# Denosumab and Odanacatib as Reference Compounds in Human Osteoclast Cultures

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**Jussi M. Halleen, Jenni Bernoulli, Jukka P. Rissanen, Katja M. Fagerlund.**
**Pharmatest Services Ltd, Turku, Finland**
*E-mail correspondence to jussi.halleen@pharmatest.com*

## Introduction

Human osteoclasts can be generated from bone marrow-derived CD34+ mesenchymal stem cells in the presence of macrophage colony-stimulating factor (M-CSF) and ligand for receptor activator of nuclear factor kappa B (RANKL).[1] The culture system can be conveniently used in preclinical efficacy studies for testing compounds targeted to inhibit osteoclast differentiation or activity.[2]

## Aim of the Study

Our goal was to optimize separate *in vitro* culture systems for determining osteoclast differentiation and activity, and validate the RANKL inhibitor denosumab and the cathepsin K inhibitor odanacatib as reference inhibitors of osteoclast differentiation and activity, respectively.

## Materials and Methods

**Cell culture**
CD34+ human osteoclast precursor cells (Poietics® Human Osteoclast Precursors, Lonza) were cultured on bovine bone slices for 7 days in the presence of M-CSF and RANKL[2] 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 cultures at day 0, and the cultures were stopped at day 7. In the osteoclast activity assay, the cells were cultured without test compounds for 7 days, followed by medium change at day 7 and addition of odanacatib (ChemieTek). The cultures were stopped at day 10.

**Endpoint measurements**
In the osteoclast differentiation assay, tartrate-resistant acid phosphatase 5b activity (TRACP 5b, BoneTRAP® assay, IDS Ltd) was measured in the culture medium collected at day 7 as an index of the number of formed osteoclasts.[2,3] In the osteoclast activity assay, C-terminal cross-linked telopeptides of type I collagen (CTX-I, CrossLaps® for culture ELISA, IDS Ltd) was measured in the culture medium collected at day 10 to quantitate bone resorption during days 7-10.

**Statistical analysis**
Statistical analysis was performed with statistical software R (version 3.1.2, www.r-project.org) using one-way ANOVA. Because statistically significant differences were observed (p<0.001 between all groups) the results of all other groups were compared separately with the results of the baseline group using Tukey's HSD test.

## Results / Osteoclast differentiation

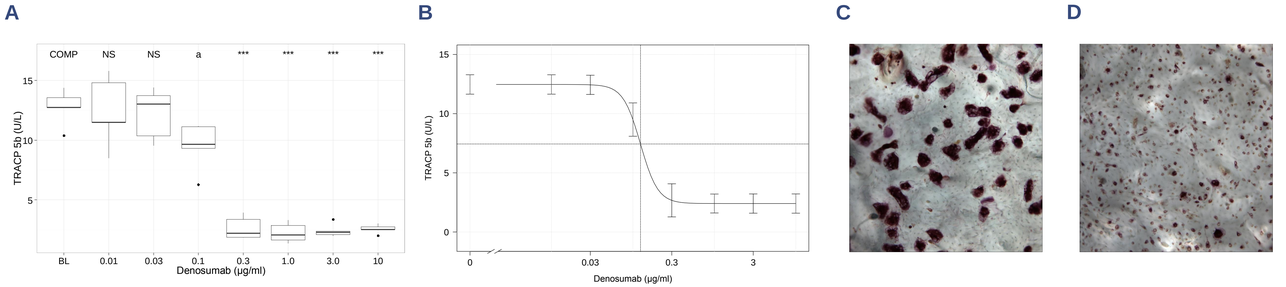


**Figure 1:** The effects of denosumab on differentiation of human osteoclasts *in vitro*. A) TRACP 5b activity (U/L) measured at day 7. COMP = Comparator group in statistics, NS = not significant, [a]statistically non-significant trend with p-value <0.1, ***p<0.001 compared to the baseline (BL) group; 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 mononuclear osteoclast precursor cells do not secrete active TRACP 5b enzyme into the culture medium.

## Results / Osteoclast activity

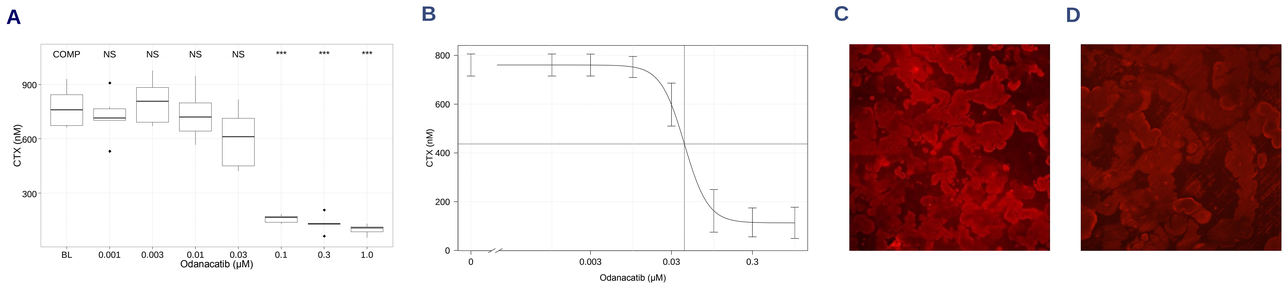


**Figure 2:** The effects of odanacatib on resorption activity of human osteoclasts *in vitro*. A) CTX values determined at the end of the resorption period at day 10. COMP = Comparator group in statistics, NS = not significant, ***p<0.001 compared to the baseline (BL) group; B) EC50 value determination (EC50 = 0.0433 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.

## Summary

- Denosumab and odanacatib showed strong concentration dependent inhibition of osteoclast differentiation and activity, respectively.
- EC50 value was 0.124 µg/ml for denosumab and 0.0433 µM for odanacatib.
- Denosumab and odanacatib are useful reference compounds of human osteoclast differentiation and activity, respectively.

## Conclusions

We conclude that we have validated denosumab as a reference compound of osteoclast differentiation and odanacatib as a reference compound of osteoclast activity in a human *in vitro* osteoclast culture system, and the culture system is a reliable and clinically predictive tool for identifying new osteoporosis drug candidates with anti-resorptive activity.

## Acknowledgements

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## References

1. Pierelli L, Scambia G, d'Onofrio G et al. Generation of multinuclear tartrate-resistant acid phosphatase positive osteoclasts in liquid culture of purified human peripheral blood CD34+ progenitors. Br J Haematol 1997;96:64-9.
2. Rissanen JP, Ylipahkala H, Fagerlund KM et al. Improved methods for testing antiresorptive compounds in human osteoclast cultures. J Bone Miner Metab 2009;27:105-9.
3. Alatalo SL, Halleen JM, Hentunen TA et al. Rapid screening method for osteoclast differentiation in vitro that measures tartrate-resistant acid phosphatase 5b activity secreted into the culture medium. Clin Chem 2000;46:1751-4.

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