Inhibition of receptor activator of nuclear factor kappa-B ligand pathway for the management of aggressive osteosarcoma

Ioannis Panagiotidis[1], Dimitrios Christoulas[2], Evangelos Terpos[1]

[1]Department of Clinical Therapeutics, Alexandra General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [2]Department of Hematology, 251 General Air-Force Hospital, Athens, Greece

Correspondence to: Evangelos Terpos. Department of Clinical Therapeutics, Alexandra General Hospital, School of Medicine, National and Kapodistrian University of Athens, 80 Vas. Sofias Avenue, 11528, Athens, Greece. Email: eterpos@med.uoa.gr; eterpos@hotmail.com.

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Receptor activator of nuclear factor kappa-B ligand (RANKL) is a member of the tumor necrosis factor receptor superfamily and is considered the most important factor for osteoclastogenesis. RANKL is encoded by a gene, which is found at chromosome 13q14 and is expressed mainly by osteocytes, activated T-cells and bone marrow stromal cells. Alternative splicing of the RANKL mRNA results either in the expression of a type-II transmembrane glycoprotein or in the expression of a soluble ligand. Soluble RANKL may also be produced from its transmembrane state by the function of metalloproteinases. RANKL acts through its binding to the receptor RANK which is on the surface of mature osteoclasts but also of osteoclast precursors and chondrocytes. The RANKL/RANK binding induces the activity of mature osteoclasts, while it inhibits their apoptosis. Furthermore, the binding of RANKL to RANK enhances the differentiation of osteoclast precursors, their fusion and the formation of mature osteoclasts through the NF-κB, c-fos and Jun N-terminal kinase pathways (1). RANKL is implicated in the pathogenesis of bone loss in several disorders including osteoporosis, malignant disorders (bone metastases of solid tumors and multiple myeloma) as well as metabolic bone diseases (1-3). Osteoprotegerin (OPG) is the decoy receptor of RANKL which is produced mainly by the osteoblasts and is often elevated to balance the RANKL overexpression in several bone malignancies. Among primary bone tumors, RANKL expression and the RANKL/OPG ratio were very high in giant cell tumor of the bone, while high RANKL mRNA expression was observed in cases of osteosarcoma, chondrosarcoma, and enchondroma, as compared to cases of multiple myeloma and bone lesions from metastatic cancer (4). More specifically for osteosarcoma, the expression of RANK and RANKL was tested in 91 human osteosarcomas tumor samples. Sixty-three osteosarcomas (69%) expressed RANK, while only 8 cases (9%) expressed RANKL. Interestingly, the expression of RANK was significantly associated with shorter disease-free survival and worse response to chemotherapy, while RANKL expression was more frequent in osteosarcoma of the lower extremity than in any other location (5). These data suggest that inhibiting RANKL seems to be a logical approach for the management of primary bone tumors. To-date, the only inhibitor of RANKL that has entered to clinical development, denosumab, has been licensed not only for the treatment of bone metastases due to solid tumors but also for the management of giant cell tumor of the bone. However, there is no data for the efficacy of denosumab in osteosarcoma patients.

In a recent study, Chen *et al*. reported that "RANKL blockade prevents and treats aggressive osteosarcomas" in animal models (6). Although osteosarcoma is a relative uncommon cancer, it is the most common malignant primary bone tumor and a main cause of cancer-related

