CLINICAL INVESTIGATION

Denosumab in the treatment of bone metastases

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Metastatic bone disease has a major impact on both the morbidity and mortality of patients. Antiresorptive bisphosphonates have revolutionized treatment and outcomes for patients with bone metastases, but pain and other skeletal complications still occur, adversely affecting quality of life and survival. Recent understanding of the bone microenvironment has highlighted the importance of RANK, its ligand (RANKL) and the decoy receptor OPG in the vicious cycle of bone resorption and destruction. Exploiting this triad led to the development of denosumab, a fully-human monoclonal antibody to RANKL. The success of this bone-directed agent in early clinical trials and favorable safety and tolerability profile led to the conduct of large multi-center randomized trials in breast, prostate, myeloma and other advanced cancers. In this review, we discuss the development of denosumab, the data that led to its licensing for patients with bone metastases and the future for bone-directed therapies.

Keywords: bisphosphonate • bone-directed therapy • bone metastases • denosumab • OPG • RANK • RANKL.

Metastatic bone disease

Many patients with advanced cancer develop skeletal involvement in the course of their disease. Untreated, bone metastases can result in a substantial burden of bone pain and other skeletal complications, which have a major impact on quality of life [1] and possibly also survival [2]. Up to 70% of patients with advanced prostate or breast cancer will develop bone metastases and the figure approaches 100% in myeloma. However, survival in patients with bone metastases is often longer than in patients with metastases at other sites and may be measured in years. For example, in a population of women with bone metastases from breast cancer, median survival was approximately 2–3 years [3] and, indeed, in patients with bone only metastases, approximately 20% of patients survive for 5 years or more [4]. Great efforts have therefore been made to prevent, minimize and treat complications related to osseous metastases, termed skeletal-related events (SREs): fracture, spinal cord compression, hypercalcemia and surgery or radiotherapy to bone for bone pain. Without bone-directed therapy, a patient with breast cancer and bone metastases can experience up to an average of 4 SREs per year [5].

The occurrence of SREs is primarily due to the increased bone resorption that occurs as metastatic tumors develop. Although bone pain is commonly treated on a multi-disciplinary basis, which may involve analgesic medications, chemotherapy, endocrine therapy, external beam radiotherapy and surgery, pain can be refractory to these measures and it is now accepted that drug-based therapies that reduce bone resorption (antiresorptive drugs) are a crucial component of the management of such patients. Several biomarkers that are readily measured in urine or serum have been developed to 'report' on the bone resorption status of patients. Examples are urinary (u) *N*-telopeptide of type I collagen (NTX) and

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C-telopeptide of type I collagen, which are formed during the degradation of type I collagen during normal bone turnover, but which may be substantially elevated in metastatic bone disease under the influence of the vicious cycle. Another bone resorption biomarker, TRAP-5b is sometimes used as a more direct measure of osteoclast activity and is unaffected by renal dysfunction. Such biomarkers have found increasing use in the prediction of risk of SREs and in monitoring the effectiveness of anti-bone resorptive drugs in clinical trials.

Bisphosphonates

Bisphosphonates are a class of bone antiresorptive drugs that have revolutionised the management of bone metastases and have become established in routine clinical practice, based on extensive clinical studies in a variety of cancer types. Nitrogen-containing bisphosphonates such as zoledronic acid and ibandronate are significantly more potent bone resorption inhibitors than the earlier non-nitrogen agents such as clodronate [6]. In breast cancer, for example, oral ibandronate significantly reduced the risk of SRE compared with placebo (Hazard ratio [HR] 0.62; 95% CI: = 0.48, 0.79, p = 0.0001) while zoledronic acid has shown superiority over the less potent pamidronate (4 mg zoledronic acid vs 90 mg pamidronate; time to first SRE 310 days vs 174 days; p = 0.0134) [7]. In hormone-refractory prostate cancer, zoledronic acid 4 mg reduced SREs by 11% (p = 0.021) and median time to first SRE (p = 0.011) compared with placebo [8]. Intravenous (iv.) zoledronic acid 4 mg significantly increased time to first SRE (230 vs 163 days; p = 0.023) compared with placebo in a group of mixed solid tumors [9,10].

