Critical Reviews in Oncology / Hematology 133 (2019) 85–91

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

# RANK/RANKL signaling inhibition may improve the effectiveness of checkpoint blockade in cancer treatment

Peter A. van Dam[a,b,*], Yannick Verhoeven[a,b], Xuan B. Trinh[a,b], An Wouters[b], Filip Lardon[b], Hans Prenen[a,b,c], Evelien Smits[b], Marcella Baldewijns[d], Martin Lammens[d]

[a] Multidisciplinary Oncologic Centre Antwerp (MOCA), Antwerp University Hospital, Edegem, B2650, Belgium
[b] Center for Oncological Research (CORE), University of Antwerp, Wilrijk, B2610, Belgium
[c] Fase 1 Unit of Experimental Oncology, Antwerp University, Edegem, B2650, Belgium
[d] Department of Histopathology, Antwerp University Hospital, Edegem, B2650, Belgium

ARTICLE INFO

*Keywords:*
RANK
RANKL
Immunotherapy
Checkpoint inhibition
Solid tumors
Microenvironment

ABSTRACT

Binding between the receptor activator of nuclear factor-kB (RANK) and its ligand (RANKL) triggers recruitment of TNF receptor associated factor (TRAF) adaptor proteins and activation of downstream pathways. RANK/RANKL signaling is controlled by a decoy receptor called osteoprotegerin (OPG) which interacts with RANKL. Additional networks regulating RANK/RANKL signaling are active in a context specific manner. RANK/RANKL signaling is essential for the differentiation of bone-resorbing osteoclasts, and is deregulated in pathological processes such as postmenopausal osteoporosis or cancer induced bone destruction. Cells expressing RANK and RANKL are commonly found in the tumor microenvironment. The RANKL/RANK pathway is often overexpressed in tumors of the breast, prostate, endometrium, cervix, stomach, oesophagus and bladder, thyroid and correlated with poor prognosis. RANK signaling plays an important role in the innate and adaptive immune response as it generates regulatory T (Treg) cells and increases production of cytokines. RANK expression induces chemoresistance in vitro through the activation of multiple signal transduction pathways. RANKL blockade improves the efficacy of anti-CTLA-4 monoclonal antibodies against solid tumors and experimental metastases. As RANK inhibition enhances the immune response there is an increasing interest in combining it with immune therapy in an attempt to sensitize immune resistant tumors to immune therapies. Several studies are ongoing to assess this concept. The role of RANK/RANKL inhibition should be further pursued as an immunomodulatory strategy in combination with other treatment modalities.

## 1. Background

Immune therapies have revolutionized oncology over the last years, particularly in patients with melanoma, Hodgkin lymphoma, thoracic end urothelial cancer and mismatch repair deficient tumors (Aspelagh et al., 2018). However, with the exception of the first two diseases, only a minority of patients responds to immune therapy. Factors influencing the beneficial effect of immune therapies include mutational burden and neoantigen load, quality and clonality of neoantigens, expression of antigen presenting molecules and immune checkpoints, interferon gamma responsiveness and composition of the microenvironment (hot versus cold tumors (Aspelagh et al., 2018). Although combinations of immune therapy (eg CTL4 and PDL1 blocking) can be synergistic they do not resolve this problem and often induce significant additional toxicity (Santana-Davila and Show, 2018). Tumor cells have multiple mechanisms for evading immune surveillance such as immunological processes influencing the regulatory T cell function, antigen presentation, modifying the production of immune suppressive mediators (eg VEGF, IKK2), tolerances and immune deviation (Vinay et al., 2015; Tilborghs et al., 2017; van Dam et al., 2016). Therefor there is an increasing interest in combining immune therapy with immune modulating drugs in order to sensitize immune resistant tumors to immune therapies (Casey et al., 2015). Recent data are suggesting that RANK/RANKL inhibition may be an attractive approach to increase the effectiveness of immunotherapy. Signaling between the receptor activator of nuclear factor-kB (RANK) and its ligand (RANKL) is essential for the differentiation of bone-resorbing osteoclasts, and is deregulated in pathological processes such as postmenopausal osteoporosis or cancer induced bone destruction (Santana-Davila and Show, 2018). However, cells expressing RANK and RANKL are also commonly found in the

* Corresponding author at: Multidisciplinary Oncologic Centre, Antwerp University Hospital, Wilrijkstraat 10, B2650, Edegem, Belgium.
*E-mail address:* peter.vandam@uza.be (P.A. van Dam).

https://doi.org/10.1016/j.critrevonc.2018.10.011
Received 3 August 2018; Received in revised form 12 October 2018; Accepted 28 October 2018

tumor microenvironment. RANK signaling plays an important role in the innate and adaptive immune response as it generates regulatory T (Treg) cells and increases production of cytokines. Denosumab is a fully human monoclonal antibody that binds the cytokine RANKL and blocks the interaction of RANKL with RANK (Hanley et al., 2012; Lacey et al., 2012). Denosumab is approved for the "prevention of skeletal related events (pathological fracture, radiation to bone, spinal cord compression or surgery to bone in adults with bone metastases from solid tumours" and for the treatment of osteoporosis (Castellano et al., 2011; Wada et al., 2006). The drug has been widely used alone and in combination with chemotherapy, radiotherapy and targeted drugs. It has an acceptable toxicity profile, making it attractive to be combined with other drugs. Side effects are absent or mild and in most cases easy to manage. Hypocalcemia and hypophosphatemia, muscle cramps, cellulitis, numbness and rarely osteonecrosis of the jaw have been described (Castellano et al., 2011) In the present paper the effects of RANK/RANKL signaling inhibition on the microenvironment of malignant tumors are reviewed. It is hypothesized that this approach may be used to improve the response to immunotherapy.

