When tumor cells home to bone they secrete factors that stimulate osteoclast formation, resulting in increased bone resorption. This increases the release of factors from bone matrix that stimulate the growth of tumor cells, leading to a vicious cycle characterized by extensive bone loss and enhanced tumor growth in bone. Thus, factors that inhibit osteoclast differentiation or activity may have the potential to inhibit the vicious cycle. The RANKL inhibitor denosumab is approved for treatment of bone metastases from solid tumors, and the cathepsin K inhibitor odanacatib has been shown to suppress bone resorption in breast cancer patients with bone metastases.

**Materials and Methods**

Cell culture

- Osteoclasts were cultured on Secure bone matrix for 7 days in the presence of M-CSF and RANKL, and allowed to differentiate into bone-resorbing osteoclasts. After completion of osteoclast differentiation at day 7, the culture medium was removed and new culture medium was added into the wells. The mature osteoclasts were then cultured for an additional 3 days, allowing them to resorb bone. In the osteoclast differentiation assay, denosumab (Prolia, Amgen) was added in the culture medium at day 0, and the cultures were stopped at day 7. In the osteoclast activity assay, the cells were cultured without test compounds for 4 days, followed by medium change at day 7 and addition of odanacatib (CintraTec). The cultures were stopped at day 10.

**Results / Osteoclast differentiation**

![Figure 1: The effects of denosumab on differentiation of human osteoclasts in vitro. A) TRACP 5b activity (UL) measured at day 7. NS = not significant, p<0.05. **p<0.001 compared to the baseline (BL) group. B) EC50 value determination (EC50 = 0.124 µg/ml). C) and D) Representative microscopic images of the effects of denosumab on formation of TRACP-positive multinuclear osteoclasts at day 7. C) Baseline (no added compounds), D) Denosumab, 0.3 µg/ml, magnification x 100. Denosumab blocks osteoclast differentiation at a stage where the mononuclear osteoclast precursor cells are TRACP positive, and the multinuclear osteoclast precursor cells do not secrete active TRACP 5b enzyme into the culture medium.](image)

**Results / Osteoclast activity**

![Figure 2: The effects of odanacatib on resorption activity of human osteoclasts in vitro. A) CTX values determined at the end of the resorption period at day 10. NS = not significant. **p<0.001 compared to the baseline (BL) group. B) EC50 value determination (EC50 = 0.483 µM). C) and D) Representative microscopic images of the effects of odanacatib on resorption pit formation (TRITC-labeled wheat germ agglutinin lectin staining) at day 10. C) Baseline (no added compounds), D) Odanacatib 0.1 µM, magnification x 100. The strong inhibition of bone resorption by odanacatib is partly due to decreasing the depth of the formed resorption pits, which is not seen in the microscopic images, indicating that CTX measurements detect more reliably total effects on resorption.](image)