Cancer Treatment Reviews 62 (2018) 18–28

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevierhealth.com/journals/ctrv

Anti-Tumour Treatment

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

A.F. de Groot [a], N.M. Appelman-Dijkstra [b], S.H. van der Burg [a], J.R. Kroep [a,*]

[a] Department of Medical Oncology, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands
[b] Department of Endocrinology, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 21 September 2017
Received in revised form 25 October 2017
Accepted 25 October 2017

Keywords:
Denosumab
RANK
RANKL
Anti-tumor
Immunity
Breast cancer

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-

* Corresponding author at: Department of Medical Oncology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands.
E-mail address: J.R.Kroep@lumc.nl (J.R. Kroep).

https://doi.org/10.1016/j.ctrv.2017.10.010
0305-7372/

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 "Interven tion/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

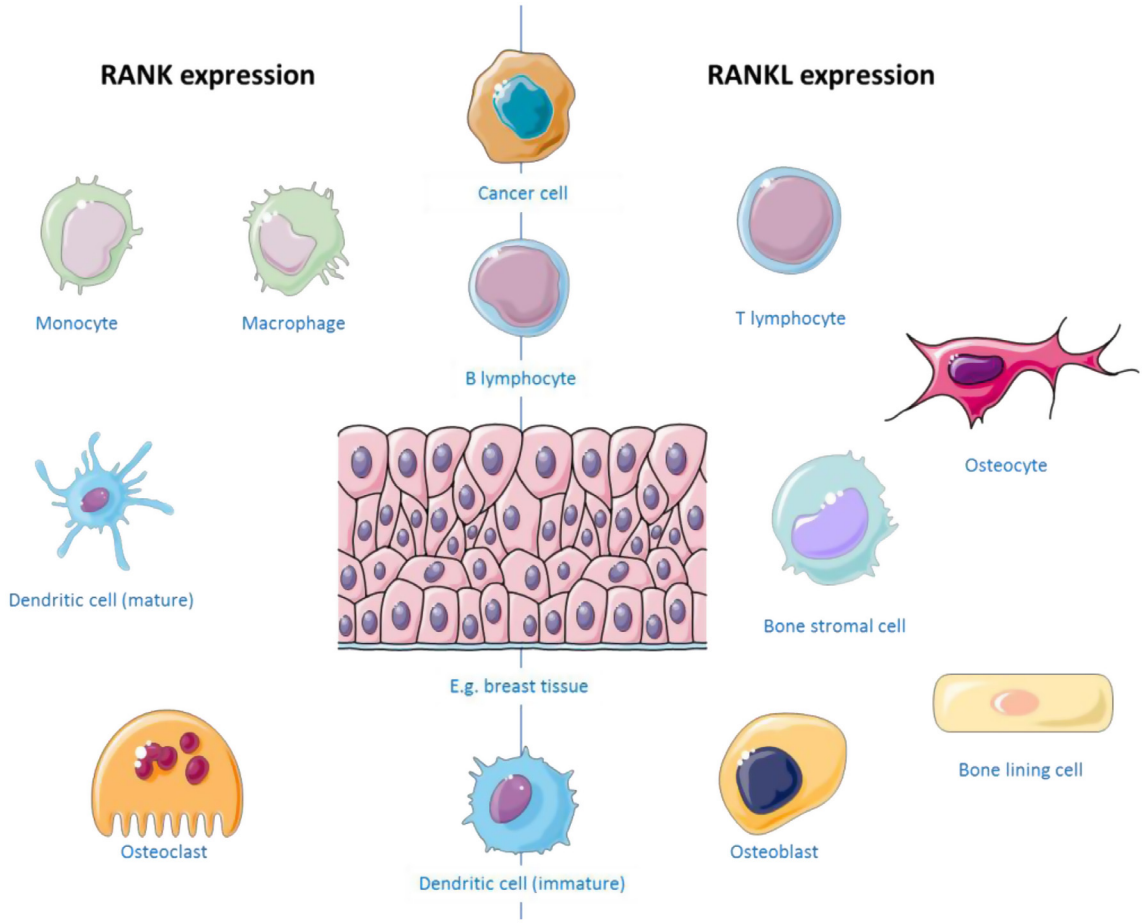

Fig. 1. Expression of RANK and RANKL. RANK and RANKL are expressed on different cell types, from cells in healthy tissues to cancerous cells and immune cells. The figure was created using adapted images from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License (available at http://smart.servier.com/).

Table 1
Mouse models studying the effect of RANKL inhibition on bone metastasis and survival. STB = Skeletal tumor burden, OS = Overall survival.

Cancer type	Intervention	Effect	Reference
Breast cancer	OPG-Fc	↓ STB ↑ OS	[24]
Breast cancer	OPG-Fc	↓ STB	[25]
Breast cancer	OPG-Fc/zoledronic acid	↓ STB	[26]
Breast cancer	OPG-Fc	↓ STB	[27]
Breast cancer	OPG/ibandronate	↓ STB	[28]
Prostate cancer	OPG-Fc	↓ STB	[29]
Prostate cancer	OPG-Fc	↓ STB	[30]
Prostate cancer	OPG-Fc	↓ STB	[31]
Prostate cancer	RANK-Fc	↓ STB	[32]
Prostate cancer	OPG-Fc	↓ STB	[33]
Colon cancer	OPG-Fc	↓ STB	[27]
Melanoma	OPG-Fc/zoledronic acid	↓ STB	[34]
Non-small cell lung cancer	OPG-Fc/zoledronic acid	↓ STB	[35]
Non-small cell lung cancer	RANK-Fc	↓ STB	[36]
Non-small cell lung cancer	OPG-Fc + docetaxel	↓ STB ↑ OS	[37]
Prostate cancer	OPG-Fc + docetaxel	↓ STB ↑ OS	[38]
Prostate cancer	RANK-Fc + docetaxel	↓ STB	[39]
Epidermoid carcinoma	OPG-Fc + panitumumab	↓ STB	[40]
Breast cancer	RANK-Fc + rhApo2L/TRAIL/dulanermin	↓ STB	[41]
Breast cancer	OPG-Fc + tamoxifen	↓ STB	[42]

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 insulin-like growth factor-1 (IGF-1) are released, which then stimulate cancer cells, homed to places with a high bone turnover [43], to proliferate and to release bone resorbing factors like parathyroid hormone-related protein (PTHrP) and interleukin-6 (IL-6), eventually stimulating RANKL producing cells to secrete more RANKL [44,45] (Fig. 2). In accordance with Pagets "seed and soil" theory [46], altering the microenvironment of the bone by inhibition of RANKL makes the bone less attractive for tumor cells as a site for metastasis and consequently, this prevents and decreases tumor outgrowth in the bone.