## 2. RANK/RANKL signaling

RANKL, a tumor necrosis factor alpha superfamily cytokine, was originally identified in T-cells and dendritic cells (DC). It is a type II homotrimeric membrane protein that is encoded by the TNFS11 gene in humans and is also known as TNF-related activation induced cytokine (TRANCE), osteoprotegerin ligand (OPGL) or osteoclast differentiation factor (ODF). RANKL has three isoforms due to alternative splicing of the same gene (Ikeda et al., 2001; Lam et al., 2001; Luan et al., 2012). The full length RANKL is called RANKL1, while RANKL2 misses a part of the intracellular domain, and in RANKL3 the N-terminal part is deleted. RANKL can be present in a membrane or soluble form (Sigl et al., 2016). It is the only known ligand binding to a membrane receptor named receptor activator of nuclear factor-kB (RANK), a member of the TNF receptor superfamily (TNFRSF11 A). RANK is a type I transmembrane protein with a large cytoplasmic domain containing four cysteine-rich repeat motifs and two N-glycolisation sites. Binding between RANKL and RANK induces receptor trimerization which triggers recruitment of TNF receptor associated factor (TRAF) adaptor proteins and activation of downstream signaling pathways (such as TRAF6, NF-kB, AKT/PKB, JNK, ERK, Src and the MAP kinase cascade) (Gonzalez-Suarez and Sanz-Moreno, 2016). RANK/RANKL signaling is controlled by a decoy receptor called osteoprotegerin (OPG) (TBFRSF11B) which interacts with RANKL (Fig 1). OPG lacks transmembrane or cytoplasmatic domains, but is a soluble glycoprotein which can exist either as a 60-kDa monomer or as a 120-kD dimer. The dimerization of OPG is necessary for RANK/RANKL inhibition as it increases the affinity of OPG to RANKL dramatically (Schneeweis et al., 2005). OPG expression can be upregulated by several factors such as TRAIL, IL-1beta, Wnt/beta catenin signaling, TNFalpha and estrogen, and down regulated by TGF-beta and PTH (Millian, 2015). The final inhibitory effect of OPG on RANKL is dependent on its binding to these ligands (Renema et al., 2016; Theolaire et al., 2004) Recently, a new receptor for RANKL, LGR4, has been identified (Luo et al., 2016). LGR4 competes with RANK to bind RANKL and suppresses canonical RANK signaling. RANKL binding to LGR4 activates the Gαq and GSK3-β signaling pathway, an action that suppresses the expression and activity of nuclear factor of activated T cells and cytoplasmic, calcineurin-dependent 1 (NFATC1) during osteoclastogenesis. In addition functional RANK splicing variants have been identified in normal tissues and breast cancer cell lines suggesting that a complex network of additional levels of regulation beyond RANK, LGR4 and OPG exist (Papanastasiou et al., 2012)

## 3. RANK/RANKL signaling and the microenvironment

RANK and RANKL expressing tumor cells are commonly found in the tumor microenvironment (Chu and Chung, 2014). RANKL modulates the immune response by inducing T-cell proliferation and dendritic cell survival (Wong et al., 1997). In human breast carcinomas RANKL is found in tumor infiltrating lymphocytes) (TILs) and RANK is strongly expressed in tumor associated macrophages (TAMs) (Gonzalez-Suarez et al., 2010). TAMs accumulate in the microenvironment and depending on their M2 or M1 phenotype are involved in tumor growth, angiogenesis and metastasis. RANKL acts as a chemoattractant for these cells. RANK/RANKL signaling in M2 macrophages modulates production of chemokines, promoting the proliferation of T regulating (Treg) lymphocytes thereby creating an immunosuppressive environment. As RANKL is mainly produced by Treg lymphocytes, a vicious circle is established in conjunction with the TAMS mainly expressing RANK (Tan et al., 2011). Tumor-infiltrating Treg cells have been shown to stimulate mammary cancer metastasis through RANKL-RANK signaling (Li et al., 2009). RANKL treatment enhances survival of mature dendritic cells (DCs) and triggers generation of proinflammatory cytokines (IL1, IL6, IL12) which can promote differentiation of CD4 + T cells into TH1 cells providing a major costimulatory factor for CD4 + T cell reponses (Li et al., 2009; Chen et al., 2001). RANK is also expressed on NK cells, playing an important role in immunosurveillance. RANKL/RANK is involved in cross talk between the bone and immune systems making osteoclasts to function as antigen-presenting cells thereby activating CD4+ and CD8 + T cells (Zhang et al., 2017). A similar phenomenon is likely to be present in the microenvironment of solid tumors. The crosstalk of tumor cells with the immune system is not completely understood, but the impact of RANK-RANKL signaling on the tumor immune response is likely to be context specific (Tilborghs et al., 2017). Due to sequestering OPG by tumor cells or entrapment of OPG by the proteoglycans and glycosaminoglycans of the extracellular matrix, a microenvironment is created that facilitates the expansion of the tumor cells (Goswami and Sharma-Walia, 2016). In addition OPG binds TRAIL, a key natural pro-apoptotic and anticancer factor. By blocking TRAIL activity OPG can act as an antiapoptotic and pro-proliferative stimulus for cancer cells (Renema et al., 2016; Reid et al., 2009). It has been shown that RANK/RANKL signaling can promote the initial stages of cancer development by inducing stemness and epithelial mesenchymal transition (Palifox eet al., 2012) RANKL (eg produced by osteoblasts or bone marrow stromal cells) attracts RANK-expressing cells and induces their migration by activation of specific signaling pathways such as the MAP kinase pathway (Liu et al., 2016). RANKL was also detected in endothelial cells has also been implicated in angiogenesis through a Src and phospholipase C-dependent mechanisms (Min et al., 2003)

## 4. The RANKL/RANK pathway in human cancer beyond bone metastasis

Several studies documented RANK signaling to be important in a variety of cancers. This was nicely reviewed by Renema et al and de Groot et al (Renema et al., 2016; de Groot et al., 2018). Mouse models and trandomized studies in humans have shown that combination of antibodies blocking OPG or RANK with chemotherapy, hormone therapy or targeted drugs resulted in stronger decrease of tumor burdon in the bone (de Groot et al., 2018). However, it recently became clear that inhibition of RANK signaling has a direct effect on tumor cells beyond the bone. Yamada et al could show that RANKL-overexpressing head and neck squamous cell carcinomas were grossly vascularized, and in a mouse model RANKL promoted tumor angiogenesis in a VEGF independent manner suggesting that VEGF-independent strategies blocking RANKL signaling should be taken into consideration to inhibit angiogenesis (Yamada et al., 2011). In animal non small cell lung cancer (NSCLC) models, denosumab delayed bone metastases and reduced skeletal tumor growth (De Castro et al., 2015). In a posthoc analysis of patients with lung cancer treated with denosumab because of bone metastases, denosumab prolonged overall survival by 1.2

