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Anti-Tumour Treatment

The anti-tumor effect of RANKL inhibition in malignant solid tumors – A systematic review

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ABSTRACT

At present, accumulating evidence suggests that inhibition of receptor activator of nuclear factor kappa-B ligand (RANKL) does not only induce an increase in bone mass and strength, but also has anti-tumor effects. Denosumab, an antibody targeting RANKL, is used to treat osteoporosis and to prevent skeletal related events (SREs) in patients with bone metastases originating from solid tumors. However, expression of RANKL and its receptor activator of nuclear factor kappa-B (RANK) is not solely restricted to cells involved in homeostasis of the bone and RANKL-RANK signalling appears to play a substantial role in many other processes in the body like mammary physiology, mammary tumorigenesis and the immune system. In pre-clinical models, RANKL inhibition has been shown to reduce skeletal tumor burden and distant metastases as well as to decrease mammary carcinogenesis. Clinically, RANKL inhibition improves bone-metastasis free survival in patients with prostate cancer and disease-free survival in patients with breast cancer. In addition, RANKL treatment may form a preventative strategy in patients at high risk for malignancies of the breast. Current clinical studies are evaluating the effect of denosumab on survival, the immune system and other biomarkers into a greater extent. To that purpose, a systematic review of the literature was performed and a narrative review synthesized, describing the present pre-clinical and clinical evidence of an anti-tumor effect of RANKL inhibition and the potential role of the immune system as one of the underlying mechanisms.

Introduction

It is thought that signaling induced by interaction of receptor activator of nuclear factor kappa-B ligand (RANKL), a member of the tumor necrosis factor (TNF) cytokine family [1], with its receptor, receptor activator of nuclear factor kappa-B (RANK), is involved in all steps of breast tumor development; from initial tumor formation to migration of cancer cells and subsequent metastasis [2]. Breast cancer is the most common cancer amongst women with an incidence of roughly 1.7 million new cases worldwide [WR1]. In metastatic breast cancer, the bone is the most common secondary site, which is involved in about 70% of patients [3]. Also cancers from the prostate, lung, kidney and thyroid frequently metastasise to the bone [4]. Bone metastases can cause severe morbidity and a consecutive reduced quality of life by inducing skeletal related events (SREs) [5], defined as pathological fractures, need for orthopaedic surgery, need for radiotherapy to the bone or spinal cord compression [6]. Due to the development of improved treatment options, advanced breast cancer has become a chronic illness in many patients [WR2]. The prolonged life expectancy brings along challenges in the management of advanced breast cancer and SREs. Although bisphosphonates have been used successfully for many years to prevent and manage these SREs, denosumab has also been registered for this purpose and is increasingly used.

Denosumab is a fully human IgG2 monoclonal antibody with affinity and specificity for human RANKL [WR3]. Denosumab blocks the binding of RANKL to its receptor RANK expressed on osteoclasts, causing a subsequent reduction of the formation, function and survival of these osteoclasts and as a consequence, bone resorption is reduced [WR3,7]. By binding of RANKL, denosumab mimics the action of the natural decoy receptor of RANKL called osteoprotegerin (OPG) [8]. Denosumab (at a dose of 60 mg q6 months) is currently registered for the treatment of patients at high risk for bone fractures, including postmenopausal women and men with osteoporosis, men with prostate cancer receiving hormone ablation therapy and women with breast cancer receiving aromatase inhibitor treatment [WR4]. Furthermore, deno-

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sumab (at a dose of 120 mg q4 weeks) is used to prevent SREs in patients with a solid tumor that has spread to the bone and to treat giant cell tumors of bone [WR3]. Additionally, new evidence suggests that denosumab may also have anti-tumor effects by osteoclast dependent and independent mechanisms. Anti-tumor effects of RANKL inhibition have intensively been studied preclinically. Clinically the first data are emerging. Here we summarize current pre-clinical and clinical evidence of the anti-tumor effects of RANKL inhibition in malignant solid tumors, with a special focus on breast cancer, and speculate on its potential capacity to modulate the tumor immune microenvironment.

Methods

In cooperation with a trained librarian, two search strategies were composed. The following databases were searched: PubMed, Embase (OVID-version), Web of Science, and COCHRANE Library.

The two query consisted of the combination of the following subjects:

Query 1: denosumab/RANKL inhibition and anti-cancer.
Query 2: denosumab/RANKL inhibition and immunity.

For the different concepts, all relevant keyword variations were used, not only keyword variations in the controlled vocabularies of the various databases, but the free text word variations of these concepts as well. The search strategy was optimized for all consulted databases, taking into account the differences of the various controlled vocabularies as well as the differences of database-specific technical variations (e.g., the use of quotation marks). The final search was performed on the 30th of June 2017. The bibliographic databases yielded 876 references for query number 1, and 211 references for query number 2 (English publications only). Relevant publications were also checked for related publications. For the complete search strategies, see the appendix.

On the 30th of June 2017, a systemic search in clinicaltrials.gov was performed using the following terms in the field "Intervention/Treatment": Denosumab OR PROLIA OR XGEVA OR AMG 162 OR RANKL. The search yielded 157 results.