Direct anti-tumor effects of RANKL inhibition

The above described observed effect of RANKL inhibition on bone metastases might also be partially explained by the fact that RANKL inhibition is capable of blocking the direct effect of RANKL on tumor cells expressing RANK. Studies demonstrated that RANKL can induce migration of cancer cells expressing RANK [33,34] by triggering a metastasis gene signature [33]. This phenomenon was shown to apply to many tumor types [2]. As described earlier, Jones et al. showed that RANKL inhibition curtailed the skeletal

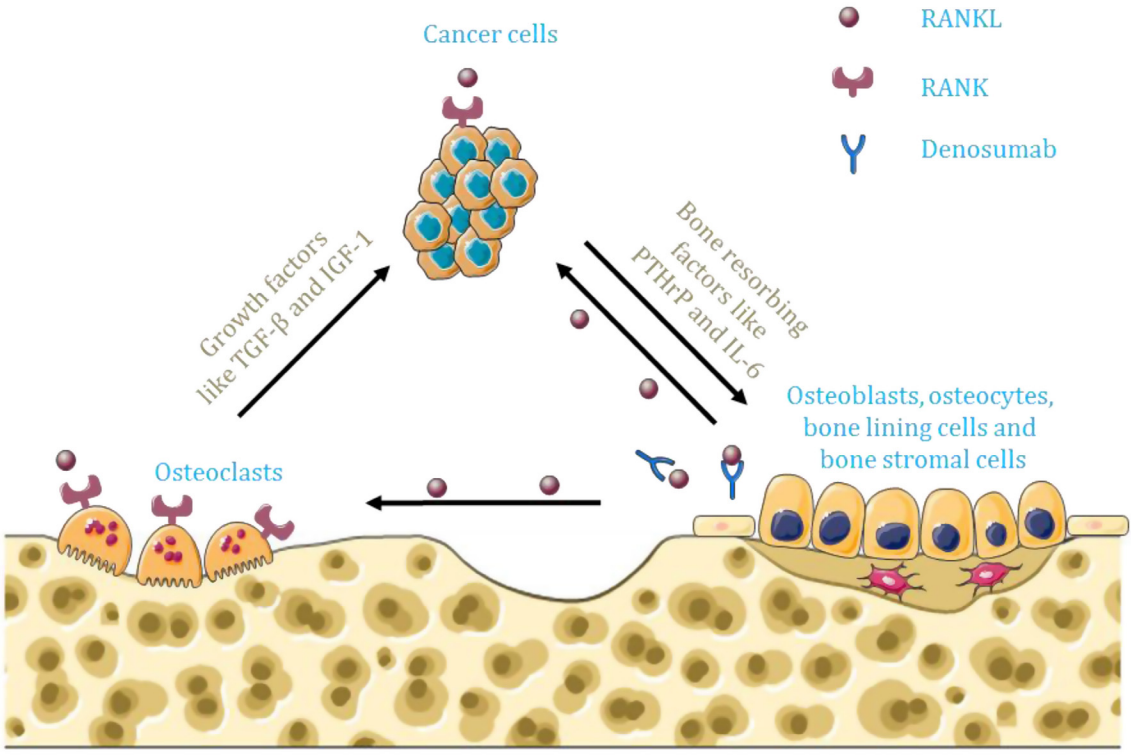

Fig. 2. The vicious cycle. RANKL is secreted by e.g. osteoblasts, osteocytes, bone lining cells and bone stromal cells, activating osteoclasts which elicits bone resorption. Growth factors like TGF-β and IGF-1 are then released, which stimulate cancer cells to proliferate and to release bone resorbing factors like PTHrP and IL-6 stimulating osteoblasts and other RANKL producing cells to secrete more RANKL. Inhibition of RANKL can interrupt this cycle. The figure was created using adapted images from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License (available at http://smart.servier.com/).

tumor burden in a mouse model of melanoma metastasis. Interestingly, zoledronic acid did not [34]. In another model for breast cancer, skeletal tumor growth was decreased upon both RANKL inhibition and treatment with zoledronic acid. However, the tumor burden was significantly more restrained with RANKL inhibition than with zoledronic acid [26]. These two models suggest a direct effect of RANKL on RANK expressing tumor cells in skeletal metastases [26,34]. More evidence of a direct RANKL effect on RANK expressing tumor cells was delivered by the studies of Gonzalez-Suarez et al. [47] and Tan et al. [48]. Using transgenic and orthotopic breast cancer mouse models that display spontaneous metastases to the lung, they were able to show that RANKL inhibition in mouse mammary tumor virus (MMTV)-*neu* mice [47], RANK knockdown within the used orthotopic mammary tumor cell line and RANKL inhibition in mice bearing tumors induced by this cell line decreased metastases in the lung [48]. Recently, Yoldi et al. demonstrated that knocking out RANK in MMTV-Polyoma Middle T mice also leads to a reduction in lung metastases as a result of increased tumor cell differentiation [49]. These data show that RANKL is directly involved in metastasis of RANK expressing tumor cells and that RANKL inhibition is capable of reducing skeletal tumor burden and visceral metastases in a direct manner.

Progestin (as used in hormone replacement therapy) has been reported to increase the risk of breast cancer, both pre-clinically and clinically [50,51]. The mechanism behind the increased incidence of breast cancer caused by progestin seems to be RANK/RANKL related and indicates that this axis may also drive tumorigenesis. A major clue in unravelling this mechanism was the discovery of the role of the RANK-RANKL axis in the formation of lobulo-alveolar mammary structures, required for lactation. Upon knockout of RANKL or RANK, mice failed to develop the mammary gland during pregnancy properly [52]. It is known that progesterone, partly via RANK/RANKL, mediates the proliferation of the mammary epithelium in mice [53] and human beings [54]; progesterone stimulated progesterone receptor (PR) positive cells, upregulate RANKL leading to RANKL stimulation of PR negative cells in a paracrine way [55]. Via the same pathway mammary tumorigenesis can be established. In a MMTV-RANK transgenic mice model, treatment with carcinogen and progesterone lead to an increase of pre-neoplasia and mammary tumor development compared to wild type mice that were treated similarly. Moreover, when these mice were treated with a RANKL inhibitor, mammary tumor development was reduced. In addition, treatment with a RANKL inhibitor also reduced mammary tumor development in MMTV-*neu* transgenic mice [47]. Furthermore, deletion of RANK in the mammary-gland epithelial cells in another study resulted in a diminished rate and a delay in medroxyprogesterone acetate (MPA, a progestin)-driven breast cancer in mice [56]. This study also observed a MPA driven expansion of mammary stem cells and a decrease of expansion upon deletion of RANK in the mammary-gland epithelial cells, in line with studies demonstrating that RANKL can mediate progesterone driven mammary stem cell expansion [57,58]. Furthermore, deletion of RANK also led to a decrease of the self-renewal capacity of mammary cancer stem cells. These two pre-clinical studies show that progesterone driven breast cancer is mediated by RANK/RANKL and that RANKL inhibition can attenuate this effect in a direct way. Additionally, inactivation of RANK leads to impaired breast cancer recurrence by eliciting differentiation of tumor cells and make tumors become more sensitive to treatment with docetaxel [49].

Two separate studies suggested that breast cancer driven by *BRCA1* mutations may also be mediated via RANK/RANKL. In mouse models mimicking *BRCA1* deficiency, RANKL inhibition resulted in diminished breast tumorigenesis [59], RANK inactivation led to a

Table 2
Finished and ongoing clinical trials investigating anti-tumor effects of denosumab. BMFS = bone-metastasis free survival, OS = overall survival, PFS = progression-free survival, DFS = disease-free survival, DTC = disseminated tumor cells, CTC = circulating tumor cells, IHC = immunohistochemistry. Studies with a NCT number can be found on https://clinicaltrials.gov and studies with an ACTRN number can be found on http://www.anzctr.org.au/default.aspx.