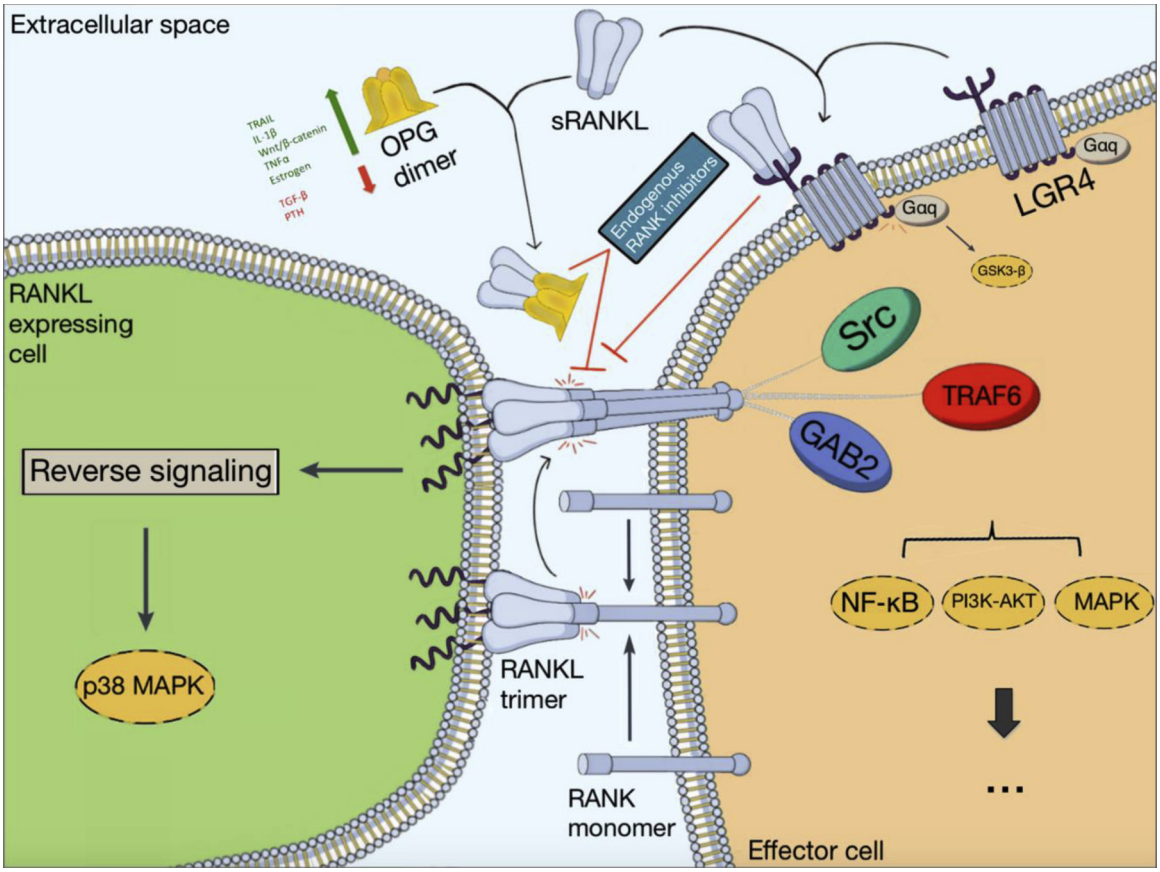


**Fig. 1. RANKL/RANK signaling and its endogenous inhibition.** Binding between RANKL and RANK induces receptor trimerization, which triggers recruitment of TNF receptor associated (TRAF) adaptor proteins and activation of downstream signaling pathways (such as NF-kB, PI3K-AKT and the MAP kinase casade) (Gonzalez-Suarez and Sanz-Moreno, 2016). This activity can be induced by either membrane-bound RANKL or soluble RANKL (sRANKL). sRANKL is derived from the membrane-bound form through alternative splicing or proteolytic cleavage (for example, by matrix metalloproteinase (MMP) or a disintegrin and metalloproteinase (ADAM) family members) RANK itself lacks kinase activity, and its signaling is initially mediated by adaptor molecules, such as TRAF proteins (including TRAF6), GRB-associated-binding protein 2 (GAB2), and SRC. The signaling cascade is controlled by a decoy receptor called osteoprotegerin (OPG) (TNFRSF11B), which interacts with RANKL. OPG lacks transmembrane or cytoplasmatic domains, but is a soluble glycoprotein, which can exist either as a 60-kDa monomer or as a 120-kD dimer. The dimerization of OPG is necessary for RANK/RANKL inhibition as it increases the affinity of OPG to RANKL dramatically (Schneeweis et al., 2005). OPG expression can be upregulated by several factors such as TRAIL, IL-1beta, Wnt/beta catenin signaling, TNFalpha and estrogen, and down regulated by TGF-beta and PTH (Millian, 2015). The final inhibitory effect of OPG on RANKL is dependent on its binding to these ligands (Renema et al., 2016; Theolaire et al., 2004). Recently, a new receptor for RANKL, leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4), has been identified (Luo et al., 2016). LGR4 competes with RANK to bind RANKL and suppresses canonical RANK signaling. RANKL binding to LGR4 activates the Gαq and GSK3-β signaling pathway, an action that suppresses the expression and activity of nuclear factor of activated T cells and cytoplasmic, calcineurin-dependent 1 (NFATC1) during osteoclastogenesis. The endogenous inhibitors target both soluble and membrane-bound forms of RANKL Additionally, reverse signaling following RANKL ligation has also been reported in studies using in vitro models of osteoblastogenesis and can modulate IFNγ secretion from CD4 + T cells through the p38 MAPK-dependent signalling pathway.

months versus zoledronic acid (De Castro et al., 2015). This issue warrants further investigation in order to elucidate whether the survival benefit is due to better control of bone metastases or related to a direct antitumor effect of denosumab. Naumik et al measured the concentration of OPG and soluble RANKL in bronchoalevolar lavage fluids of 44 NSCLC patients and 15 healthy volunteers taken as control subjects (Naumnik et al., 2018). The OPG content but not sRANKL was higher in the NSCLC group than that in controls. However, the greater the level of sRANKL in NSCLC patients, the shorter the overall survival. The RANKL/RANK pathway was variably expressed in tumors of the thyroid and increased serum OPG was also correlated with poor prognosis in gastric, cervical, oesophagal and bladder carcinoma (Heymann et al., 2008; Mizutani et al., 2004; Zhang et al., 2018; Shang et al., 2015; Ma et al., 2017). Song et al (2014) found that RANK expression was significantly higher in hepatocellular carcinoma (HCC) than in peritumoral hepatic tissue (Song et al., 2014). HCC cell lines express RANK constitutively, and activation of the RANKL-RANK axis significantly promoted migration and invasion ability of HCC cells in vitro. Recently it has been demonstrated that RANK/RANKL-expression is significantly elevated in endometrial cancer tissue, particularly in tumors of higher stage. RANK levels were positively correlated to expression of N-Cadherin and negatively with E- cadherin suggesting that RANK/RANKL expression is related to epithelial-mesenchymal transition (EMT). Cell line evidence shows that the induced EMT is mediated by the cytokine CCL20 and activation of the MAPK pathway in endometrial cancer cells (Liu et al., 2016; Wang et al., 2015). The RANK-mediated signal network was also found to drive EMT in prostate cancer cells (Chu and Chung, 2014; Christoph et al., 2018). Christoph et al could show, using a PCR after microdissection of 71 prostate cancers, that RANK, RANKL and OPG are directly expressed by prostate cancer cells in the primary tumor and showed a clear correlation with Gleason Score, serum PSA level and advanced disease (Christoph et al., 2018). Schultz et al found that osteolytic prostate cancer cells induce the expression of RANKL in a STAT3-5 dependent manner (Schultz et al., 2014). Therefor there may be a role for RANKL inhibitors to suppress this inflammatory network.

