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REVIEW

Denosumab—A Powerful RANKL Inhibitor to Stop Lytic Metastases and Other Bone Loss Actions by Osteoclasts

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Abstract Denosumab is a perfect example on the targeted anticancer therapy. The inhibition of RANKL activity suppressed the osteoclasts' resorptive function and so prevented skeletal related events. This effect is useful not only against bone metastases, but also in the treatment of other diseases caused by bone loss. In different solid tumors with bone metastasis the quality of life also improved, although the overall survival usually showed no change. On the market the main competitors for denosumab are still the bisphosphonates (questions of costs and reimbursement are not discussed) and some potential new agents e.g. Src kinases (as dasatinib, saracatinib, bosutinib), cathepsin K inhibitors, (e.g. odanacatib), and new selective estrogen receptor modulators (e.g. bazedoxifene, lasofoxifene). Nevertheless, today denosumab is one of the most powerful agents in bone-saving area.

Keywords Denosumab · RANKL-inhibitor · Osteoclasts · Metastasis

Several tumors can produce bone metastases, either lytic or plastic, due to the disturbed activity of osteoclasts and osteoblasts. In oncological settings the loss of regulation between these and other cell types responsible for the maintenance of bone integrity is partly due to the production of those factors and cytokines by tumor cells which can increase primarily the bone loss by osteoclasts. Therefore one of the main targets to prevent bone metastases or decrease the related complications is the differentiation function of osteoclasts. Today two approaches are trying to achive clinical improvements inhibiting formation and/or activity of osteoclasts: bisphosphonates (mainly zoledronic acid) and denosumab. Most of the clinical trials compare these two drugs, identifying the best way to optimize the therapy. Needless to say that many other diseases suffer from loosing the regulation of bone turnover (e.g. osteoporosis, rheumatoid arthritis) severly depressing quality of life [1, 2].

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Regulation of Bone Formation and Resorption

Osteoclasts are essential participants of skeletal growth in bone and in mineral homeostasis. The basic processes are the removal of mineralized bone by osteoclastic resorption followed by the formation and mineralization of new bone matrix with osteoblasts [3, 4]. In certain diseases the amount of the bone removed by osteoclasts exceeds bone formation resulting in decreased skeletal integrity and risk of pathological fractures. (Similar problem is created in postmenopausal osteoporosis.)

The normal bone remodeling is influenced by several factors. In the activation of osteoclast the RANKL/RANK pathway is very important. RANKL (receptor activator of nuclear factor-κB ligand) is a member of TNF (tumor necrosis family) superfamily. It is expressed primarily on osteoblasts, released into the microenvironment and by binding to transmembrane RANK receptor, activates immature osteoclasts. The balance is maintained by osteoprotegerin (OPG), a decoy receptor of RANKL, inhibiting its activity. Therefore the RANKL/OPG ratio is an important regulator to maintain bone equilibrium between bone loss (e.g. in osteoporosis) and gain (e.g. in osteopetrosis).

Osteoclast differentiation is induced by two cytokines: macrophage colony-stimulating factor (M-CSF) and RANKL. M-CSF first activates the survival and proliferation of monocyte-macrophage lineage and the expression of RANK. The differentiation from osteoclast precursors to multinucleated osteoclasts is induced by RANKL, which requires the M-CSF dependent expression of RANK at the cell surface of the precursors. M-CSF and RANKL are produced by bone marrow stromal cells and osteoblasts. RANK is also secreted by T-cells and at lower level by B-cells. Most of the cells producing RANKL, also secrete OPG, which can inactivate RANKL extracellularly. All of these molecules act paracrine manner therefore regulating bone resorption locally. However, there is a link between the local factors and endocrine regulation by several calcium-regulating hormones, e.g. sex hormones, parathyroid hormone, and vitamin D3 [5, 6]. It is also accepted that osteoclasts can release several osteoblast stimulating cytokines and growth factors from the matrix, as insulin-like growth factor I and II, TGFβ, or bone morphogenic proteins (BMP). These matrix originated factors can support/stimulate the proliferation of tumor cells, partly by niche formation producing a friendly microenvironment for the arriving tumor cells.