death in children and adolescents (7). Long-term survival for patients with localized osteosarcoma has improved over the last decades from 20%, five decades before, to 60–80%, primarily due to novel induced multi-agent chemotherapy and gradually improved surgical techniques. Despite the advances in the management of osteosarcoma, long-term overall survival for patients with metastatic osteosarcoma at diagnosis remains as low as 15–30%. Current therapeutic approaches are generally based on various factors such as tumor entity, tumor stage, age, gender, general condition, quality of life, life expectancy and others. Modern management includes surgery, chemotherapy and palliative radiotherapy. Although the standard of care combines neoadjuvant and adjuvant chemotherapy with surgical treatment, radiotherapy is administered with a surgical resection in some cases (7). The aim of surgery in osteosarcoma is to perform as much tumor removal as possible (within a wide area of normal tissue) to avoid local recurrence and improve overall survival. The extent of surgical resection and its optimal margin is determined per Enneking's tumor staging and consists of several options such as amputation (not a first choice anymore due to advances made in chemotherapy, surgical techniques, surgical devises and diagnostic methods), limb salvage with endoprosthetic or biological reconstruction, rotationplasty etc. The choice is based on tumor grade, location and response to neoadjuvant chemotherapy (8). However, despite the advantages of surgical techniques the local recurrence rate in patients with no metastatic osteosarcoma has been reported as high as 46% (9). Chemotherapy remains the most common treatment for patients with OS since 1970. The standard protocol for multi-agent chemotherapy consists of doxorubicin, cisplatin and high dose methotrexate (MTX) with leukovorin-rescue (MAP), ± ifosfamide, which provides approximately 70% overall survival for patients with primary OS (10). Novel agents such as pemetrexed, although less toxic than MTX and effective for other malignant tumors, did not induce apoptosis more effectively than MTX in osteosarcoma cell lines *in vitro*, while *in vivo* pemetrexed did not enhanced anti-tumor activity (11,12). Liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE) is the first new agent approved for the treatment of non-metastatic osteosarcoma in the last 30 years. A recent randomized trial demonstrated that L-MTP-PE with MAP and ifosfamide significantly improved the 6-year overall survival of patients with primary osteosarcoma from 70% to 78%; but this was not the case for metastatic disease (13). Conventional radiotherapy plays a minor role, since osteosarcoma is a relative radio-resistant tumor. Therefore, the use of radiotherapy is limited in the treatment of primary osteosarcoma and is usually not applied as first choice. Prophylactic radiation for lung metastasis has not demonstrated to have a clear benefit and is accompanied with high percentage of serious complications. On the other hand, radiation therapy can be used as an effective palliative option for painful bone metastases (14). For patients with metastatic osteosarcoma at diagnosis MAP ± ifosfamide remains the only effective first line treatment with no satisfactory alternative so far. Therefore, new targeted therapies are needed for the treatment of metastatic osteosarcoma.

Osteosarcoma most commonly arises in the metaphyseal region of long bones, within the medullary cavity, and penetrates the cortex of the bone to involve the surrounding soft tissues (15). A pseudocapsule is formed around the penetrating tumor. Histologically, osteosarcoma is characterized as a highly cellular tumor composed of pleomorphic spindle-shaped cells capable of producing osteoid matrix. Recent developments in molecular biology have provided insight into the molecular pathogenesis of osteosarcoma. Several tumor suppressor genes are implicated in the development of osteosarcoma. Retinoblastoma (*RB*) tumor suppressor gene and tumor protein 53 (*p53*) gene seem to play a major role in the development of osteosarcoma. Indeed, osteosarcoma is the second most common tumor in those altered by *RB*. In some studies, loss of heterozygosity of *RB* at 13q was detected in up to 60% of tumors. Moreover, *RB* has been established as a poor prognostic factor in osteosarcoma and occurs frequent in high grade osteosarcoma (16). Osteosarcoma is also the second most common cancer where *p53* mutations are detected (the rate is ranged from 40–60%) (17). Apart from gene mutations other factors seem to play a major role in the development of osteosarcoma. These include parathyroid hormone, vascular endothelial factor, fibroblast growth factor, protein kinase, insulin-like growth factor receptor, granulocyte-macrophage colony-stimulating factor, interferons, human epidermal growth factor receptor 2, platelet-derived growth factor and others (15). Several studies attempted to use the above molecules as potential therapeutic targets but unfortunately these strategies have shown very little or no therapeutic advantage (7).

Osteosarcoma cell invasion into the bones relies extensively in the increased osteoclastic activity. The substantial osteolysis which is present in some osteosarcomas

