Denosumab for bone diseases: translating bone biology into targeted therapy

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Abstract
Signalling of receptor activator of nuclear factor-κB (RANK) ligand (RANKL) through RANK is a critical pathway to regulate the differentiation and activity of osteoclasts and, hence, a master regulator of bone resorption. Increased RANKL activity has been demonstrated in diseases characterised by excessive bone loss such as osteoporosis, rheumatoid arthritis and osteolytic bone metastases. The development and approval of denosumab, a fully MAB against RANKL, has heralded a new era in the treatment of bone diseases by providing a potent, targeted and reversible inhibitor of bone resorption. This article summarises the molecular and cellular biology of the RANKL/RANK system and critically reviews preclinical and clinical studies that have established denosumab as a promising novel therapy for metabolic and malignant bone diseases. We will discuss the potential indications for denosumab along with a critical review of safety and analyse its potential within the concert of established therapies.

Introduction
Osteoporosis is a skeletal disorder characterised by low bone mass and microarchitectural deterioration, resulting in a high risk for fragility fractures (1). Osteoporotic fractures, in particular hip fractures, are associated with an increased mortality, and commonly cause an irreversible reduction of an individual’s quality of life and independence (2). Pharmacological treatment can be categorised by its mode of action into either antiresorptive or bone anabolic. While treatment options using anabolic substances are currently limited to intermittent administration of the full-length PTH (1–84) or its fragment (1–34) (3), various antiresorptive medications exist. Of these, aminobisphosphonates constitute the largest and most commonly used class that has been employed for more than two decades. However, oral bisphosphonates may cause esophageal irritation and gastrointestinal discomfort, and are associated with poor long-term compliance. Intravenous bisphosphonates avoid these obstacles, but their use may be limited in advanced renal insufficiency with a glomerular filtration rate (GFR) below 30 ml/min.

The discovery and characterisation of a novel cytokine system comprising the receptor activator of nuclear factor-κB (RANK) ligand (RANKL), its receptor RANK, and its soluble decoy receptor osteoprotegerin (OPG) has been a pivotal key towards understanding the biology of bone resorption and has paved the way for developing a targeted treatment for bone diseases characterised by excessive osteoclastic activity (4, 5). With the development of antibodies against RANKL and their successful evaluation in phase 3 studies, a highly specific and targeted therapy has become available for bone diseases. In this article, we will summarise the translation from the discovery of a new bone cytokine towards the development of a novel therapy for skeletal diseases.

RANKL/RANK/OPG pathway
The RANKL/RANK/OPG pathway mediates the differentiation and activity of osteoclasts. RANKL is primarily expressed on the surface of osteoblastic lineage cells such as mesenchymal stem cells and osteoblasts as
well as periosteal cells (6). While endothelial cells, T lymphocytes, synovial fibroblasts and various tumour cells have been shown to express RANKL (7–9), the pool of circulating RANKL is largely determined by the production within the bone compartment (10). RANKL promotes bone resorption by increasing the number and activity of functional osteoclasts through activation of its receptor RANK. RANK is mainly expressed by osteoclasts. In addition, endothelial cells, smooth muscle cells and various malignant cells express RANK (11–13), as do T and B lymphocytes, and dendritic cells. RANKL- and RANK-knockout mice lack lymph nodes (14, 15). However, while transgenic OPG rodents, in which RANKL is continuously inhibited, have an increased bone mass, no immune abnormalities were detected, indicating redundancy in the immune system (16).

The induction of osteoclastogenesis through RANKL/RANK interaction is counterbalanced by the soluble receptor antagonist OPG. Thus, RANKL and OPG represent essential factors in the regulation of differentiation, fusion, activation and survival of osteoclasts, with RANKL being a ‘gas pedal’ and OPG a ‘brake pedal’ (Fig. 1) (17). Under normal circumstances, the ratio of RANKL–OPG is carefully balanced, allowing physiological bone remodelling but preventing unwanted bone loss. Oestrogen deficiency (18), systemic glucocorticoid exposure (19), T cell activation as in rheumatoid arthritis (20) and skeletal malignancies (multiple myeloma, bone metastases) (21, 22) enhance the ratio of RANKL–OPG, promote osteoclastogenesis, and bone resorption, and induce bone loss. In addition, alterations of RANKL and OPG have been linked to vascular calcification (23).