Cancer type	Phase	Denosumab	Comparator	Primary endpoint	Secondary endpoint (s)	Status	Reference
Prostate cancer	3	120 mg every 4 weeks	Placebo	BMFS	Time to first bone metastasis	Finished	[62]
Lung cancer	3	120 mg every 4 weeks	Zoledronic acid	Time to first on-study SRE	OS (exploratory)	Finished	[65]
Lung cancer (SPLENDOUR)	3	120 mg every 3–4 weeks	No denosumab	OS	PFS	Ongoing	NCT02129699
Lung cancer	2	120 mg every 3–4 weeks	Placebo	OS	PFS	Ongoing	NCT01951586
Breast cancer (D-CARE)	3	120 mg every 4 weeks for 6 months, then every 3 months	Placebo	BMFS	OS, DFS	Ongoing	[63] NCT01077154
Breast cancer (ABCSG-18)	3	60 mg every 6 months	Placebo	Time to first clinical fracture	DFS, BMFS, OS	Ongoing	[64] NCT00556374
Breast cancer	2	120 mg every 4 weeks for 6 months, then every 3 months	No comparator	Reduction of bone marrow DTC	DTC counts	Ongoing	NCT01545648
Breast cancer	2	120 mg every 4 weeks	No comparator	Reduction of CTC	–	Ongoing	NCT01952054
Breast cancer (D-Beyond)	2	120 mg twice, one week apart	No comparator	Chang in Ki67 (IHC)	Tumor apoptosis	Ongoing	NCT01864798
Breast cancer (GeparX)	2	120 mg every 4 weeks	No denosumab	pCR rates	–	Ongoing	NCT02682693
Breast cancer	2	120 mg every 4 weeks for three months	No comparator	Fraction of patients with reduction in CTC	Percent change in CTC, PFS	Ongoing	NCT03070002
Healthy subjects - breast	1	60 mg or 120 mg once	No denosumab	Change in Ki67 (IHC)	–	Finished	NCT02099461
Breast cancer	1	120 mg once	No comparator	Pharmacodynamic markers of RANKL inhibition	–	Ongoing	NCT02900469
Breast cancer (BRCA-D)	–	120 mg every 4 weeks for three months	No comparator	Change in Ki67 (IHC)	–	Ongoing	ACTRN12614000694617
Melanoma (CHARLI)	1/2	120 mg every 4 weeks (first 4 weeks weekly)	No comparator	PFS	OS	Not yet open	NCT03161756

delay and lower incidence of breast cancer as well as limited progression of already established tumors and RANKL inhibition led to prevention of pre-neoplastic lesions [60]. In the latter study, a reduced expansion of mammary progenitor cells upon RANK inactivation was observed. Following these studies, Cuyàs et al. reported that the formation of mammospheres in *BRCA1* deficient breast cells, was impaired when cells were treated with denosumab [61].

Clinical evidence for an anti-tumor effect of denosumab

The anti-tumor effect of denosumab is tested in several trials with breast, lung, melanoma and prostate cancer patients. An overview of these clinical trials can be found in Table 2.

Denosumab and its effect on bone metastases

Only one clinical trial reported data on the effect of RANKL inhibition on bone metastases. In this phase III study, patients with castration-resistant prostate cancer (n = 1432) were randomized between either denosumab (120 mg every four weeks) or placebo. Treatment with denosumab prolonged the bone-metastasis free survival (29.5 versus 25.2 months; hazard ratio (HR) = 0.85; 95% confidence interval (CI) = 0.73–0.98; p = .028) which was the primary endpoint. Furthermore, denosumab delayed the time to first bone metastasis as compared to placebo (33.2 versus 29.5 months; HR = 0.84; 95% CI = 0.71–0.98; p = .032) [62]. These modest but promising results make that the data of the ongoing D-CARE study in patients with high risk early breast cancer and the same primary endpoint, are eagerly awaited. In this phase III study, patients (n = 4509) receiving standard of care (neo)adjuvant therapy are randomized between adjuvant denosumab (120 mg every four weeks for six months, then every three months) or placebo for the duration of five years [63].

Denosumab and its effect on survival

Interestingly, some clinical trials, although not primarily designed to study an effect on survival, did find a survival advantage of denosumab as compared to either zoledronic acid or placebo.

The interim analysis of the ABCSG-18 trial, a phase III trial with postmenopausal breast cancer patients receiving adjuvant aromatase inhibition (n = 3425) and randomizing between adjuvant denosumab (60 mg every 6 months) and placebo, showed an improved disease-free survival in the patients treated with denosumab (HR = 0.816; p = .051) [64]. More long term data from this trial and data on disease-free and overall survival from the above mentioned D-CARE trial are awaited [63].

An anti-tumor effect of denosumab was observed not only in breast cancer patient but also in patients with lung cancer. First, in an exploratory and hypothesis-generating subgroup analysis of a phase III trial, where lung cancer patients with bone metastases (n = 811) were randomized between denosumab (120 mg every four weeks) and zoledronic acid (4 mg every four weeks), denosumab gave an increased overall survival compared to zoledronic acid (8.9 versus 7.7 months; HR = 0.80; 95% CI = 0.67–0.95; p = .01) [65]. To validate these findings, the randomized, open-label phase III SPLENDOUR trial (Survival imProvement in Lung cancEr iNduced by DenOsUmab theRapy) is investigating the effect of denosumab (120 mg every 3–4 weeks) as an addition to standard first-line chemotherapy in patients with advanced non-small cell lung cancer (n = 1000). The primary outcome measure is overall survival. Secondary outcome includes progression free survival [WR5]. In addition, a second randomized controlled phase II trial in patients with advanced non-small cell lung cancer (n = 226), examines the effect of denosumab (120 mg every 3–4 weeks) compared to placebo (2:1 randomization) in addition to standard first

line chemotherapy [WR6]. Also the retrospective analysis of another trial in 149 patients with non-squamous non-small cell lung cancer and bone metastases, roughly one third of whom received denosumab, one third received zoledronic acid and one third received no bone targeting agents, suggested that treatment with denosumab is associated with a better overall survival [66].

Denosumab and its effect on breast tissue in BRCA1 mutation carriers

Two of the above described pre-clinical studies, also tested the effect of denosumab in patients with a *BRCA1* mutation. In the first study, using *BRCA1* mutation carriers derived three dimensional breast organoid systems, it was found that the hyper responsiveness to progesterone of this tissue is abated upon denosumab treatment. Furthermore, three *BRCA1*-mutation carriers were treated with denosumab where after a decrease in proliferation, as measured by Ki67 expression, of the breast epithelial cells was observed when comparing pre- and posttreatment biopsies [59]. These patients are part of the BRCA-D trial, which is currently recruiting pre-menopausal women carrying a *BRCA1* or *BRCA2* mutation and pre-menopausal women with no mutation but at high risk for breast cancer (n = 40). The primary endpoint is change in Ki67 expression in the breast epithelium [WR7]. In the other study, the expansion of mammary progenitor cells was reduced when these cells were treated with denosumab [60].

An anti-tumor effect of RANKL inhibition mediated via the immune system

The involvement of RANK-RANKL signalling in the immune system is indisputable. The pathway is known to play a role in the development of the immune system as reviewed by Cheng et al. [10] and Ferrari-Lacraz et al. [67]. The RANK/RANKL pathway plays an essential role in both stimulation of the immune system (e.g. lymph-node, B- and T-cell development) and inhibition of the immune system (e.g. generation of regulatory T-cells and induction of T-cell tolerance) [10,67].

Although RANK, RANKL and OPG knockout mice have a clearly disrupted immune phenotype (e.g. lymph node agenesis, impaired T or B cell development) [67], the effect of mutations in these genes in humans is less well studied and the clinical relevance is uncertain. So far, a study in humans carrying mutations in both *TNFRSF11A* (RANK) genes showed a defect in immunoglobulin production in three out of eight studied patients and two of these patients failed to respond to tetanus vaccination [68], while a study in humans carrying mutations in both *TNFSF11* (RANKL) genes did not reveal any evident immune defects [69]. In addition, the last group of patients did not seem to have an increased risk of infections or immune defects [69]. The difference in phenotype between mice models and humans might be explained by species-specific differences or by residual function of RANK or RANKL despite the mutation [10,67,69]. Blocking of the RANK-RANKL axis is not expected to have severe immune mediated side effects since RANKL inhibition did not overtly affect the immune response [67,70–73], nor did it impair local or systemic inflammation parameters [74] in animal models. Also, clinical trials suggest no significant increase in infections or development of (new) neoplasms in patients treated with denosumab compared to placebo/no comparator (60 mg regime) [75,76] or zoledronic acid (120 mg regime) [77]. This might be explained by the existence of the redundant CD40-CD40L axis [67,78].