Activation of RANK signaling promotes mammary tumorigenesis as MMTV-RANK transgenic mice are prone to develop mammary tumors

(Pellegrini et al., 2013). It has been postulated that paracrine signaling through RANK/RANKL is responsible for the expansion of mammary stem cells observed during pregnancy and luteal cycles (Gonzalez-Suarez and Sanz-Moreno, 2016; Cross et al., 2006). Pharmacologic inhibition of RANKL or genetic ablation of RANK reduces (particularly estrogen and progesterone receptor negative) mammary tumor and metastasis development in animal models (Ma et al., 2017). Breast cancer cells are able to produce RANKL and stimulate osteoclast differentiation when co-cultured with bone marrow stromal cells (Papanastasiou et al., 2012; Cross et al., 2006; Park et al., 2003). In human breast tumors, high RANK expression levels are associated with altered mammary differentiation, suggesting that increased RANK signaling may contribute to breast carcinogenesis (Gonzalez-Suarez and Sanz-Moreno, 2016). In a large prospective study including 1976 invasive breast cancer cases serum soluble RANKL (sRANKL) was quantified using an ELISA and serum OPG using an electrochemiluminescent assay. No solid evidence was found for an association between sRANKL and breast cancer risk (Sarink et al., 2017). However, tissue levels and not serum levels of components of the RANK/RANKL signaling network may be crucial factors involved. High levels of RANK were found in human primary breast adenocarcinomas that lack expression of the hormone receptors, in tumors with high pathologic grade and proliferation index, and are significantly associated with metastatic tumors. These results suggest that RANK expression in primary breast cancer is associated with poor prognosis (Pellegrini et al., 2013). The RANKL/RANK pathway also plays an important role in breast cancer progression (Palafox et al.,2012). It has been shown in vitro that HIF-1 alpha induced expression of RANKL initiates increased migration of breast cancer cells via PI3K/AKT signaling (Tang et al., 2011). Zhang et al studied the effect of Casitas B-lineage lymphoma (Cbl-b, an essential regulator of the RANKL/RANK pathway) on the prognosis of RANK-expressing breast cancer patients, as well as on RANKL/RANK pathway (Zhang et al., 2015). The results showed that RANK and Cbl-b expression was separately detected in 154 (154/300, 51.3%) and 165 (165/300, 55.0%) breast cancer tissue samples. In RANK-expressing breast cancer patients, Cbl-b expression was correlated with significantly lower metastasis rate, better disease-free survival (DFS) and breast cancer-specific survival (BCSS). To explore underlying mechanisms these authors performed functional in vitro studies revealing that Cbl-b down-regulated RANK protein expression and inhibited RANKL-induced breast cancer cell migration by negatively regulating the Src-Akt/ERK pathway. This suggests that Cbl-b improves the prognosis of RANK-expressing breast cancer patients by inhibiting RANKL-induced breast cancer cell migration and metastasis. Survival data of a group of 248 breast cancer patients from TCGA, revealed an $EGFR^{hi}/RANK^{hi}$ subpopulation that showed a statistically significant ($p = 0.001$) reduced overall survival when compared to $EGFR^{low}/RANK^{low}$ group of patients (Papanastasiou et al., 2018). EGFR and RANK combinatorial in vitro analyses revealed a significant upregulation of AKT and ERK signaling after EGF stimulation in cell lines and also an increase of breast cancer cell invasiveness.

The adjuvant effect of RANK/RANKL inhibition in patients with breast cancer remains unclear. In a recent analysis of the ASBSG-18 study (a prospective study of 3425 patients with hormone sensitive early breast cancer treated with an adjuvant aromatase inhibitor which were randomized to receive placebo or denosumab 60 mg subcutaneously every six months for 5 years) it was shown that disease free survival was significantly better (p 0.026) in the denosumab group at 5 (89.2% vs 87.3%) and 8 years follow-up (80.6% vs 77.5%) (Gnant et al., 2018). A cohort study including 37.604 female MEDICARE beneficaries confirmed that patients receiving at least 6 months of an oral bisphosphonate, two doses of ibandronate or one dose of zoledronate or denosumab at doses equivalent or higher to those approved for osteoporosis, during the first two years of BC diagnosis, had a significantly better overall survival (81% vs 77%) (Herrera Pena et al., 2018). In the DCARE study 4509 patients with high risk early breast cancer had either standard (neo)adjuvant therapy with or without denosumab (120 mg SC every month for 6 months, then 3 monthly up to 5 years). Twenty percent of these patients were HER-2 positive, and 95.9% of the recruited population had anthracycline and/or taxane chemotherapy. Denosumab did not improve bone metastasis free survival, disease free- or overall survival in the postmenopausal subset (Coleman et al., 2018). In summary, current data suggest that in a postmenopausal population which is only treated with an aromatase inhibitor there is a clear benefit of treatment with denosumab, but this is not the case when adjuvant chemotherapy is given. Mechanistically one can hypothesize that chemotherapy reduces some of tumor suppressive effects of RANK/RANK inhibition in the cancer microenvironment.

## 5. RANK/RANKL signaling and chemo- or radiotherapy

The role of the RANK/RANK signalling in drug resistance remains unclear. Tsubaki et al found that treatment with RANKL induced drug resistance in RANK-expressing but not RANK-negative cell lines (Tsubaki et al., 2016). RANKL stimulation of RANK-expressing cells increased multidrug resistance protein 1 (MDR1), breast cancer resistance protein (BCRP), and lung resistance protein 1 (LRP1) expression and decreased BIM expression through various signaling molecules. RNA silencing of BIM expression induced drug resistance, but the RANKL-mediated drug resistance could not be overcome through the RNA silencing of MDR1, BCRP, and LRP1 expression. These results indicate that the RANK/RANKL system induces chemoresistance through the activation of multiple signal transduction pathways (Sisay et al., 2017). In a mouse model RANKL blockade increases the efficacy of cisplatin chemotherapy (Faget et al., 2018). Thimodeaou et al evaluated early breast cancer patients, who had been treated with anthracycline-based chemotherapy within two randomized trials, by quantitative reverse transcription–polymerase chain reaction on 819 formalin-fixed paraffin-embedded tumor tissue samples for mRNA expression of RANK, OPG, and RANKL (Timotheadou et al., 2017). In the univariate analysis, low RANKL mRNA expression was found to be an unfavorable factor for DFS [hazard ratio (HR) = 1.33, 95% confidence interval (CI) 1.05–1.68, Wald's P = .018] and bone metastasis–free survival (HR = 1.67, 95% CI 1.09–2.56, P = .018), although it did not retain its significance in the multivariate analysis. RANK/OPG, RANKL/ RANK, and RANKL/OPG ratios (using the median value as a cutoff) were not associated with DFS, OS, or bone metastasis–free survival. One can conclude that at the moment there is no objective evidence that RANK/RANL signaling inhibition has an influence on the effectivity of chemotherapy in humans.

