# *In Vitro* Osteoblast Assays for Studying the Effects of Cancer Therapeutics on Bone Biology

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## Introduction

Bone cell activity is an important factor in regulating bone metastases from tumor cells in the skeletal environment. When cancer cells home to bone they secrete factors that stimulate osteoclast activity leading to increased bone resorption. Bone then releases stimulatory factors that in turn promote the growth and proliferation of cancer cells. This process is called the vicious cycle often leading to osteolytic lesions in bone when osteoclastic bone resorption exceeds osteoblastic bone formation. Osteolytic lesions are common in breast and lung cancer and multiple myeloma. There is a vicious cycle between cancer cells and bone cells also in the context of osteoblastic lesions. In osteoblastic metastases, the vicious cycle observed in osteolytic disease takes place but in addition to this cancer cells produce osteoblast-stimulating factors including bone morphogenetic protein (BMP), epidermal growth factor (EGF) and platelet derived growth factor (PDGF). Osteoblasts also influence osteoclasts by producing RANKL, which stimulates osteoclast differentiation. Many cancer patients with bone metastasis have both osteolytic and osteoblastic lesions. This is common for example in prostate cancer.

The aim of the study was to establish an *in vitro* cell culture model to study the effects of compounds on osteoblast differentiation and activity.

## Materials and Methods

A mouse osteoblast progenitor cell line KS483 (1, 2) was used in the study. BMP-2 (Peprotech) and 17β-estradiol (E2; Sigma-Aldrich) were used as test substances and were added in the beginning of the culture and simultaneously with culture medium change every 3-4 days. Ascorbic acid was added into the cell culture medium at day 4. In the osteoblast differentiation assay, the activity of alkaline phosphatase (ALP) in cell lysates was measured at day 8 as a marker of osteoblast differentiation (3). In the osteoblast activity assay, KS483 mouse osteoprogenitor cells were cultured for 13 days and N-terminal propeptide of type I procollagen (PINP) secreted into the culture medium was determined at day 11 (Rat/Mouse PINP EIA, IDS Ltd) to demonstrate effects on organic bone matrix formation. β-glycerophosphate was added to the culture at day 11. The cultures were stopped at day 13 by removing the culture media from the wells and adding hydrochloric acid. Calcium deposition, a marker of inorganic bone formation, was determined at the end of the study (Calcium Assay Kit, Cayman Chemical).