Despite the benefits of bisphosphonates, SREs still occur with consequential impact on morbidity and mortality. Therefore, research has been directed at the development of alternative agents that can further improve the reduction of SREs. Denosumab is a new bone-modifying drug that has shown much promise in this setting.

Development of denosumab

The vicious cycle

Healthy bone is not an inert organ, but is constantly being remodeled within a dynamic, but tightly controlled microenvironment containing osteoblasts (responsible for bone formation), osteoclasts (responsible for bone resorption) and other cells such as osteocytes, along with mineralized bone matrix. Metastatic tumor growth within this environment causes disruption of the balanced remodeling, resulting in increased bone resorption [11,12]. Tumor cells secrete a great variety of proteins that interact with the local cells and pathways, increasing resorption, which releases further growth factors into the system. This is turn feeds tumor growth in the so-called 'vicious cycle' of resorption and bone destruction [13]. An understanding of these interactions is crucial to the development of bone-directed therapies with several emerging treatments exploiting the array of potential targets [14].

RANK/RANKL/OPG triad

The communications between osteoclasts and osteoblasts have been studied extensively, identifying the RANK/RANKL/OPG triad as having a principal role. RANKL, expressed by bone marrow stromal cells, activated T cells and osteoblasts, is essential for promoting osteoclastogenesis, committing a precursor to the osteoclast phenotype [15]. Its receptor, RANK, a member of the TNF receptor superfamily, is found on the surface of osteoclast precursors, chondrocytes and mature osteoclasts [16]. The binding of RANKL to RANK induces osteoclast differentiation, fusion and formation of mature osteoclasts, increases their activity and blocks apoptosis. OPG, also a member of the TNF family, is the decoy receptor for RANKL, blocking the RANKL–RANK interactions and the aforementioned processes [17]. The RANK/RANKL/OPG pathway is an integral component of bone turnover, regulated by several cytokines and chemokines secreted within the bone microenvironment. Therefore, targeting the RANK–RANKL interaction as the principal driver of bone turnover became an attractive opportunity.

Preclinical evidence for targeting RANK and its ligand

Early work *in vitro* using hematopoietic bone marrow precursors co-cultured with T cells confirmed the ability of RANKL to induce osteoclastogenesis, which could be blocked in the presence of OPG [18]. Further studies *in vivo* went on to show how inhibition of OPG manifests across many tumor sites (predominately prostate, myeloma and breast cancer cell lines). Blockade of OPG using soluble recombinant OPG (ligand-binding domain of human OPG fused to the Fc domain of human IgG; rOPG-Fc) inhibited tumor growth in bone [19,20], inhibited osteoclastogenesis [19,21–24], increased bone mineral density (BMD) [23,24] and increased tibial cancerous bone [23,24]. Several studies showed this to be a dose-dependent effect. Additionally, it has been shown that administration of rOPG-Fc can improve survival [20,25]. There was also some early evidence that OPG may be superior to zoledronic acid in terms of its effects on osteoclastogensis [26]. Alternative agents of inhibition were investigated by means of soluble murine RANK-Fc (sRANK-Fc) in

a prostate cancer model [27]. sRANK-Fc diminished osteoblastic lesions, suppressed bone turnover markers and decreased tumor burden in bone.

The above studies all highlighted the therapeutic potential of OPG blockade and subsequently further investigation in combination with chemotherapy was undertaken [28,29]. These studies suggest that the combination results in increased tumor cell apoptosis and decreased tumor burden in bone compared with either treatment alone.

The first clinical study to incorporate this approach was a Phase I trial of a recombinant OPG molecule [30]. Dose-dependent inhibition of bone resorption was demonstrated but development was halted because of the occasional development of antibodies to synthetic OPG, which had the potential to inhibit endogenous OPG as well.