Treatment with RANKL resulted in marked protection from cell death in response to γ-irradiation and doxorubicin (Schramek et al., 2010). Schramek et al could show that γ-irradiation-induced upregulation of the pro-apoptotic molecules BIM, PUMA and NOXA did not occur in the presence of RANKL. Loss of RANK expression on mammary epithelial cells abrogated the protective effects of MPA on γ-irradiation-induced cell death (Schramek et al., 2010). There are no clinical studies addressing the adjunct effect of RANKL inhibitors to radiotherapy. Although associated with several methodological, practical and ethical challenges, randomized controlled trials are needed.

## 6. RANK/RANKL signaling and checkpoint inhibition

In 2014 Smyth et al described a case of a rapidly advancing metastatic melanoma with aggressive and symptomatic bone metastases requiring treatment with the anti-RANKL antibody denosumab for palliation in a patient who was concomitantly treated with the anti-CTLA-4 antibody ipilimumab (Smyth et al., 2016). She had a dramatic partial response and was alive at 62 weeks. Using the B16F10 melanoma preclinical model of experimental metastases, these authors could demonstrate that anti-CTLA-4 and anti-RANKL monoclonal antibodies (mAbs) have modest antimetastatic activities when used as

monotherapy, but metastases suppression was considerably enhanced when these drugs were combined at the time of intravenous melanoma inoculation. The combined effect of anti-CTLA-4 and anti-RANKL is dependent on lymphocytes, as treatment was completely ineffective in RAG2−/−γc−/− mice lacking all lymphocytes and in mice specifically depleted of natural killer cells. Based on the above observations, Afzal and Shirai evaluated in a retrospective analysis the synergistic effect of immune checkpoint inhibitors and denosumab in metastatic melanoma patients (Afzal and Shirai, 2018). Eleven (29.72%) out of 37 patients assessed received immune checkpoint inhibitors and denosumab, and the others only immune checkpoint inhibitors. Median progression-free and overall survival in the cohort having the combination treatment respectively was 11.6 and 57 months compared with 4.15 and 22.8 months in the controls. Although there are potential biases, this suggests that adding denosumab to immune checkpoint inhibitors may have a beneficial effect on outcome. In an attempt to unravel the underlying mechanisms of this observation, Ahern et al studied the efficacy of a combination of RANKL and CTLA-4 blockade by analysis of tumor-infiltrating lymphocytes, tumor growth, and metastasis in a model using a variety of neutralizing antibodies and gene-targeted mice (Ahern et al., 2017). RANKL blockade improved the efficacy of anti-CTLA-4 mAbs against solid tumors and experimental metastases. Treg-depleting anti-CTLA-4 mAbs of the mouse IgG2a isotype showed the highest combinatorial activity. The optimal combination depended on the presence of activating Fc receptors and lymphocytes (particularly natural killer and $CD8^+$ T cells), whereas anti-RANKL alone did not require Fc receptors. The significantly higher T-cell infiltration into solid tumors post anti-RANKL and anti-CTLA-4 was accompanied by increased T-cell effector function. In the recently opened CHARLI trial (NCT03161756) and KEYPAD (NCT03280667) study denosumab in combination with immune checkpoint inhibitors will be evaluated in patients with unresectable or metastatic melanoma and renal cell carcinoma respectively. Two translational studies are under way to study the impact of denosumab on the systemic immunity and local immunologic microenvironment in detail: the PERIDENO (NCT03532087) study in postmenopausal patients with HER2 negative breast cancer in combination with AC-T chemotherapy and our neoadjuvant study with denosumab monotherapy in cervical cancer (ISS 20,177,041).

It is clear that RANK/RANKL inhibition has multiple important effects on the immune environment which may be context specific and difficult to entangle. Neoadjuvant studies could be crucial to enravel its role in a clinical context (van Dam et al., 2017). Immunotherapy can induce spectacular responses, often lasting for a considerable time, but is effective in only in a minority of patients. One of the major challenges for future research is to increase the applicability and effectiveness of this attractive treatment modality by making the tumor more immunogenic. Induction of the abscopal effect by radiotherapy, the use of medication changing the microenvironment and the development of new biomarkers are amongst the potential options which should be explored (van Dam et al., 2018; Tuyaerts et al., 2018).

## 7. Conclusion

The role of RANK/RANKL inhibition should be further pursued as an immunomodulatory strategy in combination with other treatment modalities. The effects of RANK/RANKL signaling inhibition on the microenvironments needs further attention beyond the bone. As denosumab has clear immune stimulating effects and an interesting toxicity profile the drug has an interesting potential to be explored for this indication.

## Authors' contributions

PAvD and designed the paper and performed the data acquisition and analysis. Interpretation of the data and drafting and revision of the manuscript was performed by all authors.

## Funding

None declared.

## Ethics approval and concent to participate

Not relevant for this paper.

## Availability of data and material

References available.

## Concent for publication

All authors concent for publication.

## Competing interests

PvD has received a grant from Amgen to perform an academic trial on denosumab in cervical cancer. The other authors have no disclosures.

## References

Afzal, M.Z., Shirai, K., 2018. Immune checkpoint inhibitor (anti-CTLA-4, anti-PD-1) therapy alone versus immune checkpoint inhibitor (anti-CTLA-4, anti-PD-1) therapy in combination with anti-RANKL denosumuab in malignant melanoma: a retrospective analysis at a tertiary care center. Melanoma Res. 22 (4), 341–347. https://doi.org/10.1097/CMR.0000000000000459. [Epub ahead of print].

Ahern, E., Harjunpää, H., Barkauskas, D., Allen, S., Takeda, K., Yagita, H., Wyld, D., Dougall, W.C., Teng, M.W.L., Smyth, M.J., 2017. Co-administration of RANKL and CTLA4 antibodies enhances lymphocyte-mediated antitumor immunity in mice. Clin. Cancer Res. 23 (19), 5789–5801. https://doi.org/10.1158/1078-0432.CCR-17-0606.

Aspelagh, S., Morel, D., Soria, J.C., Postel-Vinay, S., 2018. Epigenetic modifiers as new immunomodulatory therapies in solid tumors. Ann. Oncol. 29, 812–824. https://doi.org/10.1093/annonc/mdy50.

Casey, S.C., Amedei, A., Aquilano, K., Azmi, A.S., benecia, F., Bhakta, D., Bilsland, A.E., Boosani, C.S., Chen, S., Ciriolo, M.R., Crawford, S., Fujii, H., Georgakilas, A.G., Guha, G., et al., 2015. Cancer prevention and therapy through the modulation of the tumor microenvironment. Sem. Cancer Biol. 35, S199–S223.