## References

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## Results

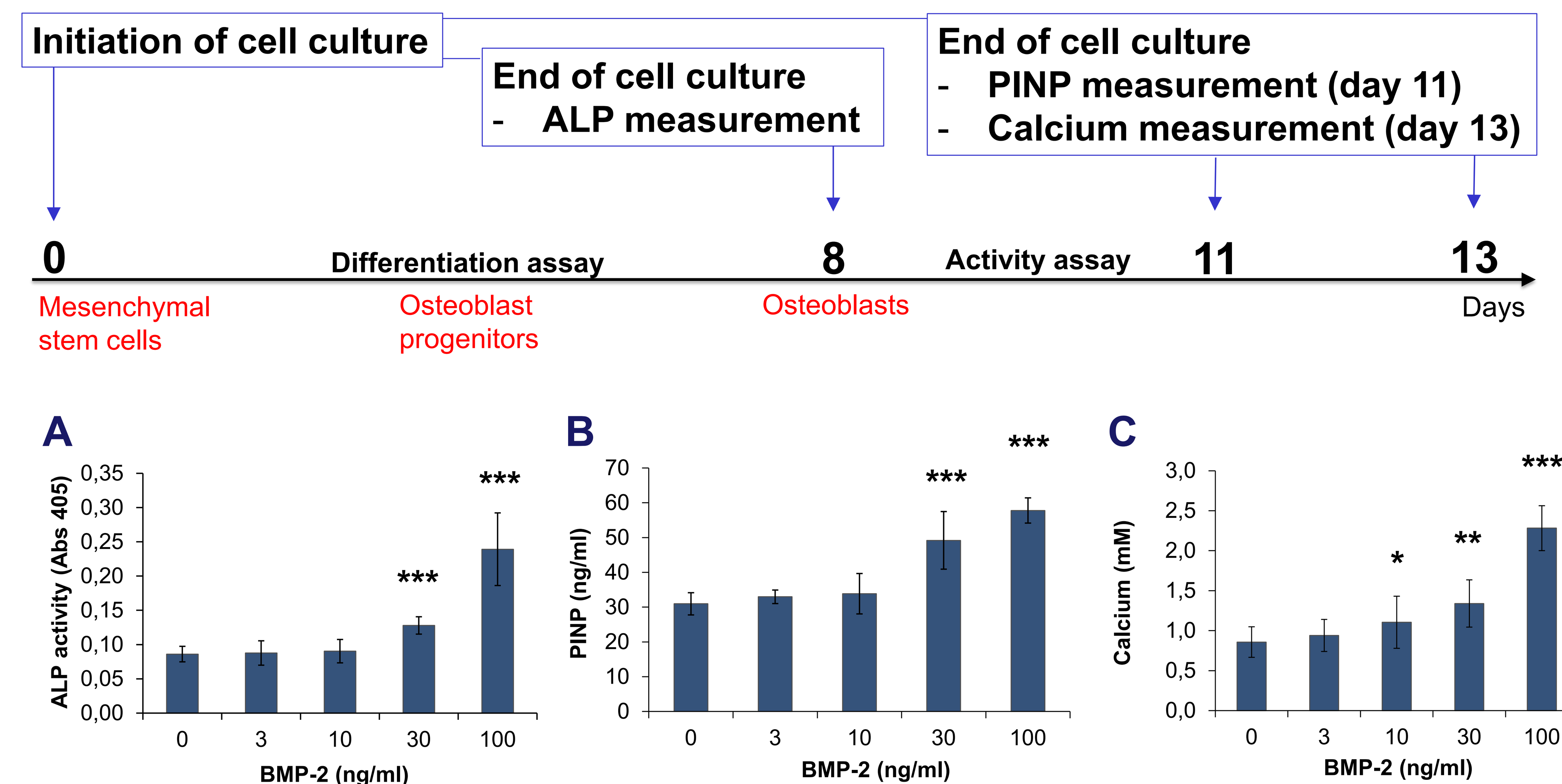


**Figure 1.** Effects of BMP-2 on osteoblast differentiation and activity. A) The results of osteoblast differentiation assay are shown as cellular ALP activity (Abs 405 nm) measured from cell lysates at day 8. B) PINP secreted into the culture medium at day 11 indicates organic bone formation, and C) calcium deposition at day 13 is a measure of inorganic bone formation. * p < 0.05; ** p < 0.01; *** p < 0.001.

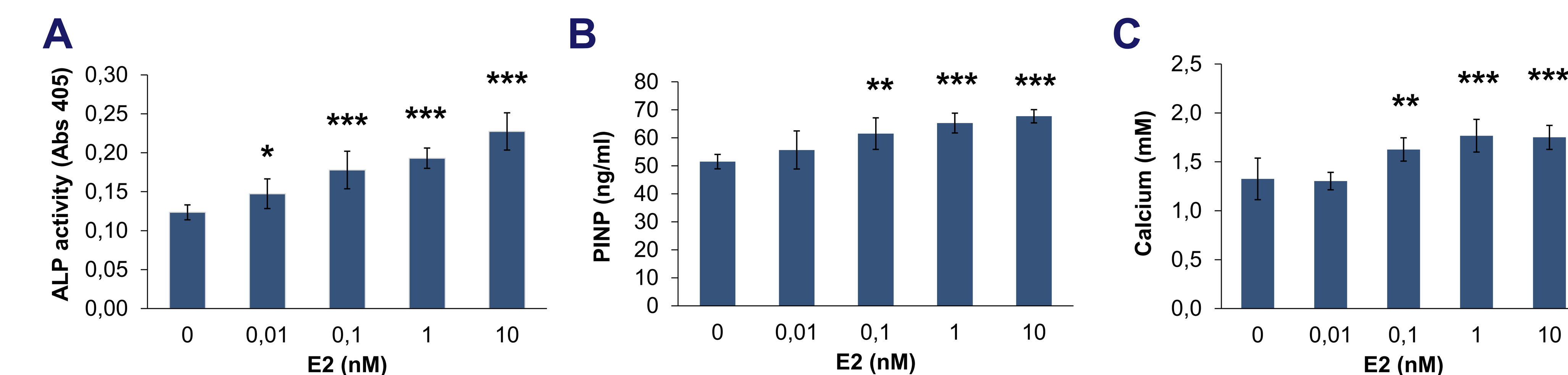


**Figure 3.** Effects of E2 on osteoblast differentiation and activity. A) The results of osteoblast differentiation assay are shown as cellular ALP activity (Abs 405 nm) measured from cell lysates at day 8. B) PINP secreted into the culture medium at day 11 indicates organic bone formation, and C) calcium deposition at day 13 is a measure of inorganic bone formation. * p < 0.05; ** p < 0.01; *** p < 0.001.