Interestingly, there is some pre-clinical evidence that does point at an immune mediated anti-tumor effect via RANKL inhibition. Tumor infiltrating regulatory T cells expressing RANKL (Fig. 1), were shown to drive pulmonary metastases mediated via RANK-RANKL in a breast cancer model [48]. One can imagine a vicious cycle in which regulatory T cells expansion is driven by M2 type tumor associated macrophages present in the tumor, whereupon RANKL produced by these regulatory T cells attracts more M2–macrophages. Due to this immunosuppressive microenvironment tumor cell growth is sustained and eventually metastases may arise [2]. Blockade of the RANK/RANKL axis might interfere in this process. Indeed, a reduction in regulatory T cells was observed in a mouse model of type 1 diabetes upon blockade of the RANK/RANKL axis [79]. Furthermore, Khan et al. demonstrated that inhibition of RANKL transiently blocked T cell tolerance, leading to an increased number of melanoma specific T cells and an improved anti-tumor response [80]. Studies of the immune modulatory effect of RANKL inhibition in the clinical setting are limited. In line with the role of regulatory T cells in metastases, a case report described a patient with metastatic melanoma with a remarkable response upon treatment with the combination of denosumab and the CTLA4-specific antibody ipilimumab. Albeit that it was not yet clear if this was the underlying mechanism [81]. Following this case study, recently it was shown that combination of RANKL inhibition and anti-CTLA4 is indeed more effective than either one of the agents alone in different tumor mouse models. An effect on lung metastases was reported to depend on natural killer cells and an effect on subcutaneous tumors on CD8 + T cells. The effect of the combinational therapy was not related to an increased depletion efficacy of regulatory T cells [82]. The combinatorial effect of anti-RANKL and immune checkpoint inhibitors in patients will be investigated in the CHARLI trial, which is a phase 1/2 study examining the effect of denosumab in combination with nivolumab (anti-PD1 specific antibody) with or without ipilimumab and is about to start recruiting patients soon (Table 2).

Two clinical studies investigated the effect of denosumab on lymphocyte counts with conflicting outcomes. While one study (n = 49) reported no changes in T and B cell counts [83], the other (n = 10) demonstrated an upregulation (defined as the percent change versus baseline) of T and B cells upon treatment with denosumab [84]. However, it must be noted that the number of patients and dosing regimen in the two studies was different; the first study administered a single dose (either 0.01, 0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg) whereas the second study administered 60 mg every 6 months for one year.

In conclusion, although an immune mediated anti-tumor effect of denosumab is well conceivable, strong pre-clinical and clinical evidence currently is lacking and results of the new trials have to be awaited.

Discussion and future perspectives

RANK-RANKL interaction does not only influence bone homeostasis but plays a critical role in numerous processes in the body. At present, pre-clinical and clinical evidence pointing towards an anti-tumor effect of RANKL inhibition is expanding. Several mechanisms underlying this effect have been proposed and investigated; this includes direct and indirect, osteoclast mediated and osteoclast independent effects. The role of the RANK/RANKL axis in primary breast cancer development and the observed decrease in proliferation of mammary tissue of patients at increased risk for breast cancer upon treatment with denosumab, indicate that RANKL inhibition might also be used as prevention in patients at high risk for breast cancer. We speculate that anti-RANKL therapy has an effect on the immune system and that modulation of the immune system via RANKL might induce an anti-tumor effect. However, current conclusions are still premature and more data has to be generated. To get more insight in the immune modulation of RANKL inhibition and a possible dose dependent effect of denosumab, we have initiated a phase II study, PERIDENO, in which

the effect of denosumab treatment on the systemic and local immune environment will be determined in patients with early breast cancer [WR8].

In order to determine if certain groups of patients might benefit particularly from denosumab as an anti-cancer agent, future studies should also focus on menopausal status. In postmenopausal women lacking oestrogen, RANKL is upregulated, resulting in accelerated bone resorption and a consequent risk of osteoporosis [85]. It can also be considered that RANKL upregulation is disadvantageous in terms of tumor growth; RANKL upregulation may result in a more favourable microenvironment in the bone and have a direct effect on RANK expressing cancerous cells, as outlined in this review. This indicates that postmenopausal women, in the therapeutic cancer setting, might benefit more from anti-RANKL therapy than pre- or premenopausal women. This concept was shown to be true for bisphosphonates. When given as adjuvant treatment, bisphosphonates curtail the recurrence rate, the distant recurrence rate as well as decrease the bone recurrence rate in patients with early breast cancer and improve breast cancer survival. However, this effect was only seen in postmenopausal and not in premenopausal women [86]. In the neoadjuvant setting, a similar trend was observed in postmenopausal patients [87].

Denosumab is considered to be an effective and safe drug. Denosumab has shown to be superior to zoledronic acid in terms of SRE prevention in a combined analysis, where three randomized phase III trials with a similar set-up were included [77]. In these trials either patients with bone metastases as a result of advanced breast cancer [88], prostate cancer [89] or other solid tumors or multiple myeloma [90] were included. Patients were either treated with denosumab (120 mg) or zoledronic acid (4 mg) every 4 weeks. Denosumab was shown to be superior in diminishing the risk of a first SRE by 17% and first and subsequent SRE by 18% compared to zoledronic acid [77]. Furthermore, no major differences were observed in the number of adverse and serious adverse events between the two groups. Particularly, the number of cases with osteonecrosis of the jaw, infections and new malignancies were similar between the groups. There were more cases of hypocalcaemia, 9.6% in the denosumab group versus 5.0% in the zoledronic acid group, and less cases of renal toxicity, 9.2% versus 11.8%, respectively, as expected [91,92]. In the open-label extension of two of the included trials, patients with advanced breast cancer [88] and patients with advanced prostate cancer [89] were offered to continue denosumab or switch from zoledronic acid to denosumab for an additional period up to two years. The data from this extension period, up to 5 years in the breast cancer group and 5.6 years in the prostate cancer group, confirmed the findings of the safety profile of denosumab [93]. For the treatment of osteoporosis, the registration of denosumab (60 mg) was based on robust data in postmenopausal woman with osteoporosis showing a significant risk reduction in (non-)vertebral and hip fractures [94]. Although it is thought this dosage of denosumab can be administered safely [76,95], limited comparative data on safety and effectiveness between denosumab and zoledronic acid are available at present and no conclusions on superiority of one agent over the other can be drawn yet [96]. Regarding osteonecrosis of the jaw in earlier mentioned studies, this adverse event occurred in up to 6.9% in the open-label extension trial of Stopeck et al. [93], in six cases in the crossover group (2207 patients enrolled) and in seven cases in the long-term group (2343 patients enrolled) in the open-label extension of the FREEDOM trial of Bone et al. [76], while in the ABCSG-18 trial of Gnant et al., no cases of osteonecrosis of the jaw were reported [95]. Therefore, with these safety data, utilizing denosumab in the future as an anti-tumor agent should be considered without major risks.