Osteclast-osteoblast coupling and communication is also very important in the regulation of bone remodeling. Several factors have been mentioned as part of this interaction, e.g. Wnt 10a and sphingosine-1-phosphate (SIP) derived from the osteoclasts with the capacity to activate bone formation, or ephrin B4 using ephrin B2 receptor [7, 8].

The RANKL/OPG ratio can be perturbed in cancer patients [9]. The consequences are manyfold. The local balance of these factors can influence bone metastatic lesions:to be lytic or blastic. The change in the balance is partly due to the factors produced by the tumor cells and bone cells in the bone microenvironment interacting with the hemopoietic stem-cell niche, and partly as a result of the anticancer therapy. Moreover, there are data that RANKL/RANK pathway could be involved in mammary carcinogenesis. Some experiments revealed that bone marrow derived RANKL increased the bone tropism in RANK expressing tumor cells. In animal models RANKL-inhibition decreased the invasion and metastasis formation of osteosarcoma cells [10, 11].

Drugs Against Bone Resorption—Inhibition of Osteoclasts

It is obvious that inhibition of osteoclasts may have therapeutic advantage. The currently used main strategy covers bisphosphonates, but a new wave is represented by denosumab inhibiting RANKL.

Bisphosphonates

Bisphosphonates (BPs)—analogs of pyrophosphate, a normal component of bone matrix - have the ability to bind strongly to bone mineral and also to inhibit mature osteoclasts. Once taken up by osteoclasts the non-nitrogen-containing BPs are metabolized in the cytosol to ATP analogues inhibiting cell functions and inducing apoptosis. The nitrogen-containing BPs, however, inhibit farnesyl isophosphate (FPP) synthase, a member in the mevalonate pathway, which prevent the prenylation of small GTP-binding proteins, that are essential for osteoclast survival and function [12].

Denosumab

Different attempts were made to inhibit RANKL using Fc fusion proteins. With Fc-OPG and OPG-Fc, OPG was fused to the Fc portion of human immunoglobulin G1. Later, extracellular domain of RANK was fused to the Fc part of IgG1. Last, a fully human monoclonal antibody, denosumab (AMG162—Xgeva®, Amgen) was introduced with binding capacity to RANKL—serving as an exogenous OPG - and as a result RANKL is unable to bind to RANK. The full consequences are the block in development, activation and survival of osteoclasts.[13]

Comparisons

A major difference between denosumab and BPs on osteoclasts is that BPs should be taken up by the cells, while denosumab act in the extracellular space. RANKL inhibition prevents the differentiation of monocyte-macrophage lineage into multinucleated osteoclasts, whereas long-term treatment with BPs leads to the accumulation of osteoclasts, including those multinucleated cells which detached from the surface and finally are eliminated by apoptosis [14].

The distribution of denosumab and BPs could be different in the bone. Denosumab as an antibody is expected to be present throughout the extracellular area without sustained binding to the bony surface. In healthy postmenopausal women the pharmacokinetic response of denosumab was non-linear and dose-dependent, with a half-life of approximately 26 days. Denosumab is cleared by the reticuloendothelial system, independently from the renal clearance. The pharmacodynamics showed that 1 mg/kg single s.c. dose (clinically relevant) resulted a rapid decrease in the urinary N-telopeptide/creatinine [15]. In contrast, some of the BPs adsorbed to the mineral surface will be trapped and buried within the original site of resorption by newly synthetized bone. Using different BPs in long-term clinical studies a new steady state in bone turnover was achieved up to 6 months and the decreased level of the resorption remained

constant as long as treatment continued. It means that BPs buried in bone does not effect resorption for at least as it remains buried there.

The pharmacokinetic half-lives for the elimination of BPs that are retained in the bone are thus not equivalent to the half-lives of their biochemical effects. The elimination of BPs from bone greatly depends on remodeling and resorption. After release from the skeleton, mainly as a result of resorptive actions, BPs excreted via the kidney. Small amount of BPs can be measured over many months or even years after stopping BPs treatment [16, 17]. It means that BPs must be present in the circulation and available for recycling to bone mineral surface which explains the long duration of action of zoledronate acid (Zometa®, Novartis), in particular, and also explains why the effect of BPs are less rapidly reversible after stopping treatment than those of denosumab.