In an alternative approach, denosumab (AMG162) was developed as a fully humanized synthetic, specific IgG_2 antibody [28]. It binds with a high affinity to RANKL and, critically for clinical development, does not induce a host antibody reaction [31]. Using 'knock-in' methods, exchanging murine RANKL for human RANKL, studies showed that treatment with subcutaneous (sc.) denosumab decreases trabecular osteoclast surfaces, increases bone density and volume, and decreases resorption [28]. In preclinical primate studies, denosumab showed a specific, dose-dependent inhibition of bone resorption and increase in BMD [32]. Owing to the actions of denosumab in potently inhibiting mature osteoclast function as well as osteoclast differentiation, it was anticipated that denosumab would inhibit the interactions between tumor cells and osteoclasts, suppress bone turnover and potentially inhibit the development of malignant bone lesions [33].

Clinical trials with denosumab

Phase I & early Phase II studies

The initial toxicity and dose-limiting effects of denosumab were investigated amongst postmenopausal women and reported effective serum NTX suppression with a single sc. injection [34]. The toxicity profile was very favorable with no serious adverse events. The first study of efficacy and safety in cancer patients (24 myeloma and 29 breast cancer patients with bone metastases), compared denosumab and pamidronate in a randomized, double-blind, double-dummy trial [35]. In the denosumab arm, maximum suppression of bone resorption (70%) from a single dose (1 or 3 mg) was attained after 7 days and maintained for the 84 days of the study. Although a similar suppression was seen with pamidronate, it was not maintained beyond 28 days. The ability of denosumab to suppress uNTX and serum NTX was confirmed, as was its tolerability profile.

These encouraging first-in-human studies gave rise to a randomized Phase II trial in 255 patients with bone metastases from breast cancer [36]. Within these 255 patients, five cohorts were treated with varying doses of denosumab (4 weekly sc. 30, 120 or 180 mg or 12 weekly 60 or 180 mg) blinded to dose and frequency, while one cohort received open-label iv. bisphosphonate four-times weekly weekly (pamidronate, ibandronate or zoledronic acid). Significant reductions in uNTX were reported in 74% patients receiving denosumab compared with 63% receiving bisphosphonate. For first on-study SRE, 9 versus 16% experienced an SRE for denosumab and bisphosphonate respectively. Regarding safety, hypocalcemia was more common and more severe amongst those patients receiving denosumab compared with bisphosphonate. Taking into account the need to maximize bone resorption as measured by uNTX, this study led to the selection of sc. administration of 120 mg denosumab every 4 weeks as the dose for subsequent Phase III studies in patients with metastatic bone disease.

In a population of prostate patients with bone metastases, denosumab significantly suppressed the bone turnover markers uNTX and TRAP5b, regardless of previous bisphosphonate exposure [37,38]. Similar effects on bone turnover markers (in this case serum *C*-telopeptide of type I collagen) were reported in a Phase II study of myeloma patients, again regardless of previous iv. bisphosphonate exposure [39].

Randomized Phase III trials

Three identically designed Phase III trials for treatment of bone metastases from breast cancer, from castration-resistant prostate cancer and from other solid tumors and myeloma have each compared denosumab to zoledronic acid 4 mg iv., using the current standard of treatment, as an active control. Trials of zoledronic acid versus placebo (or, in the case of breast cancer vs pamidronate) in the same settings have already been referred to. Stopeck *et al.* randomized 2046 breast cancer patients with radiologically-confirmed bone metastases to receive either sc. denosumab 120 mg and iv. placebo or iv. zoledronic acid 4 mg and sc. placebo every 4 weeks [3]. Patients were allowed cancer-specific therapies except for iv. bisphosphonates (previous oral bisphosphonates were allowed) and were strongly recommended to be prescribed nutritional supplementation with calcium and vitamin D. The primary end point was to confirm noninferiority of denosumab in time to first on-study SRE; superiority was a secondary end point. Denosumab significantly delayed time to first on-study SRE by 18% compared with zoledronic acid (HR: 0.82; 95% CI: 0.71–0.95; p = 0.001 noninferiority; p = 0.01 superiority). The median to first on-study SRE

was 26.4 months in the zoledronic acid group and was not reached by the denosumab group.