Interestingly, recently a new RANKL receptor, the leucine-rich repeat-containing G-protein-coupled receptor 4 (LRG4) was discovered [97]. LRG4 induces, when bound by RANKL, inhibition of osteoclast differentiation which is in contrast with the effect of binding of RANKL to RANK. Although the exact relationship between LRG4 and cancer is not clear yet, it was shown that it is involved in the proliferation of several tumor cell lines [98]. Hence, future studies should also bear in mind the potential interactions between RANKL and LRG4.

In conclusion, pre-clinical and clinical studies suggest that denosumab possesses an anti-tumor effect but more research is needed and trial results are awaited to confirm current evidence.

Conflict of interest

J.R. Kroep has participated on behalf of the Leiden Center for Bone Quality in expert panel meetings for denosumab and received a research grant from Amgen and Novartis. N.M. Appelman-Dijkstra has participated on behalf of the Leiden Center for Bone Quality in expert panel meetings for Prolia in the Netherlands. The other authors declare no conflict of interest.

Acknowledgement

We are indebted to the librarian of The Walaeus Library of the Leiden University Medical Center for his help with the construction of the search strategies.

Appendix

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

Database	Search Strategy	Number of references	Number of unique references
PubMed	((“Denosumab”[majr] OR “denosumab”[tiab] OR denosumab*[tiab] OR “Prolia” [tiab] OR “Xgeva”[tiab] OR “AMG 162″[tiab] OR ”AMG-162″[tiab] OR “RANKL inhibitor”[tiab] OR “RANKL inhibitors”[tiab] OR “rank l inhibitor”[tiab] OR “anti RANKL”[tiab] OR “anti RANK L”[tiab] OR “RANKL inhibition”[tiab] OR “rankl inhibiting”[tiab] OR “RANKL-inhibitor”[tiab] OR “RANKL-inhibitors”[tiab] OR “ra nk-l-inhibitor”[tiab] OR “anti-RANKL”[tiab] OR “anti-RANK-L”[tiab] OR “RANKL-inhibition”[tiab] OR “rankl-inhibiting”[tiab]) AND (“anti-tumor”[tw] OR “anti-tumor”[tw] OR “anti-cancer”[tw] OR “anti-neoplastic”[tw] OR “anti tumor”[tw] OR “anti tumor”[tw] OR “anti cancer”[tw] OR “anti neoplastic”[tw] OR “antitumor” [tw] OR “antitumour”[tw] OR “anticancer”[tw] OR “antineoplastic”[tw] OR anti-tumor*[tw] OR anti-tumor*[tw] OR anti-cancer*[tw] OR anti-neoplastic*[tw] OR	637	637

Appendix (*continued*)

Database	Search Strategy	Number of references	Number of unique references
	anti tumor*[tw] OR anti tumor*[tw] OR anti cancer*[tw] OR anti neoplastic*[tw] OR antitumor*[tw] OR antitumour*[tw] OR anticancer*[tw] OR antineoplas*[tw] OR "Drug Screening Assays, Antitumor"[Mesh] *OR "Antineoplastic Agents"[mesh] OR "Antineoplastic Agents"[Pharmacological Action] OR "Neoplasms/drug therapy"[majr] OR "Tumor Microenvironment"[Mesh] OR* "Survival Rate"[mesh] OR "survival"[tw] OR "Survival"[mesh] OR "Mortality"[mesh] OR "mortality"[tw] OR "mortality"[Subheading]))		
Embase (OVID-version)	(("Denosumab"/OR "denosumab".ti,ab OR denosumab*.ti,ab OR "Prolia".ti,ab OR "Xgeva".ti,ab OR "AMG 162".ti,ab OR "AMG-162".ti,ab OR "RANKL inhibitor".ti,ab OR "RANKL inhibitors".ti,ab OR "rank l inhibitor".ti,ab OR "anti RANKL".ti,ab OR "anti RANK L".ti,ab OR "RANKL inhibition".ti,ab OR "rankl inhibiting".ti,ab OR "RANKL-inhibitor".ti,ab OR "RANKL-inhibitors".ti,ab OR "rank-l-inhibitor".ti,ab OR "anti-RANKL".ti,ab OR "anti-RANK-L".ti,ab OR "RANKL-inhibition".ti,ab OR "rankl-inhibiting".ti,ab) AND (exp "antineoplastic activity"/ OR "anti-tumor".ti,ab OR "anti-tumor".ti,ab OR "anti-cancer".ti,ab OR "anti-neoplastic".ti,ab OR "anti tumor".ti,ab OR "anti tumor".ti,ab OR "anti cancer".ti,ab OR "anti neoplastic".ti,ab OR "antitumor".ti,ab OR "antitumour".ti,ab OR "anticancer".ti,ab OR "anti neoplastic".ti,ab OR anti-tumor*.ti,ab OR anti-tumor*.ti,ab OR anti-cancer*.ti,ab OR anti-neoplastic*.ti,ab OR anti tumor*.ti,ab OR anti tumor*.ti,ab OR anti cancer*.ti,ab OR anti neoplastic*.ti,ab OR antitumor*.ti,ab OR antitumour*.ti,ab OR anticancer*.ti,ab OR antineoplas*.ti,ab *OR* "Survival Rate"/OR "survival".ti,ab OR exp "Survival"/ OR exp "Mortality"/OR "mortality".ti,ab)) NOT conference review.pt	549	174
Web of Science	((TI = ("Denosumab" OR "denosumab" OR denosumab* OR "Prolia" OR "Xgeva" OR "AMG 162" OR "AMG-162" OR "RANKL inhibitor" OR "RANKL inhibitors" OR "rank l inhibitor" OR "anti RANKL" OR "anti RANK L" OR "RANKL inhibition" OR "rankl inhibiting" OR "RANKL-inhibitor" OR "RANKL-inhibitors" OR "rank-l-inhibitor" OR "anti-RANKL" OR "anti-RANK-L" OR "RANKL-inhibition" OR "rankl-inhibiting") AND TS = ("antineoplastic activity" OR "anti-tumor" OR "anti-tumor" OR "anti-cancer" OR "anti-neoplastic" OR "anti tumor" OR "anti tumor" OR "anti cancer" OR "anti neoplastic" OR "antitumor" OR "antitumour" OR "anticancer" OR "antineoplastic" OR anti-tumor* OR anti-tumor* OR anti-cancer* OR anti-neoplastic* OR anti tumor* OR anti tumor* OR anti cancer* OR anti neoplastic* OR antitumor* OR antitumour* OR anticancer* OR antineoplas* *OR* "Survival Rate" OR "survival" OR "Survival" OR "Mortality" OR "mortality")) OR (TS = ("Denosumab" OR "denosumab" OR denosumab* OR "Prolia" OR "Xgeva" OR "AMG 162" OR "AMG-162" OR "RANKL inhibitor" OR "RANKL inhibitors" OR "rank l inhibitor" OR "anti RANKL" OR "anti RANK L" OR "RANKL inhibition" OR "rankl inhibiting" OR "RANKL-inhibitor" OR "RANKL-inhibitors" OR "rank-l-inhibitor" OR "anti-RANKL" OR "anti-RANK-L" OR "RANKL-inhibition" OR "rankl-inhibiting") AND TI = ("anti neoplastic activity" OR "anti-tumor" OR "anti-tumor" OR "anti-cancer" OR "anti-neoplastic" OR "anti tumor" OR "anti tumor" OR "anti cancer" OR "anti neoplastic" OR "antitumor" OR "antitumour" OR "anticancer" OR "antineoplastic" OR anti-tumor* OR anti-tumor* OR anti-cancer* OR anti-neoplastic* OR anti tumor* OR anti tumor* OR anti cancer* OR anti neoplastic* OR antitumor* OR antitumour* OR anticancer* OR antineoplas* *OR* "Survival Rate" OR "survival" OR "Survival" OR "Mortality" OR "mortality")))	178	43
Cochrane Library	(("Denosumab" OR "denosumab" OR denosumab* OR "Prolia" OR "Xgeva" OR "AMG 162" OR "AMG-162" OR "RANKL inhibitor" OR "RANKL inhibitors" OR "rank l inhibitor" OR "anti RANKL" OR "anti RANK L" OR "RANKL inhibition" OR "rankl inhibiting" OR "RANKL-inhibitor" OR "RANKL-inhibitors" OR "rank-l-inhibitor" OR "anti-RANKL" OR "anti-RANK-L" OR "RANKL-inhibition" OR "rankl-inhibiting") AND ("antineoplastic activity" OR "anti-tumor" OR "anti-tumor" OR "anti-cancer" OR "anti-neoplastic" OR "anti tumor" OR "anti tumor" OR "anti cancer" OR "anti neoplastic" OR "antitumor" OR "antitumour" OR "anticancer" OR "antineoplastic" OR anti-tumor* OR anti-tumor* OR anti-cancer* OR anti-neoplastic* OR anti tumor* OR anti tumor* OR anti cancer* OR anti neoplastic* OR antitumor* OR antitumour* OR anticancer* OR antineoplas* *OR* "Survival Rate" OR "survival" OR "Survival" OR "Mortality" OR "mortality")):ti,ab,kw	71	22
Total		1.435	876