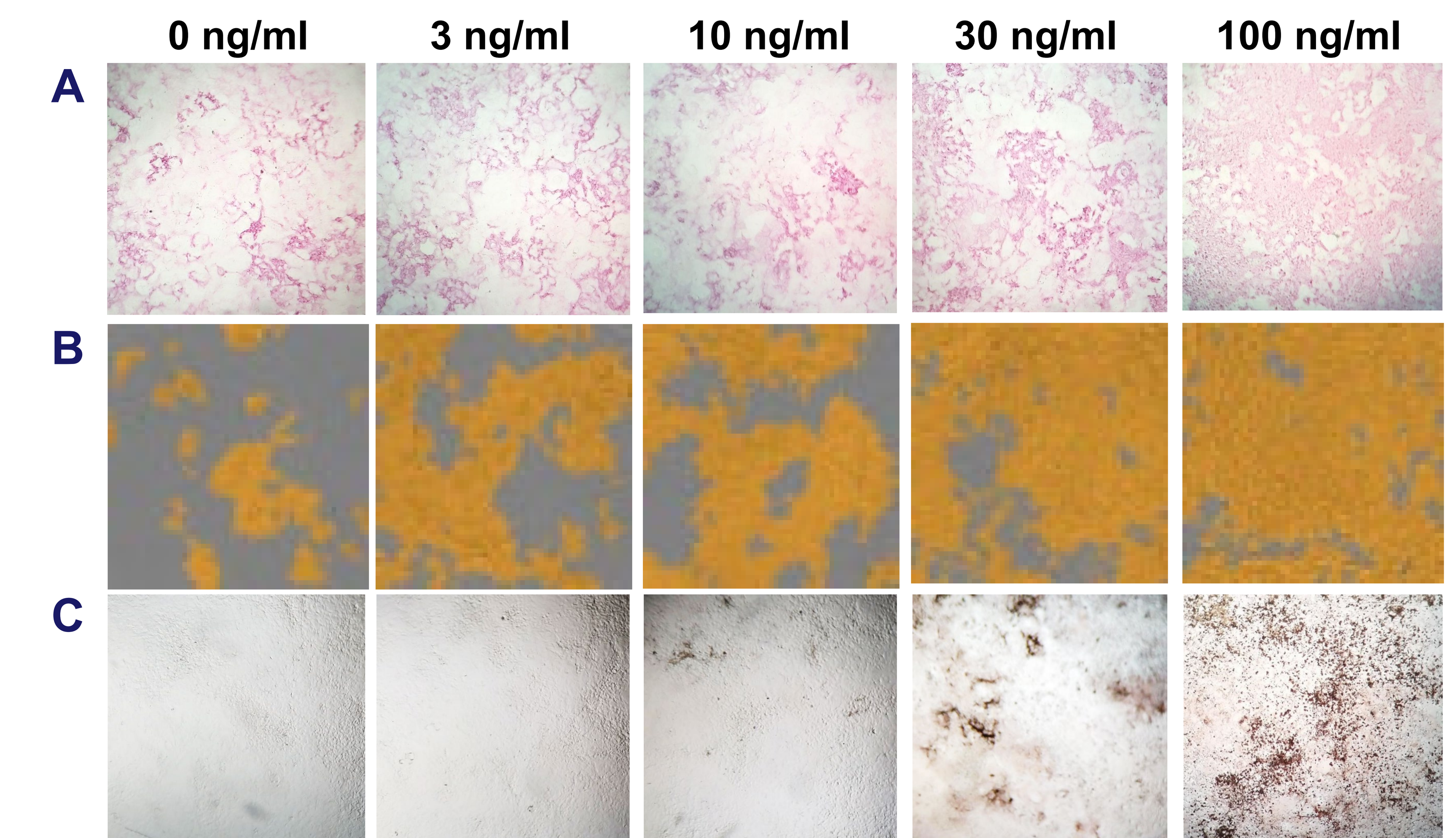


**Figure 2.** Effects of BMP-2 on osteoblast differentiation, nodule formation and mineralization. After 8 or 13 days of culture, the cells were fixed with 3% PFA. A) Osteoblast differentiation was visualized by ALP staining (Sigma-Aldrich) at day 8. B) At day 13, osteoblast cultures were imaged by IncuCyte live cell imager (Sartorius) to visualize bone nodule formation. The orange color indicates the masking of bone nodules. C) Mineralization at day 13 was visualized by von Kossa method.

## Conclusions

**BMP-2 and E2 stimulated osteoblast differentiation and activity shown by the increase in ALP, PINP and calcium levels. The results suggest that the KS483 cell line can be used for setting up reliable *in vitro* models of osteoblast differentiation and activity. These osteoblast assays can be used for studying the effects of compounds on bone formation and cancer related bone events.**

## Acknowledgements

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