In a similar study conducted in men with castration-resistant prostate cancer and bone metastases (n = 1904), superiority of denosumab over zoledronic acid in time to first on-study SRE was confirmed (median time to first on-study SRE was 20.7 months [95% CI: 18.8–24.9] with denosumab compared with 17.1 months [15.0–19.4] with zoledronic acid [HR: 0.82, 95% CI: 0.71–0.95; p = 0.0002 for noninferiority; p = 0.008 for superiority]) [40]. Both studies also report greater suppression of bone turnover markers with the monoclonal antibody.

A third trial in cancer patients with solid tumors (nonbreast, nonprostate, n = 1596) or myeloma (n = 180), found that denosumab was noninferior to zoledronic acid (HR: 0.84; 95% CI: 0.71–0.98; p = 0.0007), but just failed to meet statistical significance to confirm superiority (p = 0.06) [41].

Because the three studies had identical design, it was possible to perform a preplanned integrated analysis, including the evaluation of safety and efficacy. Overall, denosumab was superior to zoledronic acid in reducing risk of a first SRE by 17% (HR: 0.83; 95% CI: 0.76–0.90; p < 0.0001), with a median delay of 8.2 months [42].

These large Phase III studies have now led to marketing authorization by the US FDA and European Medicines Agency for denosumab (as XGEVA) for the prevention of SREs in patients with bone metastases from solid tumors (but not myeloma). A further larger trial looking only at the benefits of denosumab in myeloma patients is currently underway.

■ Denosumab in other oncology indications

Although this review is focused on metastatic bone disease, denosumab is also being developed for other areas of oncological bone disease. In a study of 252 women with early breast cancer, receiving aromatase inhibitor therapy, denosumab (60 mg sc., every 6 months) produced significant increases in BMD compared with placebo [43]. Denosumab is currently being further evaluated in this setting in approximately 3400 postmenopausal women in the ongoing ABCSG-18 (NCT00556374). This study will yield information on the effects of denosumab treatment on fracture rate, disease recurrence rates and long-term safety. Similarly, in men with prostate cancer receiving androgen deprivation therapy, which is known to produce a rapid fall in BMD and increase in fracture rate, denosumab, 60 mg sc. every 6 months, reduced the incidence of new vertebral fractures (1.5 vs 3.9%; RR: 0.38; 95% CI: 0.19–0.78; p = 0.006) and induced an increase in BMD compared with placebo [44]. Denosumab (Prolia) has now been approved by the FDA for cancer treatment-induced bone loss.

Denosumab (120 mg, sc. every 4 weeks) has also been investigated in early cancer trials for the prevention of development of metastases. In a study of 1432 men with nonmetastatic prostate cancer, but at high risk of developing bone metastases, denosumab significantly increased the bone-metastasis-free survival by a median of 4.2 months compared with placebo (HR: 0.85; 95% CI: 0.73–0.98; p = 0.028) [45]. Although there are not yet any corresponding data for prevention of metastases by denosumab in breast cancer, a large placebo-controlled study is currently underway (D-CARE – EUDRACT 2009-011299-32). A summary of key clinical studies with denosumab is included in Table 1.

Safety profile

In recent years, osteonecrosis of the jaw (ONJ) has emerged as the most significant adverse event associated with bisphosphonate therapy. The Phase III studies of denosumab compared with zoledronic acid offered a key opportunity to better define the incidence of ONJ and to prospectively study this using rigorously defined procedures to compare denosumab with zoledronic acid. All three studies reported cases of ONJ with a total over 3 years of 52 in the denosumab arms (1.8%) and 37 (1.3%) in the zoledronic acid arms, but the difference was not significant (p = 0.13) [46,47]. These data were reassuring, since some literature data from smaller, less well-controlled studies had reported higher rates for bisphosphonates. Also, most cases were relatively mild and >95% were able to be treated conservatively, that is, without invasive surgery.