Clinical Activity of Anti-Osteoclast Agents—An Emphasis on Denosumab

In the past decades bisphophonates have been the central drugs for both benign and malignant bone diseases. The increased knowledge on the regulation of bone metabolism, especially the importance of RANKL/RANK/OPG interaction stimulated the use of certain inhibitory agents in order to decrease bone loss and pain in oncological settings. The first study to decrease the RANKL activity on osteoclast function used a recombinant OPG (AMGN-0007) without clinical success [18]. One of the problems was the host antibody production against the drug. That important side effect was prevented by another antibody, a fully human, synthetic IgG that binds to RANKL with high affinity inhibiting its interaction with RANK. This drug is denosumab.

In preclinical models denosumab dose-dependently decreased bone resorption and increased bone mineral density (BMD). In 2004 a single dose of denosumab (3 mg/kg) in healthy postmenopausal women effectively decreased the urinary NTX (N-telopeptide of type I collagen, a marker of bone resorption) level for a long pertiod (6 months) without serious side effects (with an exception of a transient increase of parathyreoid hormone level).

In a double-blind clinical trial with 54 cancerous patients (breast cancer and myeloma) denosumab (s.c.) and pamidronate (i.v.) were compared. Both drugs caused a suppression in urinary NTX level, but the action of denosumab lasted much longer [19]. In a further study with a wide range of different doses and intervals in patients with breast cancer and bone metastases, the most reliable schedule - showing the best balance of efficacy and tolerability and suggested for further trials - was 120 mg s.c. every four weeks [20].

Treatment Induced Bone Loss

In postmenopausal women with early-stage breast cancer reciving aromatase inhibitor were given 60 mg denosumab s.c. twice yearly (HALT-BC study; this dosage was used in postmenopausal osteoporosis). The BMD significantly improved in lumbar spine and also in forearm in denosumab-treated compared to placebo-treated patients [21]. A further trial (ABCSG-18) currently study the fracture rate in about 3.500 postmenopausal patients treated with adjuvant aromatase inhibitor. Retrospective analyses revealed that the fracture risk increased during ADT (androgen-deprivation therapy) in prostate cancer patients [22, 23]. In a placebo-contolled trial 1.468 men with non-metastatic prostate cancer reciving ADT were treated with denosumab (36 months) and the incidence of new vertebral fractures decreased significantly (1.5 % in denosumab-treated and 3.9 % in placebo-treated group). Besides, fractures at any site decreased also (5.2 % vs 7.2 %) but not significantly [24]. The BMD improved substantially. Denosumab was well tolerated, although an increase in cataracts requires further study to evaluate the safety of denosumab in this respect.

Prevention of Metastasis

Certain disease-free survival benefit of adjuvant bisphosphonates in early stage breast cancer were reported in some clinical trials, but not in all. To date there are no data in this patients group concerning the effect of denosumab. The potential antimetastatic action will be evaluated in a large, international, placebo-controlled study (D-CARE, NCT01077154), at a dose of 120 mg denosumab once a months for 6 months and every 3 months thereafter.

Although prostate cancer has a tendency to metastatize into the bones, the informations on the antimetastatic action of BPs are very limited, due to the underpowered studies. Recently, 1.432 men with nonmetastatic, castration-resistant prostate cancer, with high risk of bone metastasis, were randomized to receive either denosumab (120 mg monthly, s.c.) or placebo. The primary endpoint was the bone-metastasis-free survival. Denosumab significantly increased the survival by a median of 4.2 months compared to placebo. This advantage, however, did not resulted into improvements in overall survival. Denosumab was well tolerated in general, but episodes of hypercalcemia and osteonecrosis of jaw occurred [25].

In a randomized trial (phase III) in a subset of 702 NSCLC (non-small cell lung cancer) patients with bone metastasis the overall median survival was 1.4 months longer in denosumab compared with zoledronic acid treated patients (9.5 months vs 8.1 months) [26].

Prevention Skeletal Morbidity

In patients with bone metastases skeletal morbidity is estimated by skeletal-related events (SRE). (SRE include pathological fractures, need for either radiotherapy or surgery to bone, and spinal cord compression.) In a phase II study performed in patients with bone metastases produced by different solid tumors, the effect of denosumab (180 mg s.c.every 4 weeks or every 12 weeks for 25 weeks) and BPs (pamidronate or zoledronic acid, every 4 weeks, i.v.) was compared. All patients had NTX over 50 nmol/mmol creatinine despite the ongoing treatment with BPs. The primary endpoint was the normalization of urinary NTX level, which was achieved much better in denosumab-treated than BPs-treated patients (71 % vs 29 %). The normal level of NTX was maintained at 25 weeks in 64 % vs 37 %, and SRE (secondary end point) appeared in 8 % vs 17 % of the patients, respectively [27].