Castellano, D., Sepulveda, J.M., Garcia-Escobar, I., Rodriguez-Antolin, A., Sundlov, A., Cortes-Funes, H., 2011. The role of RANK-ligand inhibition in cancer: the story of denosumab. Oncologist 16 (2), 136–145.

Chen, N.J., Huang, M.W., Hsieh, S.L., 2001. Enhanced secretion of IFN-gamma by activated Th1 cells occurs via reverse signaling through TNF-related activation-induced cytokine. J. Immunol. 166 (1), 270–276.

Christoph, F., König, F., Lebentrau, S., Jandrig, B., Krause, H., Strenziok, R., Schostak, M., 2018. RANKL/ RANK/OPG cytokine receptor system: mRNA expression pattern in BPH, primary and metastatic prostate cancer disease. World J. Urol. 36 (February (2)), 187–192. https://doi.org/10.1007/s00345-017-2145-y. Epub 2017 Dec 4.

Chu, C.Y., Chung, L.W., 2014. RANK-mediated signaling network and cancer metastasis. Cancer Metastasis Rev. 33, 497–509.

Coleman, R.E., Finkelstein, D., Martin, M., Iwata, H., Glaspy, J.A., Zhou, Y., Jandial, D., Chan, A., 2018. Adjuvant denosumab in early breast cancer: first results from the international multicenter randomized phase III placebo controlled study. J. Clin. Oncol. 36, S501.

Cross, S.S., Harrison, R.F., Balasubramanian, S.P., Lippitt, J.M., Evans, C.A., Reed, M.W., Holen, I., 2006. Expression of receptor activator nuclear factor kappa beta ligand (RANKL) and tumor necrosis factor related apoptosis inducing ligand (TRAIL) in breast cancer, and its relations with osteoprotegrin, estrogen receptor and clinicopathological variables. J. Clin. Pathol. 59, 716–720.

De Castro, J., Garcia, R., Garrido, P., Isla, D., Massuti, B., Blanca, B., Vazquez, J., 2015. Therapeutic potential of denosumab in patients with lung cancer: beyond prevention of skeletal complications. Clin. Lung Cancer 16, 431–446.

de Groot, A.F., Appelman-Dijkstra, N.M., van der Burg, S.H., Kroep, J.R., 2018. The anti-tumor effect of RANKL inhibition in malignant solid tumors - A systematic review. Cancer Treat. Rev. 62 (January), 18–28. https://doi.org/10.1016/j.ctrv.2017.10.010. Epub 2017 Nov 2. Review.

Faget, J., Contat, C., Zangger, N., Peters, S., Meylan, E., 2018. RANKL signaling sustains primary tumor growth in genetically engineered mouse models of lung adenocarcinoma. J. Thorac. Oncol. 13 (March 3), 387–398. https://doi.org/10.1016/j.jtho.2017.11.121.

Gnant, M., Pfeiler, G., Steger, G., Egle, D., Greil, R., Fitzal, F., Wette, V., Haslbauer, F., Melbiner, E., et al., 2018. Adjuvant denosumab in early breast cancer: Disease-free survival analysis of 3425 postmenopausal patients in the ABCSG-18 trial. J. Clin. Oncol. 218 (36), S500.