In preclinical studies, an altered RANKL/OPG ratio has been clearly linked to bone loss in metabolic, immune-mediated and malignant bone diseases providing proof of principle that this pathway is essential for skeletal homoeostasis. The significance of the RANKL/RANK pathway in osteoclastogenesis and metabolic bone diseases has rendered it a prime therapeutic target. Initially, RANKL was antagonised by a chimeric OPG-Fc fusion protein. However, in light of unfavorable pharmacokinetics, necessitating injections every 4 weeks, cross-reactivity with TRAIL, another TNF ligand member and the development of neutralising antibodies this strategy was not further pursued. Instead, denosumab, a fully human MAB against RANKL was developed. Unlike OPG-Fc, denosumab does not cross-react with any other TNF ligands and has not been associated with the appearance of neutralising antibodies (24).

**Preclinical data**

The importance of the RANK/RANKL/OPG system in the pathogenesis of metabolic bone diseases has been demonstrated in various animal models of human disease (Table 1). The pivotal role of OPG in bone metabolism was established in studies with OPG-deficient mice. These mice suffered from osteoporosis and spontaneous fractures along with vascular calcification (25). The skeletal, but not the vascular phenotype could be rescued by concurrent overexpression of the OPG transgene from mid-gestation (26). By contrast, OPG-transgenic mice displayed osteopetrosis with an increased bone mass and bone density resulting from absent functional osteoclasts (27). These data underline the critical role of OPG and RANKL in bone remodelling (28).

Various animal models of human diseases display alterations of the RANKL/OPG homoeostasis (Table 1). Thus, a murine model of rheumatoid arthritis is associated with an increased RANKL–OPG ratio that is caused by a T cell-mediated release of RANKL (29). Similarly, androgen ablation in rats caused an increase of RANKL expression within the bone marrow (30). Myeloma, breast and prostate cancer cells, express RANKL or up-regulate local RANK production, and can provoke bone destruction through local activation of osteoclasts. In a mouse model of bone metastases due to breast cancer, RANKL expression was higher compared with healthy bone and was inhibited by a recombinant OPG-Fc protein (31), as was the case for bone metastases due to colon cancer (32).

**Clinical data**

**Postmenopausal osteoporosis**

The phase 1 trial to evaluate the antiresorptive activity and safety of denosumab was a randomised, double-blind, placebo-controlled, dose escalation study in 49 postmenopausal women (Table 2). In this study, urinary
N-telopeptide, a bone resorption marker was decreased by 81% with denosumab (3 mg/kg) compared with a 10% decrease in the placebo group (24). In the subsequent phase 2 study on 412 postmenopausal women with low bone mass, denosumab given at a dose of 60 mg every 6 months over 1 year increased the lumbar spine bone mineral density (BMD) by 6.7% compared with a loss of 0.6% in the placebo group (33). These effects were sustained in a 12 months extension of this trial (34). Continuation of the trial for another 24 months using a number of different treatment regimens, including discontinuation and restarting therapy, showed that long-term treatment with denosumab (for up to 48 months) increased BMD at the lumbar spine and at the total hip of 11.8 and 6.1% respectively. Of note, discontinuation of denosumab resulted in a decrease of BMD by 6.6% at the lumbar spine within 12 months. When restarted, BMD increased again by 9% from original baseline values, showing that effects of denosumab are reversible when treatment is discontinued, but that the bone remains susceptible to denosumab when treatment is resumed. Bone turnover markers increased to levels above baseline at 6 months after discontinuation of denosumab. In contrast, discontinuation of alendronate treatment resulted in only a modest decrease of lumbar spine BMD, and bone resorption markers remained below baseline through month 48 (35). In a 24 months extension of this trial, BMD continued to increase in patients on denosumab (36). Also, patients that had been previously allocated to a different treatment also showed increases of BMD at all measured sites demonstrating sustained responsiveness to denosumab.

The phase 3 study to establish the efficacy of denosumab in the treatment of postmenopausal osteoporosis was the FREEDOM trial in which 7868 women received either biannual s.c. injections of 60 mg denosumab or placebo for 3 years. In the treatment group, the incidence of radiomorphometric vertebral fractures, clinical hip and clinical non-hip, non-vertebral fractures was decreased by 68, 40 and 20% respectively (37). Importantly, denosumab reduced vertebral fracture risk independently of kidney function, even in women with a GFR of 15–29 ml/min without causing more adverse events (38). It should be noted that bone biopsy data from patients with advanced renal insufficiency receiving denosumab have not been published. In patients with pre-existing adynamic bone disease, treatment with a highly potent antiresorptive such as denosumab may in fact be harmful.