Query 2: denosumab/RANKL inhibition and immunity.

Database	Search Strategy	Number of references	Number of unique references
PubMed	((“Denosumab”[majr] OR “denosumab”[tiab] OR denosumab*[tiab] OR “Prolia” [tiab] OR “Xgeva”[tiab] OR “AMG 162″[tiab] OR ”AMG-162″[tiab] OR “RANKL inhibitor”[tiab] OR “RANKL inhibitors”[tiab] OR “rank l inhibitor”[tiab] OR “anti RANKL”[tiab] OR “anti RANK L”[tiab] OR “RANKL inhibition”[tiab] OR “rankl inhibiting”[tiab] OR “RANKL-inhibitor”[tiab] OR “RANKL-inhibitors”[tiab] OR “ra nk-l-inhibitor”[tiab] OR “anti-RANKL”[tiab] OR “anti-RANK-L”[tiab] OR “RANKL-inhibition”[tiab] OR “rankl-inhibiting”[tiab]) AND (“Immunity”[mesh] OR “immunity”[tw] OR “immunology”[Subheading] OR “immunology”[tw] OR “Immune System”[mesh] OR “immune system”[tw] OR “Lymphocytes”[mesh] OR “lymphocytes”[tw] OR “Lymphocyte Count”[mesh] OR “lymphocyte”[tw] OR immunologic*[tw] *OR “Immunologic Techniques”[Mesh] OR “Immune System Phenomena”[Mesh] OR “immune”[tw] OR “Immune System Processes”[Mesh]*))	172	172
Embase (OVID-version)	((*“Denosumab”/ OR “denosumab”.ti,ab OR denosumab*.ti,ab OR “Prolia”.ti,ab OR “Xgeva”.ti,ab OR “AMG 162″.ti,ab OR ”AMG-162″.ti,ab OR “RANKL inhibitor”.ti,ab OR “RANKL inhibitors”.ti,ab OR “rank l inhibitor”.ti,ab OR “anti RANKL”.ti,ab OR “anti RANK L”.ti,ab OR “RANKL inhibition”.ti,ab OR “rankl inhibiting”.ti,ab OR “R ANKL-inhibitor”.ti,ab OR “RANKL-inhibitors”.ti,ab OR “rank-l-inhibitor”.ti,ab OR “anti-RANKL”.ti,ab OR “anti-RANK-L”.ti,ab OR “RANKL-inhibition”.ti,ab OR “rankl-inhibiting”.ti,ab) AND (exp *“Immunity”/ OR “immunity”.ti,ab OR exp *“immunology”/OR “immunology”.ti,ab OR exp *“Immune System”/OR “immune system”.ti,ab OR exp *“Lymphocyte”/OR “lymphocytes”.ti,ab OR *“Lymphocyte Count”/OR “lymphocyte”.ti,ab OR immunologic*.ti,ab *OR* exp *“*immunological procedures*”/*OR “immune*”.*ti,ab*)) NOT conference review.pt	88	25
Web of Science	TS = ((“Denosumab” OR “denosumab” OR denosumab* OR “Prolia” OR “Xgeva” OR “AMG 162″ OR ”AMG-162″ OR “RANKL inhibitor” OR “RANKL inhibitors” OR “rank l inhibitor” OR “anti RANKL” OR “anti RANK L” OR “RANKL inhibition” OR “rankl inhibiting” OR “RANKL-inhibitor” OR “RANKL-inhibitors” OR “rank-l-inhibitor” OR “anti-RANKL” OR “anti-RANK-L” OR “RANKL-inhibition” OR “rankl-inhibiting”) AND (“Immunity” OR “immunity” OR “immunology” OR “immunology” OR “Immune System” OR “immune system” OR “Lymphocyte” OR “lymphocytes” OR “Lymphocyte Count” OR “lymphocyte” OR immunologic* *OR “immunological procedures*” *OR “immune*”))	69	13
Cochrane Library	((“Denosumab” OR “denosumab” OR denosumab* OR “Prolia” OR “Xgeva” OR “AMG 162″ OR ”AMG-162″ OR “RANKL inhibitor” OR “RANKL inhibitors” OR “rank l inhibitor” OR “anti RANKL” OR “anti RANK L” OR “RANKL inhibition” OR “rankl inhibiting” OR “RANKL-inhibitor” OR “RANKL-inhibitors” OR “rank-l-inhibitor” OR “anti-RANKL” OR “anti-RANK-L” OR “RANKL-inhibition” OR “rankl-inhibiting”) AND (“Immunity” OR “immunity” OR “immunology” OR “immunology” OR “Immune System” OR “immune system” OR “Lymphocyte” OR “lymphocytes” OR “Lymphocyte Count” OR “lymphocyte” OR immunologic* *OR “immunological procedures*” *OR “immune*”))	6	1
Total		335	211

References

[1] Wong BR et al. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 1997;272 (40):25190–4.
[2] Renema N et al. RANK-RANKL signalling in cancer. Biosci Rep 2016;36:4.
[3] Lee YT. Breast carcinoma: pattern of metastasis at autopsy. J Surg Oncol 1983;23(3):175–80.
[4] Coleman RE. Skeletal complications of malignancy. Cancer 1997;80(8 Suppl):1588–94.
[5] Costa L et al. Impact of skeletal complications on patients' quality of life, mobility, and functional independence. Support Care Cancer 2008;16 (8):879–89.
[6] Gralow JR, et al. NCCN Task Force Report: Bone Health In Cancer Care. J Natl Compr Canc Netw, 2013;11(Suppl 3):p. S1–50; quiz S51.
[7] Lipton A, Goessl C. Clinical development of anti-RANKL therapies for treatment and prevention of bone metastasis. Bone 2011;48(1):96–9.
[8] Schieferdecker A et al. Denosumab mimics the natural decoy receptor osteoprotegerin by interacting with its major binding site on RANKL. Oncotarget 2014;5(16):6647–53.
[9] Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys 2008;473(2):139–46.
[10] Cheng ML, Fong L. Effects of RANKL-targeted therapy in immunity and cancer. Front Oncol 2014;3:329.
[11] Hanada R et al. Central control of fever and female body temperature by RANKL/RANK. Nature 2009;462(7272):505–9.
[12] De Castro J et al. Therapeutic potential of denosumab in patients with lung cancer: beyond prevention of skeletal complications. Clin Lung Cancer 2015;16(6):431–46.
[13] Anderson DM et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997;390(6656):175–9.
[14] Wong BR et al. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J Exp Med 1997;186 (12):2075–80.
[15] Cremer I et al. Long-lived immature dendritic cells mediated by TRANCE-RANK interaction. Blood 2002;100(10):3646–55.
[16] Yun TJ et al. Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function. J Immunol 2001;166(3):1482–91.
[17] Manabe N et al. Connection between B lymphocyte and osteoclast differentiation pathways. J Immunol 2001;167(5):2625–31.