Gonzalez-Suarez, E., Sanz-Moreno, A., 2016. RANK as a therapeutic target in cancer. FEBS J. 283, 2018–2033.
Gonzalez-Suarez, E., Jacob, A.P., Jones, J., Miller, R., Roudier-Meyer, M.P., Erwert, R., et al., 2010. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature 468, 103–107.
Goswami, S., Sharma-Walia, N., 2016. Osteoprotegerin rich tumor microenvironment: implications in breast cancer. Oncotarget 5; 7 (July 27), 42777–42791.
Hanley, D.A., Adachi, J.D., Bell, A., Brown, V., 2012. Denosumab: mechanism of action and clinical outcomes. Int. J. Clin. Pract. 66 (December12), 1139–1146.
Herrera Pena, R., Lei, X., Gregor, Chavez-Mac, Zhao, H., Hermes Giordano, S., Suarez-Almazor, 2018. Overall survival in female Medicare beneficiaries with early stage breast cancer receiving bisphosphonates or denosumab. J. Clin. Oncol. 36, S530.
Heymann, M.F., Riet, A., Le Goff, B., Battaglia, S., Paineau, J., Heymann, D.O.P.G., 2008. RANK and RANK ligand expression in thyroid lesions. Regul. Pept. Suppl. 148, 46–53.
Ikeda, T., Kasai, M., Utsuyama, M., Harikowa, K., 2001. Determination of three isoforms of the receptor activator of nuclear factor-kappa B ligand and their differential expression in bone and thymus. Endocrinology 142, 1419–1426.
Lacey, D.L., Boyle, W.J., Simonet, W.S., Kostenuik, P.J., Dougall, W.C., Sullivan, J.K., et al., 2012. Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab. Nat. Rev. Drug Discov. 11 (5), 401–419.
Lam, J., Nelson, C.A., Ross, F.P., Teitelbaum, S.L., Fremont, D.H., 2001. Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity. J. Clin. Invest. 108 (7), 971–979.
Li, H., Hong, S., Qian, J., Zheng, Y., Yang, J., Yi, Q., 2009. Cross talk between the bone and immune systems: osteoclasts function as antigen-presenting cells and activate CD4+ and CD8+ T cells. Blood 116, 210–217.
Liu, Y., Wang, J., Ni, T., Wang, L., Wang, Y., Sun, X., 2016. CCL20 mediates RANK/RANKL-induced epithelial-mesenchymal transition in endometrial cancer cells. Oncotarget 18, 25328–25339. https://doi.org/10.18632/oncotarget.8291.
Luan, X., Lu, Q., Jiang, Y., Zhang, S., Wang, Q., Yuan, H., et al., 2012. Crystal structure of human RANKL complexed with its decoy receptor osteoprotegerin. J. Immunol. 189 (1), 245–252.
Luo, J., Yang, Z., Ma, Y., Yue, Z., Lin, H., Qu, G., Huang, J., Dai, W., Li, C., Zheng, C., Xu, L., Chen, H., Wang, J., Li, D., Siwko, S., Penninger, J.M., Ning, G., Xiao, J., Liu, M., 2016. LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. Nat. Med. 22, 539–546. https://doi.org/10.1038/nm.4076. Epub 2016 Apr 11.
Ma, D., Chang, L.Y., Zhao, S., Zhao, J.J., Xiong, Y.J., Cao, F.Y., Yuan, L., Zhang, Q., Wang, X.Y., Geng, M.L., Zheng, H.Y., Li, O., 2017. KLF5 promotes cervical cancer proliferation, migration and invasion in a manner partly dependent on TNFRSF11a expression. Sci. Rep. 16;7 (November1), 15683. https://doi.org/10.1038/s41598-017-15979-1.
Millian, M.M., 2015. The role of estrogen receptor in bone cells. Clin Rev. Bone Mineral Metab. 13, 105–112.
Min, J.K., Kim, Y.M., Kim, Y.M., Kim, E.C., Gho, Y.S., Kang, I.J., LEE, S.Y., Kong, Y.Y., Kwon, Y.G., 2003. Vascular endothelial growth factor upregulates expression of receptor activator of NF-kB (RANK) in endothelial cells: concomitant increase of angiogenic responses to RANK ligand. J. Biol. Chem. 278, 39548–39557.
Mizutani, Y., Matsubara, H., Yamamoto, K., Nan Li, Y., Mikami, K., Okihara, K., Kawauchi, A., Bonavida, B., Miki, T., 2004. Prognostic significance of serum osteoprotegrin levels in patients with bladder carcinoma. Cancer 101, 1794–1802.
Naumnik, W., Płońska, I., Ossolińska, M., Nikliński, J., Naumnik, B., 2018. Prognostic Value of Osteoprotegerin and sRANKL in Bronchoalveolar Lavage Fluid of Patients with Advanced Non-small Cell Lung Cancer. Adv. Exp. Med. Biol. 1047, 1–6. https://doi.org/10.1007/5584_2017_111.
Palafox, M., Ferrer, I., Pellegrini, P., Vila, S., Hernadez-Ortega, S., Urricoechea, A., Climent, F., Soler, M.T., Munoz, P., Vinals, F., Tometsko, M., Branstetter, D., Dougall, W.C., Gonzalez-Suarez, E., 2012. RANK induces epithelial-mesenchymal transition and stem ness in human mammary epithelial cells and promotes tumorigenesis and metastasis. Cancer Res. 72 (11), 2678–2688.
Papanastasiou, A.D., Sirinian, C., Kalofonos, H.P., 2012. Identification of novel human receptor activator of NF-kB isoforms generated through alternative spicing: implications in breast cancer cell survival and migration. Breast Cancer Res. 1, R112.
Papanastasiou, A.D., Sirinian, C., Plakoula, E., Zolota, V., Zarkadis, I.K., Kalofonos, H.P., 2018. RANK and EGFR in invasive breast carcinoma. J. Cancer Genet. Biomark. 216-217, 61–66. https://doi.org/10.1016/j.cancergen.2017.07.004. Epub 2017 Jul 26.
Park, H.R., Min, S.K., Cho, H.D., Kim, D.H., Shin, H.S., 2003. Expression of osteoprotegerin and RANK ligand in breast cancer bone metastasis. J. Korean Med. Sci. 18 (4), 541–546.
Pellegrini, P., Cordero, A., Gallego, M.I., Dougall, W.C., Munoz, P., Pujana, M.A., Gonzalez-Suarez, E., 2013. Constitutive activation of RANK disrupts mammary cell fate leading to tumorigenesis. Stem Cells 31, 1954–1965.
Reid, P.E., Brown, N.J., Holen, I., 2009. Breast cancer cells stimulate osteoprotegerin production by endothelial cells through direct cell contact. Moll Cancer 8 (49). https://doi.org/10.1186/1476-4598-8-9.
Renema, N., Navet, B., Heymann, M.F., Lezot, F., Heymann, D., 2016. RANK-RANKL signaling in cancer. Biosci. Rep. 36https://doi.org/10.1042/BSR20160150. e00366/.
Santana-Davila, R., Show, L.Q., 2018. The use of combination immunotherapies as frontline therapy for non-small cell lung cancer. Future Oncol. https://doi.org/10.2217/fon-2017-0124.
Sarink, D., Schock, H., Johnson, T., Overvad, K., Holm, M., Tjønneland, A., Boutron-Ruault, M.C., His, M., Kvaskoff, M., Boeing, H., Lagiou, P., Papatesta, E.M., Trichopoulou, A., Palli, D., Pala, V., Mattiello, A., Tumino, R., Sacerdote, C., Bueno-de-Mesquita, H.B.A., van Gils, C.H., Peeters, P.H., Weiderpass, E., Agudo, A., Sánchez, M.J., Chirlaque, M.D., Ardanaz, E., Amiano, P., Khaw, K.T., Travis, R., Dossus, L., Gunter, M., Rinaldi, S., Merritt, M., Riboli, E., Kaaks, R., Fortner, R.T., 2017. Circulating RANKL and RANKL/OPG and breast Cancer risk by ER and PR subtype: results from the EPIC cohort. Cancer Prev. Res. Phila. (Phila) 10, 525–534. https://doi.org/10.1158/1940-6207.CAPR-17-0125.
Schneeweis, L.A., Willard, D., Milla, M.E., 2005. Functional dissection of osteoprotegerin and its interactions with receptor activator of NF-kappaB ligand. J. Biol. Chem. 280, 41155–41164.