In a head-to-head phase trial comparing denosumab to alendronate in 1189 postmenopausal women over

### Table 1 Animal and clinical studies that have highlighted the role or therapeutic potential of OPG and receptor activator of NF-κB ligand inhibition.

<table>
<thead>
<tr>
<th>Model/disease</th>
<th>Animal study</th>
<th>Human study</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPG deficiency</td>
<td>OPG−/− mice (25)</td>
<td>Juvenile Paget’s disease (54)</td>
</tr>
<tr>
<td>OPG overabundance</td>
<td>OPG-transgenic mice (27)</td>
<td>No equivalent</td>
</tr>
<tr>
<td>Oestrogen deficiency</td>
<td>OVX rats (51)</td>
<td>Postmenopausal women (18)</td>
</tr>
<tr>
<td>Androgen ablation</td>
<td>ORX rats (30)</td>
<td>Androgen ablation for prostate cancer (41)</td>
</tr>
<tr>
<td>GIO</td>
<td>Prednisolone-treated mice (52)</td>
<td>Not performed</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Arthritis model in rat (29)</td>
<td>Rheumatoid arthritis (55)</td>
</tr>
<tr>
<td>Myeloma</td>
<td>Murine model of human myeloma (53)</td>
<td>Osteolytic lesions from multiple myeloma (44)</td>
</tr>
<tr>
<td>Bone metastases</td>
<td>Murine model of bone metastases (32)</td>
<td>Bone metastases from breast cancer (42)</td>
</tr>
</tbody>
</table>

GIO, glucocorticoid-induced osteoporosis; OPG, osteoprotegerin; ORX, orchietomy; OVX, ovarietomy.

### Table 2 Denosumab studies.

<table>
<thead>
<tr>
<th>Name</th>
<th>Phase</th>
<th>n</th>
<th>BTM</th>
<th>BMD</th>
<th>Fx/SRE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-dose placebo-controlled in postmenopausal women</td>
<td>1</td>
<td>49</td>
<td>↓</td>
<td>NA</td>
<td>NA</td>
<td>(24)</td>
</tr>
<tr>
<td>Efficacy and safety in postmenopausal women with low BMD</td>
<td>2</td>
<td>412</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>(33)</td>
</tr>
<tr>
<td>Treatment of postmenopausal osteoporosis (FREEDOM)</td>
<td>3</td>
<td>7868</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>(37)</td>
</tr>
<tr>
<td>Prevention of postmenopausal osteoporosis</td>
<td>3</td>
<td>332</td>
<td>↓</td>
<td>↑</td>
<td>NA</td>
<td>(35)</td>
</tr>
<tr>
<td>Comparison with alendronate in postmenopausal women with low BMD (DECIDE)</td>
<td>3</td>
<td>1189</td>
<td>↓</td>
<td>↑</td>
<td>NA</td>
<td>(39)</td>
</tr>
<tr>
<td>Treatment of bone loss in men on androgen deprivation treatment for non-metastatic prostate cancer (HALT)</td>
<td>3</td>
<td>1468</td>
<td>NA</td>
<td>↑</td>
<td>↓</td>
<td>(41)</td>
</tr>
<tr>
<td>Treatment of bone loss in women on aromatase inhibitors for non-metastatic breast cancer</td>
<td>3</td>
<td>252</td>
<td>NA</td>
<td>↑</td>
<td>NA</td>
<td>(40)</td>
</tr>
<tr>
<td>Denosumab versus zoledronic acid for the treatment of bone metastases in advanced breast cancer</td>
<td>3</td>
<td>2046</td>
<td>NA</td>
<td>↑</td>
<td>↓</td>
<td>(42)</td>
</tr>
<tr>
<td>Denosumab versus zoledronic acid for the treatment of bone metastases in castration-resistant prostate cancer</td>
<td>3</td>
<td>1904</td>
<td>↓</td>
<td>NA</td>
<td>↓</td>
<td>(43)</td>
</tr>
</tbody>
</table>

BMD, bone mineral density; BTM, bone turnover marker; Fx, fracture; NA, not available; SRE, skeletal-related event.
12 months (DECIDE), denosumab was significantly more effective in increasing BMD than alendronate at the total hip (3.5 vs 2.6%) and at all other measured skeletal sites (femoral neck, trochanter, lumbar spine and distal radius) (39).