Owing to renal toxicity, zoledronic acid requires monitoring for renal function before each treatment. In this respect, denosumab offers a distinct advantage, since it is not associated with renal toxicity and no such monitoring is necessary. Also, in the Phase III studies, denosumab exhibited fewer acute phase reactions in the first three days of drug initiation (8.7% of patients) than zoledronic acid (20% of patients). Whilst hypocalcemia was more frequent in the denosumab arm than the zoledronic acid arm (9.6 vs 5.0%), in most cases this was mild or asymptomatic, easily managed and caused no deaths.

Future perspective

The field of research into bone-directed therapies is particularly dynamic, especially in breast and prostate cancer, with the bisphosphonates well-established and denosumab now entering routine clinical practice for the treatment of bone metastases. While the bone-metastasis prevention trials investigating bisphosphonates have recently reported interesting

Table 1. Summary of denosumab clinical trials in cancer patients.

Study	Study design	Cancer site	Enrolled patients (n)	Primary end point result	Refs
Yonemori *et al.* 2008	Phase I open-label, dose-ascending	Breast	19	Incidence of AE: one treatment-related SAEs (G4 myositis). Common AEs were fatigue, anorexia, headache, malaise and nausea	[48]
Body *et al.* 2006	Randomized double-blind, double-dummy, active-controlled (pamidronate)	Breast Myeloma	54 (29 breast, 25 myeloma)	Safety: no drug-related SAEs. 20–25% reported fatigue in the breast cancer stratum and 20% reported asthenia in the multiple myeloma stratum. Efficacy: confirmed suppression of u and sNTX	[35]
Lipton *et al.* 2007	Phase II randomized, active-controlled multi-dose (iv. BP)	Breast	255	Median % change from baseline to week 13 of uNTX: 71% denosumab arms versus 79% iv. BP arm	[36]
Vij *et al.* 2009	Phase II open-label, single-arm	Myeloma	96 (53 relapse, 43 plateau-phase)	Suppression of serum M-protein levels: no CR, PR, MR Suppression of serum CTX: relapsed patients median 69.5% at cycle 4; plateau patients median 46.5% at cycle 4	[39]
Fizazi *et al.* 2009	Phase II randomized open-label (iv. BP)	Advanced carcinoma or myeloma	111	Proportion of patients with uNTX <50 at week 13: 71% denosumab arm versus 29% BP ($p < 0.001$)	[37]
Ellis *et al.* 2008	Phase III randomized double-blind, placebo-controlled	Early breast, receiving AI with low BMD	252	% Change from baseline LS BMD at 12 months: +4.8% denosumab arm versus -0.7% placebo arm; $p < 0.0001$)	[43]
Smith *et al.* 2009	Phase III randomized double-blind, placebo-controlled	Non-metastatic prostate cancer on androgen-deprivation therapy	1468	% Change from baseline LS BMD at 12 months: +5.6% denosumab arm versus -1.0% placebo arm; $p < 0.001$)	[44]
Stopeck *et al.* 2010	Phase III randomized, double-blind, double-dummy (zoledronic acid)	Bone metastatic breast cancer	2046	Time to first on-study SRE (noninferiority): HR: 0.82; 95% CI: 0.71–0.95; $p < 0.001$)	[3]
Fizazi *et al.* 2011	Phase III randomized, double-blind, double-dummy (zoledronic acid)	Bone metastatic castration-resistant prostate cancer	1904	Time to first on-study SRE (noninferiority): HR: 0.82; 95% CI: 0.71–0.95; $p = 0.0002$)	[40]
Henry *et al.* 2011	Phase III randomized, double-blind, double-dummy (zoledronic acid)	Non-breast, nonprostate bone metastatic carcinoma or myeloma	1776	Time to first on-study SRE (noninferiority): HR: 0.84; 95% CI: 0.71–0.98; $p = 0.0007$)	[41]
Smith *et al.* 2011	Phase III randomized double-blind versus placebo	Prevention of SREs in nonmetastatic prostate cancer	1432	Bone metastasis-free survival increased by 4.2 months versus placebo	[45]