As a further step phase III trials studied the efficacy of denosumab in BP-naiv patients (total more than 5.500), with bone metastasizing solid tumors [28–30]. Denosumab was given once in four weeks, s.c., while zoledronic acid i.v. (4 mg) with supplements of calcium and vitamin D. The primary end point was the time to the first SRE. In case of breast cancers denosumab did better, since the median time for the appearance of the first SRE was 26.4 months using zoledronic acid, but the median was not reached after denosumab-treatment (till the time of evaluation). In prostate cancer these values were 17.1 vs 20.1 months. In patients with other solid tumors and myeloma denosumab was not inferior to zoledronic acid but failed to show superiority in overall. Myeloma patients, however, unlike the results in all other primary tumor types, had no beneficial effect from denosumab-treatment. A large randomized trial (NCT01345019) is trying to evaluate the potencial effect of denosumab in multiple myeloma.

The SREs spared by denosumab were usually pathological fracture and radiation to bone, but denosumab-treatment delayed all types of SREs. None of the studies showed difference in the overall survival (except NSCLC—27) comparing the two treatment groups, but the quality of life was better after denosumab-treatment.

Upon these results *denosumab was authorized for marketing both in USA (2010) and in Europe (2011) for the prevention of SREs in adult patients with solid tumors*. But how the clinicians can make the choice between denosumab and zoledronic acid? Upon the toxicities and efficacy to save SRE is the most reliable option. It seems today that denosumab is superior to zoledronic acid in prostate cancer. breast cancer and NSCLC. In other tumors the differences are simply too close and the therapeutic decisions should be made case by case. As an example of specific use, there is a peculiar bone tumor, GCT (giant cell tumor of the bone) which is rich in active osteoclasts producing severe lytic lesions, and rarely metastases, mainly into the lung. It seems that GCT could be a good therapeutic target and the first encouraging clinical results are already reported [31, 32].

Safety

In clinical use the dose of denosumab ranges from 60 mg once every 6 months (treating osteoporosis) to 120 mg every 4 weeks (treating cancer patients). The safety of the drug is better assessed in phase III trials where the highest doses are used. One of the most important adverse effect, similarly to zoledronic acid, is ONJ (osteonecrosis of jaw). In the three phase III trials, mentioned above, the frequency of ONJ in the two treatment groups was 1.3 % (denosumab) and 1.7 % (zoledronic acid). ONJ can develop practically any time (4–30 months) with a median of 14 months. The severity of ONJ is usually mild (grade 1–3 and conservative treatment can do the job. It seemed that an important difference between denosumab and zoledronic acid, that the former has no effect on renal function. Acut phase reactions (fever, myalgia, bone pain) were recorded within the first three days in about 8.7 % in the denosumab-treated goup, while about 20 % is zoledronic acid-treated group. Hypocalcemia was measured in 9.6 % vs 5.0 %, although all patients were advised to take calcium and vitamin D (especially patients with impaired renal function). Hypocalcemia rarely required hospitalization. The incidence of infectious periods (all immunocompromised patients were excluded from the studies) were similar in the two treatment groups.

Summary

Denosumab is a perfect example on the targeted anticancer therapy [33–35]. The inhibition of RANKL activity suppressed the osteoclasts' resorptive function and so prevented skeletal related events. This effect is useful not only against bone metastases, but also in the treatment of other diseases caused by bone loss. In different solid tumors with bone metastasis the quality of life also improved, although the overall survival usually showed no change. On the market the main competitors for denosumab are still the bisphosphonates (questions of costs and reimbursement are not discussed) and some potential new agents e.g. Src kinases (as dasatinib, saracatinib, bosutinib), cathepsin K inhibitors, (e.g. odanacatib), and new selective estrogen receptor modulators (e.g. bazedoxifene, lasofoxifene). Nevertheless, today denosumab is one of the most powerful agents in bone-saving area.

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