Treatment-induced bone loss

Androgen deprivation therapy and aromatase inhibition represent established adjuvant therapies for hormone-sensitive prostate and breast cancer, but are associated with rapid bone loss and increased fragility fractures. The beneficial effect of denosumab in these conditions was demonstrated in two phase 3 trials. Biannual treatment of 252 women with non-metastatic breast cancer receiving aromatase inhibitors with 60 mg denosumab over 12 months showed a 5.5% increase of BMD at the lumbar spine compared with placebo (40).

In a randomised controlled phase 3 trial (HALT study), 1468 men with non-metastatic prostate cancer on androgen deprivation therapy received either denosumab (60 mg every 6 months) or placebo. After 36 months, BMD had increased in the denosumab group by 5.6% compared with a loss of 1.0% with placebo. Importantly, the rate of new vertebral fractures was reduced by 62% (3.9% in placebo versus 1.5% in denosumab) (41).

Bone metastases due to breast and prostate cancer

An extensive study programme has evaluated the efficacy of denosumab in malignant bone diseases (Table 2). Two phase 3 studies in patients with bone metastases secondary to breast or prostate cancer confirmed the potential of denosumab in preventing skeletal-related events (SREs). Both studies were designed as non-inferior studies comparing the efficacy of denosumab against the standard treatment with zoledronic acid. In patients with metastasised breast cancer, monthly treatment with 120 mg denosumab was superior to treatment with 4 mg zoledronic acid per month in delaying the time to the first SRE by 18%, the time to first and subsequent SRE by 23%, and a greater reduction in bone turnover markers (at week 13, uNTX/Cr −80% in DMAB versus −68% in ZOL). However, disease-free survival and overall survival were not significantly different (42). To determine whether denosumab may prevent the occurrence of bone and other metastases, a study is currently conducted in women with early high-risk breast cancer (NCT010777154).

In men with castration-resistant prostate cancer, the median time to first study SRE was 20.7 months in the group receiving 120 mg denosumab every 4 weeks compared with 17.1 months for the group treated with monthly infusions of 4 mg zoledronic acid (43).

Myeloma bone disease and others

The most comprehensive data on denosumab for the treatment of multiple myeloma comes from a randomised, double-blind study in which denosumab was compared with zoledronic acid in patients with confirmed bone metastases from solid tumours or multiple myeloma. Of note, patients with bone lesions secondary to breast or prostate cancer were not included in this study. Of the 1776 patients enrolled in this trial, 180 suffered from multiple myeloma. With monthly injections of 120 mg denosumab, the median time to first on study SRE was 21 months, compared with 16 months in patients receiving monthly injections of zoledronic acid, resulting in a non-inferiority of denosumab compared with zoledronic acid. However, denosumab lacked superiority, after adjustment for multiple comparisons (44) and has not received approval for treatment of myeloma bone disease. In light of the clinical hypocalcaemic effects of denosumab (39, 42, 43) and evidence from preclinical animal models of RANKL blockade (45–48), hypercalcaemia associated with malignancies might be another indication for denosumab, although this required formal testing in clinical trials.

Adverse effects and safety considerations

Denosumab in osteoporosis

The recommended and approved dose of denosumab in the osteoporosis setting is 60 mg every 6 months. The parenteral mode of administration avoids gastrointestinal side effects, a common disadvantage of oral bisphosphonates. The s.c. injection is generally well tolerated. The FREEDOM study indicated a higher incidence of eczema (3.0 vs 1.7%) and cellulitis (0.3 vs <0.1%) in patients on denosumab compared with placebo requiring hospitalisation (37). However, overall incidence of cellulitis (1.2% with denosumab versus 0.9% with placebo) was comparable, not related to the injection site, and no unusual pathogens or opportunistic infections were noted. In men receiving androgen ablation therapy for non-metastatic prostate cancer (HALT study), more patients (4.7%) developed cataracts after 3 years in the denosumab group versus 1.2% in the placebo group (41).

Mild and asymptomatic hypocalcaemia has been reported in 0.1% (vs 0% in the placebo group) in the HALT study (41) and in 0% (vs 0.1% in the placebo group) in the DECIDE study (39). Patients with impaired renal function are particularly prone to hypocalcaemia after denosumab administration. Hypocalcaemia is commonly followed by an increase in PTH levels that may persist for several months (24) and is generally more pronounced compared with that observed after use of intravenous bisphosphonates. Clinically, denosumab should be administered after adequate vitamin D and
calcium repletion. In the osteoporosis setting with a denosumab dosing of 60 mg every 6 months, the risk of osteonecrosis of the jaw appears very low and was not observed during the initial evaluation of the FREEDOM and HALT study. In the open label extension of the FREEDOM trial, two cases of osteonecrosis of the jaw were reported in patients who were transitioned from placebo to denosumab that healed without complication.

