RANKL inhibitors for osteosarcoma treatment: hope and caution

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Chen and collaborators propose RANKL blockade for the treatment and prevention of aggressive RANKL-overexpressing osteosarcoma (OS) in humans. Here we comment the main findings, putative applications and concerns.

OS is the most common primary bone cancer, which occurs primarily in children and adolescents, severely affecting survivors' quality of life. Current treatment for newly diagnosed OS includes three main components: preoperative chemotherapy, surgical resection, and postoperative chemotherapy (1). This strategy has improved the outcome of patients with localized OS. However, patients with advanced, metastatic, and recurrent OSs continue to experience a quite poor prognosis. After aggressive treatment with both surgery and chemotherapy, the 5-year survival rate for OS patients with localized disease is about 65%, whereas it is less than 20% for patients with metastases. The use of adjuvant chemotherapy provides no survival advantage for patients with pulmonary metastases (2). So that, it is of particular importance to develop molecularly targeted therapy to treat patients with this metastatic bone malignancy, on the basis of in-depth understanding of signaling mechanisms involved in OS tumorigenesis.

Bone growth and turnover are regulated by receptor-activator of nuclear kappa B (RANK) and its ligand (RANKL). RANKL is expressed by cells of the osteoblast lineage in the bone stroma as well as osteocytes; it binds its cognate receptor, RANK, expressed on osteoclasts and osteoclast precursors and induces osteoclastogenesis. Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL which acts as a dominant negative of the pathway. During bone metastasis, tumor cells that invade the bone secrete factors that stimulate the production of RANKL by stromal cells and osteoblasts. RANKL-RANK signaling in osteoclast precursors triggers osteoclastogenesis and bone destruction, which in turn releases growth factors to attract and stimulate tumor cell growth, propagating a "vicious cycle" between bone destruction and tumor expansion. Multiple clinical trials demonstrate that Denosumab, a fully human monoclonal antibody specific to RANKL, prevents and attenuates bone metastasis by interfering with this cycle (3).

In this work Chen and colleagues have generated a series of genetically engineered mouse models (GEMMs) combining deletions of retinoblastoma (RB), tumor protein p53 (TP53) and Prkar1a. The group of Dr. Khokha had previously shown that Prkar1a acts as a tumor suppressor in OS pathogenesis, and its deletion drives RANKL overexpression during OS tumorigenesis (4). Different combination of deletions of these three genes generated different grade of aggressiveness in OS. The most aggressive tumors showed high levels of RANKL and low expression of RANK in tumor cells; therefore RANKL was postulated to play a critical role in aggressive osteosarcomagenesis. To test this hypothesis the authors used an additional mouse model MOTO GEMM, in which SV40 T antigen oncoprotein binds and inactivates RB and p53. This model develops aggressive OS similar to those developed after Prkar1a deletion. From this GEMM a new model MOTO-Rankl$^{-/-}$ was generated and compared with its control MOTO-Rankl$^{+/+}$. The absence of RANKL caused a marked suppression of OS development, indicating that RANKL

is a critical protein for OS tumorigenesis. RANK deletion in the osteoblast lineage, where the osteoblastic RANKL expression remains intact, exerts a negligible effect on OS; while RANK deletion in osteoclast, led to delayed tumor initiation, prolonged life span, and fewer metastatic nodules in the lung. These data indicate that blockage of RANK/RANKL would have an indirect effect on tumor development through of inhibition of osteoclasts. Moreover, Chen *et al*. demonstrated that PTEN is modulated by RANKL-RANK signaling through NF-kB in OS cells supporting a direct effect of RANK signaling blockage in OS tumor development. The therapeutic benefit of RANKL inhibition in OS was tested pharmacologically using RANK-Fc, which binds RANKL and inhibits the pathway. RANK-Fc administered immediately after detection of the tumor, prolonged and extended survival and decreased lung metastasis. RANK-Fc administration before tumors detection blocked tumor development. However, upon termination of RANK, osteoclastogenesis was reinitiated and tumors developed implying that treatment cannot be interrupted.

In view of these encouraging results the authors suggest the use of RANKL inhibitors, such as Denosumab for the treatment of aggressive RANKL-overexpressing OS and prevention of heritable OS in children bearing germline mutations in the *TP53* and *RB* genes. However, it is important to take into account that a fine regulation of RANK pathway is essential for normal bone physiology, which is particularly critical in children and adolescents who are the ones suffering OS. The effect of Denosumab on the developing skeleton has not been established. RANKL signaling blockage could provoke severe growth defects in this age. As shown in this manuscript long-term RANK-Fc treatment prevented tumor formation, but also increased bone density, indicative of osteopetrosis. Osteopetrosis is a bone disease that makes bones abnormally dense and prone to breakage. Thus, using Denosumab in this age group could lead to undesired side effects, more aggravated than those observed in adults. The optimal use and long-term effects of Denosumab in the young population primarily affected by OS needs to be defined. Denosumab brings hope for patients with OS, but side effects should be carefully evaluated, doses readjusted and a personalized exhaustive control should be kept.

Moreover, it has been recently published that although RANKL expression was observed in 68% of human OS using IHC, the staining intensity was relatively low and only 37% of samples exhibited equal or mayor 10% RANKL positive tumor cells; moreover, RANK expression was not observed in OS tumor cells (5). These data cast doubt on the effectiveness of RANKL blockage in human OS. The intensity and frequency of RANKL and RANK staining in OS samples were substantially lower than that observed in giant cell tumor of bone samples, in which RANKL expression and the therapeutic efficacy of Denosumab have recently been reported (6). All results showed in the paper were based in mouse models that exhibited OS with high expression of RANKL. Therefore, before supplying this treatment in humans the expression of RANKL should be assessed in each case, which leads to a personalized treatment again. It is possible that only tumors showing high expression of RANKL would be susceptible to be treated with Denosumab. The anti-RANKL therapy may be especially important for protection against bone pathologies driven by RANKL-expressing OS tumor cells able to mediate an osteoclastic response (5). However, the absence of RANK expression in primary human OS cells suggests that an autocrine RANKL/RANK signaling in human OS tumor cells is not operative, and that the anti-RANKL therapy would not directly affect the tumor cells. This seems a different scenario to that observed in breast cancer where RANK expression on tumor cells is found in a significant percentage of patients (7,8). In this setting, the preventive and therapeutic benefit of RANKL inhibition is explained by its direct effects on RANK-expressing tumor cells. RANK-Fc treatment reduces proliferation and survival of mammary epithelial cells in early stages of tumorigenesis and causes tumor cell differentiation in aggressive adenocarcinomas (9,10).

Denosumab is now to be tested in a phase 2 trial in patients with relapsed or refractory OS (https://clinicaltrials.gov/ct2/show/NCT02470091) and compared to historical Children's Oncology Group experience. This trial will reveal if the use of this antibody could improve the prognosis of this disease, either indirectly by blocking osteoclastogenesis, or directly, impacting tumor cells. Results from this clinical trial will shed light into the risk-benefit balance of Denosumab as a treatment option in OS.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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