<td>Total hip</td>
<td>−1.89 ± 0.81</td>
<td>−1.89 ± 0.81</td>
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<tr>
<td>Femoral neck</td>
<td>−2.15 ± 0.72</td>
<td>−2.17 ± 0.71</td>
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<tr>
<td>Prevalent vertebral fracture, no. (%)</td>
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<td></td>
</tr>
<tr>
<td>yes</td>
<td>929 (23.8)</td>
<td>915 (23.4)</td>
</tr>
<tr>
<td>no</td>
<td>2864 (73.4)</td>
<td>2854 (73.1)</td>
</tr>
<tr>
<td>Unreadable or missing data</td>
<td>109 (2.8)</td>
<td>137 (3.5)</td>
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<tr>
<td>Serum 25-hydroxyvitamin D, ng/ml(^1)</td>
<td>23.1 ± 11.7</td>
<td>22.9 ± 11.3</td>
</tr>
</tbody>
</table>

\(^1\) Subjects with outlier values of more than 200 ng per milliliter were excluded from this analysis.
Legends

Figure 1:
Change in porosity of the distal radius after 12 months compared to baseline [Seeman]. In this double-blind phase 2 study, 247 postmenopausal women with low BMD were randomly assigned to denosumab (n=83), alendronate (n=82), or placebo (n=82).

Figure 2:
FREEDOM study: incidence of wrist fractures (%) after 36 months of denosumab vs. placebo [Simon]. The incidence of wrist fractures was evaluated for all FREEDOM subjects (placebo n=3906; denosumab n=3902). Figures on top of the columns indicate relative risk reduction (95% CI). High risk group with a baseline T-score at femoral neck ≤ -2.5, low risk group with a baseline T-score >-2.5.

Figure 3
Phase 2 study comparing denosumab vs. placebo: change in BMD at lumbar spine (%) and total hip (%) in the initial study (month 0-48) and extension study (month 48-96)[McClung]. After completion of the parent study (after 48 months) participants could enroll into an open-label 4-year extension study. All subjects in the extension were treated with denosumab.
Figure 1

Change in cortical porosity (%)

Placebo | Alendronate | Denosumab
--- | --- | ---
+5.2% | +2.9% | -3.0%

Figure 2

Incidence of wrist fractures after 36 months (%)

Total | High risk group | Low risk group
--- | --- | ---
Placebo | Denosumab

- Total: 16% (-11%, 37%) p=0.21
- High risk group: 40% (5%, 62%) p=0.03
- Low risk group: -4% (-51%, 28%) p=0.2

n 3906 3902 1406 1384 2500 2518
Figure 3

Initial Study (month 0-48)  Extension (month 48-96)

Change in BMD at lumbar spine (%)

Placebo (n=11)
Denosumab (n=80)

Change in BMD at total hip (%)

Initial Study (month 0-48)  Extension (month 48-96)

16.8%
6.9%