Schramek, D., Leibbrandt, A., Sigl, V., Kenner, L., Pospisilik, J.A., Lee, H.J., Hanada, R., Joshi, P.A., Aliprantis, A., Glimcher, L., Pasparakis, M., Khokha, R., Ormandy, C.J., Widschwendter, M., Schett, G., Penninger, J.M., 2010. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. Nature 468, 98–102. https://doi.org/10.1038/nature09387.
Schultz, M.A., Hagan, S.S., Datta, A., Zhang, Y., Freeman, M.L., Sikka, S.C., Abdel-Mageed, A.B., Mondal, D., 2014. Nrf1 and Nrf2 transcription factors regulate androgen receptor transactivation in prostate cancer cells. PLoS One 22;9 (January 1), e87204. https://doi.org/10.1371/journal.pone.0087204.
Shang, W.Q., Li, H., Liu, L.B., Chang, K.K., Yu JJ, XieF., Li, M.Q., Yu, J.J., 2015. RANKL/RANK interaction promotes the growth of cervical cancer cells by strengthening the dialogue between cervical cancer cells and regulation of IL-8 secretion. Oncol. Rep. 34 (6), 3007–3016.
Sigl, V., Jones, L.P., Penninger, J.M., 2016. RANKL/RANK: from bone loss to the prevention of breast cancer. Open Biol. 6 (160230). https://doi.org/10.1098/rsob.160230.
Sisay, M., Mengistu, G., Edessa, D., 2017. The RANK/RANK/OPG system in tumorigeneis and metastasis of cancer stem cells: potential targets for anticancer therapy. Onco. Ther. 10, 3801–3810.
Smyth, M.J., Yagita, H., McArthur, G.A., 2016. Combination of anti-CTL-4 and anti-RANKL in metastatic melanoma. J. Clin. Oncol. 34, e104–6. https://doi.org/10.1200/JCO.2013.51.3572.
Song, F.N., Duan, M., Liu, L.Z., Wang, Z.C., Shi, J.Y., Yang, L.X., Zhou, J., Fan, J., Gao, Q., Wang, X.Y., 2014. RANKL promotes migration and invasion of hepatocellular carcinoma cells via NFkB mediated epithelial mesenchymal transition. Plos One 9, e108507. https://doi.org/10.1371/journal.pone.
Tan, W., Zhang, W., Strasner, A., Grivennikov, S., Cheng, J.Q., Hofman, R.M., Karin, M., 2011. Tumour infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signaling. Nature 470, 548–553.
Tang, Z.N., Zhang, F., Tang, P., Qi, X.W., Jiang, J., 2011. Hypoxia induces RANK and RANKL expression by activating HIF-1 alpha in breast cancer cells. Biochem. Biophys. Res. Commun. 408, 411–416.
Theolaire, S., Wittrant, Y., Kwantat, S., Fortun, Y., Redeni, F., Heymann, D., 2004. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiologic bone remodeling. Cytokine Growth Factor Rev. 15, 457–475.
Tilborghs, S., Corthouts, J., Verhoeven, Y., Arias, D., Rolfo, C., Trinh, X.B., van Dam, P.A., 2017. The role of Nuclear Factor-kappa B signaling in human cervical cancer. Crit. Rev. Oncol. Hematol. 120, 141–150. https://doi.org/10.1016/j.critrevonc.2017.11.001. Epub 2017 Nov 7.
Timotheadou, E., Kalogeras, K.T., Koliou, G.A., Wirtz, R.M., Zagouri, F., Koutras, A., Veltrup, E., Christodoulou, C., Pentheroudakis, G., Tsiftsoglou, A., Papakostas, P., Aravantinos, G., Venizelos, V., Pectasides, D., Kosmidis, P., Karanikiotis, C., Markopoulos, C., Gogas, H., Fountzilas, G., 2017. Evaluation of the prognostic value of RANK, OPG, and RANKL mRNA expression in early breast Cancer patients treated with anthracycline-based adjuvant chemotherapy. Transl. Oncol. 10 (4), 589–598. https://doi.org/10.1016/j.tranon.2017.05.006.
Tsubaki, M., Takeda, T., Yoshizumi, M., Ueda, E., Itoh, T., Imano, M., Satou, T., Nishida, S., 2016. RANK-RANKL interactions are involved in cell adhesion-mediated drug resistance in multiple myeloma cell lines. Tumour Biol. 37 (July 7), 9099–9110. https://doi.org/10.1007/s13277-015-4761-8. Epub 2016 Jan 14.
Tuyaerts, S., Van Nuffel, A.M.T., Naert, E., van Dam, P., Vuylsteke, P., De Caluwé, A., Aspeslagh, S., Dirix, P., Lippens, L., De Jaeghere, E., Amant, F., Vandecasteele, K., 2018. Denys. PRIMMO study: a phase II study combining PD-1 blockade, radiation and immunomodulation to tackle cervical and uterine cancer. (paper submitted).
van Dam, P.A., van Dam, P.H., Rolfo, C., Giallombardo, M., Van Berckelaer, C., Trinh, X.B., Altintas, S., Huizing, M., Papadimitriou, K., Tjalma, W.A., Van Laere, S., 2016. In silico pathway analysis in cervical carcinoma reveals potential new targets for treatment. Oncotarget 7 (3), 2780–2795. https://doi.org/10.18632/oncotarget.6667.
van Dam, P.A., Rolfo, C., Ruiz, R., 2017. Neoadjuvant trials can accelerate research on novel systemic treatment modalities in cancer of the uterine cervix. Eur. J. Surg. Oncol. 43 (December 12), 2245–2247. https://doi.org/10.1016/j.ejso.2017.09.018.
van Dam, P.A., Rolfo, C., Ruiz, R., Pauwels, P., Van Berckelaer, C., Trinh, X.B., Ferri Gandia, J., Bogers, J.P., Van Laere, S., 2018. Potential new biomarkers for squamous carcinoma of the uterine cervix. ESMO Open 28;3 (June 4). https://doi.org/10.1136/esmoopen-2018-000352. e000352.
Vinay, D.S., Ryan, E.P., Pawelec, G., Talib, W.H., Stagg, J., Elkord, E., Lichtor, R., Decker, W.K., Whelan, R.L., Shanta Kumara, H.M., Signori, E., Honoki, K., et al., 2015. Immune evasion in cancer: mechanistic basis and therapeutic strategies. Seminars Cancer Biol. 35, S185–198.
Wada, T., Nakashima, T., Hiroshi, N., Penninger, J.M., 2006. RANKL-RANK signaling in osteoclastogenesis and bone disease. Trends Mol. Med. 12 (1), 17–25.
Wang, J., Zhang, H., Wang, Y., Li, Y., 2015. MPA influences tumor cell proliferation, migration and invasion induced by RANKL through PRB involving the MAPK pathway in endometrial cancer. Oncol. Rep. 33, 799–809.
Wong, B.R., Josien, R., Lee, S.Y., Sauter, B., Li, H.L., Steinman, R.M., Choi, Y., 1997. TRANCE, a new TNF family member predominantly expressed in T cells, is a dendritic cell specific survival factor. J. Exp. Med. 186, 2075–2080.
Yamada, T., Tsuda, M., Takahashi, T., Totsuka, Y., Shindoh, M., Ohba, Y., 2011. RANKL expression specifically observed in vivo promotes epithelial mesenchymal transition and tumor progression. Am. J. Pathol. 178, 2845–2856.

Zhang, L., Teng, Y., Fan, Y., Wang, Y., Li, W., Shi, J., Ma, Y., Li, C., Shi, X., Qu, X., Liu, Y., 2015. The E3 ubiquitin ligase Cbl-b improves the prognosis of RANK positive breast cancer patients by inhibiting RANKL-induced cell migration and metastasis. Oncotarget 6 (26), 22918–22933.

Zhang, S., Wang, X., Li, G., Chong, Y., Zhang, J., Guo, X., et al., 2017. Osteoclast regulation of osteoblasts via RANKRANKL reverse signal transduction in vitro. Mol. Med. Rep. 16 (4), 3994–4000.

Zhang, X., Song, Y., Song, N., Zhang, L., Wang, Y., Li, D., Wang, Z., Qu, X., Liu, Y., 2018. RANKL expression predicts poor prognosis in gastric cancer patients: results from a retrospective and single-center analysis. Braz. J. Med. Biol. Res. 11;51 (January 3), e6265. https://doi.org/10.1590/1414-431X20176265.