[18] Seshasayee D et al. A novel in vivo role for osteoprotegerin ligand in activation of monocyte effector function and inflammatory response. J Biol Chem 2004;279(29):30202–9.
[19] Dougall WC. Molecular pathways: osteoclast-dependent and osteoclast-independent roles of the RANKL/RANK/OPG pathway in tumorigenesis and metastasis. Clin Cancer Res 2012;18(2):326–35.
[20] Xiong J et al. Osteocytes, not osteoblasts or lining cells, are the main source of the RANKL required for osteoclast formation in remodeling bone. PLoS One 2015;10(9):e0138189.
[21] Hsu H et al. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 1999;96(7):3540–5.
[22] Wada T et al. RANKL-RANK signaling in osteoclastogenesis and bone disease. Trends Mol Med 2006;12(1):17–25.
[23] Schwarz EM, Ritchlin CT. Clinical development of anti-RANKL therapy. Arthritis Res Ther 2007;9(Suppl 1):S7.
[24] Canon JR et al. Inhibition of RANKL blocks skeletal tumor progression and improves survival in a mouse model of breast cancer bone metastasis. Clin Exp Metastasis 2008;25(2):119–29.
[25] Zheng Y et al. Bone resorption increases tumour growth in a mouse model of osteosclerotic breast cancer metastasis. Clin Exp Metastasis 2008;25(5):559–67.
[26] Blake ML et al. RANK expression on breast cancer cells promotes skeletal metastasis. Clin Exp Metastasis 2014;31(2):233–45.
[27] Morony S et al. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. Cancer Res 2001;61(11):4432–6.
[28] Zheng Y et al. Inhibition of bone resorption, rather than direct cytotoxicity, mediates the anti-tumour actions of ibandronate and osteoprotegerin in a murine model of breast cancer bone metastasis. Bone 2007;40(2):471–8.
[29] Zhang J et al. Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. J Clin Invest 2001;107(10):1235–44.
[30] Kiefer JA et al. The effect of osteoprotegerin administration on the intra-tibial growth of the osteoblastic LuCaP 23.1 prostate cancer xenograft. Clin Exp Metastasis 2004;21(5):381–7.
[31] Yonou H et al. Osteoprotegerin/osteoclastogenesis inhibitory factor decreases human prostate cancer burden in human adult bone implanted into nonobese diabetic/severe combined immunodeficient mice. Cancer Res 2003;63(9):2096–102.
[32] Zhang J et al. Soluble receptor activator of nuclear factor kappaB Fc diminishes prostate cancer progression in bone. Cancer Res 2003;63(22):7883–90.
[33] Armstrong AP et al. RANKL acts directly on RANK-expressing prostate tumor cells and mediates migration and expression of tumor metastasis genes. Prostate 2008;68(1):92–104.
[34] Jones DH et al. Regulation of cancer cell migration and bone metastasis by RANKL. Nature 2006;440(7084):692–6.
[35] Tannehill-Gregg SH et al. The effect of zoledronic acid and osteoprotegerin on growth of human lung cancer in the tibias of nude mice. Clin Exp Metastasis 2006;23(1):19–31.
[36] Feeley BT et al. Mixed metastatic lung cancer lesions in bone are inhibited by noggin overexpression and Rank: Fc administration. J Bone Miner Res 2006;21(10):1571–80.
[37] Miller RE et al. RANKL inhibition blocks osteolytic lesions and reduces skeletal tumor burden in models of non-small-cell lung cancer bone metastases. J Thorac Oncol 2014;9(3):345–54.
[38] Miller RE et al. RANK ligand inhibition plus docetaxel improves survival and reduces tumor burden in a murine model of prostate cancer bone metastasis. Mol Cancer Ther 2008;7(7):2160–9.
[39] Ignatoski KM et al. RANKL inhibition is an effective adjuvant for docetaxel in a prostate cancer bone metastases model. Prostate 2008;68(8):820–9.
[40] Canon J et al. Inhibition of RANKL increases the anti-tumor effect of the EGFR inhibitor panitumumab in a murine model of bone metastasis. Bone 2010;46(6):1613–9.
[41] Holland PM et al. Combined therapy with the RANKL inhibitor RANK-Fc and rhApo2L/TRAIL/dulanermin reduces bone lesions and skeletal tumor burden in a model of breast cancer skeletal metastasis. Cancer Biol Ther 2010;9(7):539–50.
[42] Canon J et al. RANKL inhibition combined with tamoxifen treatment increases anti-tumor efficacy and prevents tumor-induced bone destruction in an estrogen receptor-positive breast cancer bone metastasis model. Breast Cancer Res Treat 2012;135(3):771–80.
[43] Mishra A et al. Homing of cancer cells to the bone. Cancer Microenviron 2011;4(3):221–35.
[44] Gartrell BA, Saad F. Managing bone metastases and reducing skeletal related events in prostate cancer. Nat Rev Clin Oncol 2014;11(6):335–45.
[45] Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2002;2(8):584–93.
[46] Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev 1989;8(2):98–101.
[47] Gonzalez-Suarez E et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature 2010;468(7320):103–7.
[48] Tan W et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature 2011;470(7335):548–53.
[49] Yoldi G et al. RANK signaling blockade reduces breast cancer recurrence by inducing tumor cell differentiation. Cancer Res 2016;76(19):5857–69.
[50] Aldaz CM et al. Medroxyprogesterone acetate accelerates the development and increases the incidence of mouse mammary tumors induced by dimethylbenzanthracene. Carcinogenesis 1996;17(9):2069–72.
[51] Rossouw JE et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288(3):321–33.
[52] Fata JE et al. The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell 2000;103(1):41–50.
[53] Beleut M et al. Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. Proc Natl Acad Sci USA 2010;107(7):2989–94.
[54] Tanos T, et al. Progesterone/RANKL is a major regulatory axis in the human breast. Sci Transl Med, 2013;5(182): p. 182ra55.
[55] Kiesel L, Kohl A. Role of the RANK/RANKL pathway in breast cancer. Maturitas 2016;86:10–6.
[56] Schramek D et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. Nature 2010;468(7320):98–102.
[57] Asselin-Labat ML et al. Control of mammary stem cell function by steroid hormone signalling. Nature 2010;465(7299):798–802.
[58] Joshi PA et al. Progesterone induces adult mammary stem cell expansion. Nature 2010;465(7299):803–7.
[59] Nolan E et al. RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. Nat Med 2016;22(8):933–9.
[60] Sigl V et al. RANKL/RANK control Brca1 mutation-driven mammary tumors. Cell Res 2016;26(7):761–74.
[61] Cuyas E et al. BRCA1 haploinsufficiency cell-autonomously activates RANKL expression and generates denosumab-responsive breast cancer-initiating cells. Oncotarget 2017;8(21):35019–32.
[62] Smith MR et al. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial. Lancet 2012;379(9810):39–46.
[63] Goss PE, et al. A randomized, double-blind, placebo-controlled multicenter phase III study comparing denosumab with placebo as adjuvant treatment for women with early-stage breast cancer who are at high risk of disease recurrence (D-CARE). J Clin Oncol, 2011;29(15_suppl): p. TPS152-TPS152.
[64] Gnant M, et al. Abstract S2–02: The impact of adjuvant denosumab on disease-free survival: Results from 3,425 postmenopausal patients of the ABCSG-18 trial. Cancer Research, 2016;76(4 Suppl): p. S2–02-S2-02.
[65] Scagliotti GV et al. Overall survival improvement in patients with lung cancer and bone metastases treated with denosumab versus zoledronic acid: subgroup analysis from a randomized phase 3 study. J Thorac Oncol 2012;7(12):1823–9.
[66] Udagawa H et al. Impact of denosumab use on the survival of untreated non-squamous non-small cell lung cancer patients with bone metastases. J Cancer Res Clin Oncol 2017;143(6):1075–82.
[67] Ferrari-Lacraz S, Ferrari S. Do RANKL inhibitors (denosumab) affect inflammation and immunity? Osteoporos Int 2011;22(2):435–46.
[68] Guerrini MM et al. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. Am J Hum Genet 2008;83(1):64–76.
[69] Sobacchi C et al. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. Nat Genet 2007;39(8):960–2.
[70] Stolina M et al. Continuous RANKL inhibition in osteoprotegerin transgenic mice and rats suppresses bone resorption without impairing lymphorganogenesis or functional immune responses. J Immunol 2007;179(11):7497–505.
[71] Miller RE et al. Receptor activator of NF-kappa B ligand inhibition suppresses bone resorption and hypercalcemia but does not affect host immune responses to influenza infection. J Immunol 2007;179(1):266–74.
[72] Stolina M, et al. Long-term denosumab treatment of non-human primates had no observed effects on leukocyte subsets or T-cell-dependent immune responses. Aegean Conferences Series. 2008;vol. 35: 98 (Abstract).
[73] Stolina M et al. Regulatory effects of osteoprotegerin on cellular and humoral immune responses. Clin Immunol 2003;109(3):347–54.
[74] Stolina M et al. RANKL inhibition by osteoprotegerin prevents bone loss without affecting local or systemic inflammation parameters in two rat arthritis models: comparison with anti-TNFalpha or anti-IL-1 therapies. Arthritis Res Ther 2009;11(6):R187.
[75] Papapoulos S et al. The effect of 8 or 5 years of denosumab treatment in postmenopausal women with osteoporosis: results from the FREEDOM Extension study. Osteoporos Int 2015;26(12):2773–83.
[76] Bone HG et al. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. Lancet Diabetes Endocrinol 2017;5(7):513–23.
[77] Lipton A et al. Superiority of denosumab to zoledronic acid for prevention of skeletal-related events: a combined analysis of 3 pivotal, randomised, phase 3 trials. Eur J Cancer 2012;48(16):3082–92.
[78] Guerrini MM, Takayanagi H. The immune system, bone and RANKL. Arch Biochem Biophys 2014;561:118–23.
[79] Green EA, Choi Y, Flavell RA. Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. Immunity 2002;16(2):183–91.
[80] Khan IS et al. Enhancement of an anti-tumor immune response by transient blockade of central T cell tolerance. J Exp Med 2014;211(5):761–8.
[81] Smyth MJ, Yagita H, McArthur GA. Combination Anti-CTLA-4 and Anti-RANKL in Metastatic Melanoma. J Clin Oncol 2016;34(12):e104–6.