is the direct result of increased osteoclastic activity due to the interactions between osteosarcoma cells and osteoclasts. But is the production of RANKL by osteosarcoma cells the main mediator of osteoclastogenesis in this entity? As mentioned before, less than 10% of osteosarcoma expressed RANKL in immunohistochemistry (5). Is this enough to produce a significant and sustained osteoclast activation? In contrast to the above published results, a recent study demonstrated that RANKL expression was observed in 68% of human osteosarcomas using immunohistochemistry also (18). How can this difference between the two studies be explained? In the later study, despite the higher number of cases that expressed RANKL, the staining intensity was relatively low and only 37% of samples exhibited more than 10% RANKL positive tumor cells. Despite the low intensity of RANKL in several cases, the observation that RANKL is expressed in osteosarcoma cells themselves suggests that osteosarcoma cells may mediate an osteoclastic response, and anti-RANKL or other anti-osteoclast therapy may potentially benefit osteosarcoma patients (18). Nevertheless, bisphosphonates, a class of drugs that target mature osteoclasts and induce their apoptosis, when they were used in combination with conventional chemotherapy they did not improve the outcome of patients with osteosarcoma (7).

Denosumab is a fully human antibody that inhibits RANKL in the extracellular milieu (in a similar way to that of OPG function) and directly prevents RANKL binding to RANK at the molecular level (19). Denosumab prevents maturation of osteoclast precursors, promotes apoptosis of mature, multinucleated osteoclasts and thus inhibits the osteoclastic formation and activity. Denosumab is used for the management of postmenopausal osteoporosis and of other causes of benign osteoporosis. Furthermore, in a phase 3 study, denosumab has been shown to be more effective than zoledronic acid in the treatment of bone metastasis in patients with breast cancer (20). In another phase 3 study, denosumab was superior in comparison to zoledronic acid for the delay or prevention of skeletal-related events in patients with advanced prostate cancer. This study also showed that the RANKL/RANK pathway played a significant role in bone metastases in patients with prostate cancer even though these metastases have a predominantly osteoblastic appearance (21). Finally, in patients with lung cancer and bone metastasis denosumab was associated with significantly improved overall survival compared with zoledronic acid (22). So far, apart from osteoporosis, denosumab has been approved by FDA for the treatment of bone disease in patients with breast cancer or solid tumors and is also under investigation for bone disease in hematologic malignancies such as multiple myeloma. The results from a major phase 3 clinical study, which compared denosumab with zoledronic acid for the treatment of bone disease in patients with multiple myeloma are soon to be released. Regarding primary bone tumors, denosumab has shown remarkable effects on giant cell tumor of the bones, with objective response rates up to 88% (23,24). Thus, in giant cell tumor of the bone, which is a primary osteolytic bone tumor, rich in osteoclast like giant cells and increased RANKL expression, denosumab was granted FDA approval in June 2013 (24).

In the recent study by Chen *et al.*, the authors used RANKL blockade for the management of osteosarcoma in animal models (6). Initially, the authors showed the role of regulatory subunit *Prkar1a* gene as a major bone tumor suppressor gene. They have also shown that the loss of a single *Prkar1a* allele plays a major role in osteosarcomagenesis regardless of either RB or protein p53 status in genetically engineered mouse model (GEMM). In these models, loss of a single *Prkar1a* allele resulted in increased RANKL levels, which in turn were associated with increased osteosarcoma aggressiveness. On the other hand, GEMM with homozygous RANKL deletion exhibited delayed tumor initiation, prolonged life span and fewer metastatic nodules in the lungs, which were related to the inactivation of osteoclastogenesis in this group. These findings demonstrated the important functional contribution of RANKL/RANK induced osteoclastogenesis in osteosarcoma. Based on the above findings, the authors then tested RANKL blockade by RANK-Fc as a therapeutic agent in GEMMs with aggressive osteosarcoma. The results also included a 50% extension of life span with the RANK-Fc treatment in these highly aggressive models of osteosarcoma. In addition, spontaneous lung metastasis (the leading cause of death in patients with osteosarcoma) was more than 3 times less in RANK-Fc-treated mice.

In conclusion, the data of the study by Chen *et al.* have confirmed the role of RANKL in the natural progress of both local and metastatic disease in osteosarcoma. Furthermore, they provide solid evidence that RANKL blockade with RANK-Fc administration has therapeutics results not only in the evolution of local done disease but in the prevention of remote metastasis as well in GEMMs. In our opinion these findings rationalize the conduction of a large human trial to assess the efficacy of denosumab as targeted therapy in patients with osteosarcoma.

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Footnote

Conflicts of Interest: E Terpos has received honoraria by Amgen. The other authors have no conflicts of interest to declare.

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