Anti-tumor effects of RANKL inhibition

Over the past decades, it has become clear that the RANK-RANKL axis is not exclusively involved in bone remodelling [9], but exerts a broad range of functions in the body. The RANK-RANKL axis is known to play an important role in the immune system [10], mammary physiology, mammary tumorigenesis [2] and the central nervous system [11]. In the cancer setting, it is thought that the RANK-RANKL pathway is involved in each stage of tumorigenesis [2]. Therefore, the effect of inhibition of RANKL is expected to reach further than exclusively the inhibition of bone resorption. Combining this with emerging preclinical and clinical evidence, it is hypothesized that denosumab, by inhibiting the RANK-RANKL pathway, possesses both direct and indirect anti-tumor effects. A direct, osteoclast independent, anti-tumor effect is thought to be established by the effect of RANKL inhibition on RANK and RANKL expressing tumor cells [2,12]. Indirect anti-tumor effects are thought to be established either by changing the bone microenvironment (osteoclast dependent) or by the effect of RANKL inhibitors on non-cancerous cells like immune cells [10,12]. Obviously, all of these cells must express RANK and/or RANKL.

Expression of RANK and RANKL

RANK and RANKL are expressed on a wide variety of different cell types (Fig. 1). The interaction between T cells expressing RANKL and mature dendritic cells expressing RANK, ameliorates the growth and activation of T cells [1,13] and enhances the survival and function of dendritic cells [10,13,14]. Immature dendritic cells express both RANK and RANKL and longevity is attained in an autocrine way [15]. Both RANK and RANKL can also be found on B cells where it plays a role in the development and function of B cells [16,17]. Monocytes and macrophages express RANK and when bound by RANKL this induces effector function, antigen presentation and survival [18]. Osteoblasts, bone lining cells, bone stromal cells [19] and osteocytes [20] express RANKL, while osteoclasts express RANK [21], jointly regulating bone homeostasis upon interaction of RANK with RANKL [9]. RANK and RANKL are furthermore expressed in a wide variety of healthy tissues including breast, lymph nodes and the brain [22] and are required for normal functioning of these healthy tissues. Also, cancer cells can express both RANK and RANKL and use this expression in their advantage for survival and migration [2].

Pre-clinical evidence for an anti-tumor effect of RANKL inhibition

While in humans, inhibition of the RANK-RANKL axis can be accomplished by use of denosumab, it cannot be readily used in non-primate animal studies since denosumab recognizes primate RANKL only [WR3,WR4]. However, *in vitro* and *in vivo* non-primate animal experiments have successfully been performed with OPG-Immunoglobulin Fc segment complex (OPG-Fc) and RANK-Immunoglobulin Fc segment complex (RANK-Fc), mimicking the action of denosumab [23].

In numerous mouse models, RANKL inhibition was tested alone, as well as in combination with chemotherapeutics and targeted therapies, to evaluate the effect of RANKL inhibition on osteolytic bone lesions, bone metastasis and survival (Table 1).

In five separate mouse models of breast cancer bone metastasis, it was shown that treatment with OPG-Fc caused inhibition of the growth of skeletal metastases when given in a preventive [24–26] or therapeutic setting [24,27,28]. In one of the models, treatment with OPG-Fc resulted in a significant improvement in overall survival [24]. Complete prevention of bone metastases [29] or a decrease in tumor burden in the bone was observed when mice were treated with OPG-Fc or RANK-Fc given either after [30–33] or before [30,31] a tumor challenge with prostate cancer cells. Moreover, a reduction in skeletal tumor burden upon RANKL inhibition was observed in a colon adenocarcinoma mouse model [27], a mouse model of melanoma metastasis [34] and two non-small cell lung cancer mouse models [35,36].

A combination of OPG-Fc or RANK-Fc with chemotherapy augmented the clinical benefit in several models. In two models of non-small cell lung cancer bone metastasis, mice treated with OPG-Fc showed less skeletal tumor burden and had a higher overall survival when compared to the control arm. When docetaxel was added these clinical benefits were even more pronounced [37]. Also, two separate studies in a prostate cancer bone metastasis model revealed that while treatment with OPG-Fc or RANK-Fc suppressed skeletal tumor burden on its own, the addition of docetaxel significantly increased this effect resulting in a better median survival time in one of the studies [38,39]. Furthermore, addition of OPG-Fc or RANK-Fc to panitumumab, an antibody against the epidermal growth factor receptor, in an epidermoid carcinoma mouse model [40], to rhApo2L/TRAIL/dulanermin in a breast cancer mouse model [41] or to tamoxifen in a breast cancer mouse model [42] resulted in stronger decrease of tumor burden in the bone more than either of the targeted drugs alone.

These different mouse models show that, besides preventing excessive bone resorption, RANKL inhibition can lead to prevention of bone metastases. This might be explained by an indirect anti-tumor effect of RANKL inhibition. By blocking RANKL it is possible to disrupt the so called "vicious cycle". In this vicious cycle, RANKL is abundantly expressed by, among others, osteoblasts, inducing osteoclast mediated bone resorption. Upon bone resorption, growth factors like transforming growth factor-β (TGF-β) and