[82] Ahern E et al. Co-administration of RANKL and CTLA4 antibodies enhances lymphocyte-mediated anti-tumor immunity in mice. Clin Cancer Res 2017.
[83] Bekker PJ et al. A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J Bone Miner Res 2004;19(7):1059–66.
[84] Rossini M et al. Effects of denosumab on peripheral lymphocyte subpopulations. Endocrine 2016;53(3):857–9.
[85] Riggs BL. The mechanisms of estrogen regulation of bone resorption. J Clin Invest 2000;106(10):1203–4.
[86] Early Breast Cancer Trialists' Collaborative G. Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 2015;386(10001):1353–61.
[87] Kroep JR et al. Effects of neoadjuvant chemotherapy with or without zoledronic acid on pathological response: a meta-analysis of randomised trials. Eur J Cancer 2016;54:57–63.
[88] Stopeck AT et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol 2010;28(35):5132–9.
[89] Fizazi K et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. Lancet 2011;377(9768):813–22.
[90] Henry DH et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J Clin Oncol 2011;29(9):1125–32.
[91] Mould DR, Green B. Pharmacokinetics and pharmacodynamics of monoclonal antibodies: concepts and lessons for drug development. BioDrugs 2010;24 (1):23–39.
[92] Qi WX et al. Incidence and risk of denosumab-related hypocalcemia in cancer patients: a systematic review and pooled analysis of randomized controlled studies. Curr Med Res Opin 2013;29(9):1067–73.
[93] Stopeck AT et al. Safety of long-term denosumab therapy: results from the open label extension phase of two phase 3 studies in patients with metastatic breast and prostate cancer. Support Care Cancer 2016;24(1):447–55.
[94] Cummings SR et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med 2009;361(8):756–65.
[95] Gnant M et al. Adjuvant denosumab in breast cancer (ABCSG-18): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 2015;386(9992):433–43.
[96] Choi NK et al. Comparative safety and effectiveness of denosumab versus zoledronic acid in patients with osteoporosis: a cohort study. J Bone Miner Res 2017;32(3):611–7.
[97] Luo J et al. LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. Nat Med 2016;22(5):539–46.
[98] Zhu YB et al. GPR48 promotes multiple cancer cell proliferation via activation of Wnt signaling. Asian Pac J Cancer Prev 2013;14(8):4775–8.

Web references (WR)

[WR1] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr. [Accessed on 30/06/2017].
[WR2] Nederlandse Kankerregistratie, beheerd door IKNL ©. Available from: http://www.cijfersoverkanker.nl/. [Accessed on 30/06/2017].
[WR3] XGEVA® Product Monograph. Amgen Canada Inc. Available from: https://www.amgen.ca/products/~/media/e06e33ed57d8457c8bc1509ceced41d9.ashx, September 2016. [Accessed on 30/06/2017].
[WR4] Prolia® Product Monograph. Amgen Canada Inc. Available from: https://www.amgen.ca/products/~/media/1e79aee7d94340df88c3d97f5bb897c3.ashx, April 2017. [Accessed on 30/06/2017].
[WR5] ClinicalTrials.gov. Survival imProvement in Lung cancEr iNduced by DenOsUmab therapy (SPLENDOUR). Available from: https://clinicaltrials.gov/ct2/show/NCT02129699. [Accessed on 30/06/2017].
[WR6] ClinicalTrials.gov. Denosumab in Combination With Chemotherapy as First-line Treatment of Metastatic Non-small Cell Lung Cancer. Available from: http://clinicaltrials.gov/ct2/show/NCT01951586. [Accessed on 30/06/2017].
[WR7] ANZCTR.org.au. A pre-operative window study evaluating the biological effects of the RANK-Ligand (RANKL) inhibitor Denosumab on normal breast tissue from BRCA1 and BRCA2 mutation carriers and high risk, non-BRCA carriers. Available at: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12614000694617. [Accessed on 30/06/2017].
[WR8] Borstkanker Onderzoeks Groep. Studieoverzicht - 2017-02 PERIDENO. Available at: https://www.boogstudycenter.nl/studie/287/perideno.html. [Accessed on 15/08/2017].