AE: Adverse event; AI: Aromatase inhibitor; BP: Bisphosphonate; BMD: Bone mass density; CR: Complete response; CTX: C-telopeptide of type I collagen; G: Grade; HR: Hazard ratio; iv.: Intravenous; LS: Lumbar spine; MR: Minimal response; PR: Partial response; SAE: Serious adverse event; sNTX: Serum N-telopeptide of type I collagen; SRE: Skeletal-related events; uNTX: Urinary N-telopeptide of type I collagen.

Executive summary

Metastatic bone disease

- Bone metastases are common, especially among advanced breast and prostate cancer and myeloma patients. They carry a significant burden of pain and other complications.
- Skeletal-related events (SREs) comprise spinal cord compression, fracture, bone pain, hypercalcemia and radiotherapy or surgery to bone.
- SREs are treated with a multidisciplinary approach that now includes bone antiresorptive drugs.
- Bisphosphonates (BPs) have revolutionised the management of bone metastases.
- Nitrogen-containing BPs (zoledronic acid and ibandronate) are more potent than non-nitrogen containing BPs (e.g., clodronate).
- BPs can significantly reduce the risk of skeletal-related events (SREs) and the time to SRE in many solid tumors and myeloma.

Development of denosumab

- Understanding the bone microenvironment and vicious cycle has been the key to the development of new bone-directed therapies.
- RANKL, expressed by osteoblasts, promotes osteoclastogenesis when bound to its receptor RANK, found on the surface of osteoclasts.
- OPG blocks the RANKL–RANK interaction and osteoclastogenesis.
- *In vivo* studies show that the blockade of OPG inhibits tumor growth in bone and increases bone mineral density.

Early phase studies

- Denosumab (AMG162) was developed as a fully humanized antibody to RANKL that increases bone mineral density and suppresses bone resorption.
- The toxicity profile from early studies was favorable, with no evidence of renal toxicity and decreased acute phase reactions compared with other bone resorptive agents.
- Randomized Phase II studies reported significant reductions in bone turnover markers including *N*-telopeptide of type I collagen, *C*-telopeptide of type I collagen and TRAP5b.

Phase III studies

- Phase III studies in breast cancer, prostate cancer and other advanced malignancy confirmed denosumab's noninferiority to zoledronic acid (primary end point was time to first on-study SRE), and superiority in breast and prostate cancer.
- These studies led to approvals from the US FDA and European Medicines Agency of the use of denosumab for the prevention of SREs in patients with bone metastases from solid tumors.

Bone metastasis prevention

- In prostate cancer, denosumab increased the bone metastasis-free interval by 4.2 months compared with placebo.
- Bone metastasis prevention studies are underway in early breast cancer.

Safety profile

- Osteonecrosis of the jaw incidence was low in both zoledronic acid (1.3%) and denosumab (1.8%) arms in patients participating in the three Phase III bone metastasis trials and there was no statistically significant difference in the two arms.
- Denosumab is not associated with renal toxicity.
- Hypocalcemia occurred in 9.6% of denosumab-treated patients participating in the three Phase III bone metastasis trials. This was usually mild and often asymptomatic.

results, further prevention trials administering denosumab will report in the next few years with eagerly awaited results. Of further interest will be the results of studies of other bone-targeted agents including cathepsin K inhibitors, endothelin-receptor antagonists, SCR and other tyrosine kinase inhibitors and the radiopharmaceutical radium-223. So far they show varying degrees of promise and all must be scrutinized for their clinically-relevant outcomes and safety profiles, but there is undoubtedly an encouraging future in this area.

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