In addition, no increased incidence of atypical fractures or delayed fracture healing was reported in these large studies (37, 41).

**Denosumab in malignant conditions**

The recommended and approved dose of denosumab in the setting of bone metastases is 120 mg every 4 weeks. The most commonly used therapy under these circumstances currently includes parenteral bisphosphonates, such as pamidronate and zoledronic acid every 4 weeks. Thus, the cumulative annual denosumab in malignant bone disorders is 12 times higher compared with the osteoporosis dose.

In a head-to-head trial between denosumab and zoledronic acid in bone metastases due to breast cancer and prostate cancer, an acute-phase reaction was reported significantly less frequently in the denosumab group (10 and 8%, respectively) than in the zoledronic acid group (27 and 18% respectively). Hypocalcaemia on the other hand, occurred almost twice as often in patients receiving denosumab than in those receiving zoledronic acid for metastatic bone disease secondary to breast and prostate cancer (5 vs 3% and 13 vs 6% respectively) (42, 43). Thus, regular monitoring of serum calcium is recommended to detect and replete hypocalcaemia (49). In osteoporosis trials, hypocalcaemia due to denosumab did not pose as a relevant problem, indicating that this may be related to the higher dose used in the cancer setting.

The incidence of osteonecrosis of the jaw was similar between patients receiving bisphosphonates (1.4%) and denosumab (2.0%) for bone metastases (50). These figures are 100- to 1000-fold higher compared with the osteoporosis setting owing to the higher cumulative dose employed in oncology. The recommendations to minimise the risk of osteonecrosis of the jaw in a patient with cancer are similar for intravenous bisphosphonates and denosumab. Patients with additional systemic or local risk factors should be regularly seen by a dentist and invasive dental procedures should ideally be performed before initiating denosumab or be conducted under periprocedural antibiotic therapy (49).

**Conclusion and therapeutic role**

Denosumab has emerged as an effective therapy to reduce fractures in postmenopausal osteoporosis and male osteoporosis due to androgen ablation therapy. In addition, it delays SREs in patients with bone metastases due to breast and prostate cancer. The s.c. administration circumvents gastrointestinal discomfort associated with oral bisphosphonates. Denosumab is associated with a lower incidence of acute-phase reactions and can be used in patients with impaired renal function, an advantage over intravenous bisphosphonates in the elderly and patients with cancer. Thus, denosumab is an alternative to bisphosphonates in benign and malignant bone diseases. The safety profile is favorable, and the risk of both hypocalcaemia and osteonecrosis of the jaw can be minimised with simple measures. A unique feature of denosumab relates to its rapid off-effect, because unlike bisphosphonates it is not deposited in the skeleton. Therefore, regular administration during therapy and a long-term strategy after discontinuation of therapy are required.

In postmenopausal osteoporosis, denosumab is suitable as first-line therapy depending on local regulations after repletion with calcium and vitamin D. In addition, patients who do not tolerate oral bisphosphonates or who are incompliant may be offered this drug. In addition, patients requiring effective osteoporosis therapy, but suffer from renal insufficiency (GFR < 30 ml/min) can be treated with denosumab. For men with non-metastatic prostate cancer and osteoporosis due to androgen ablation, denosumab is the only approved therapy. Studies on bone loss associated with aromatase inhibitor therapy are ongoing. However, studies with head-to-head comparison between denosumab and other drugs with fracture endpoints are not available.

In patients with bone metastases due to breast and prostate cancer, denosumab has been approved in 2010 the US and in 2011 in Europe. The subcutaneous administration and its efficacy make it an attractive first-line therapy and reasonable alternative to parenteral bisphosphonates, in particular in patients with advanced renal insufficiency. However, the risk of hypocalcaemia needs to be minimised and the rare occurrence of osteonecrosis of the jaw needs to be considered.

Taken together, denosumab has become a novel therapeutic option for the treatment of benign and malignant bone diseases that combines efficacy with a high degree of safety and comfortable administration.

**Declaration of interest**

E Tsourdi, T D Rachner, M Rauner and C Hamann have nothing to declare. L C Hofbauer has received honoraria from Amgen, Merck, Novartis and Nycomed.

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