Introduction:
Breast cancer has a high predisposition to metastasize to bone and induces a marked osteoclast-mediated bone destruction. However, osteoblasts indirectly participate in the process of tumor-induced osteolysis. Tumor cells can activate osteoblasts notably through the secretion of PTHrP that acts on osteoblasts and increase RANKL/OPG expression that will induce osteoclast differentiation and activity.
Osteoblasts are actually vital cells for the control of bone turnover. Immature osteoblasts regulate the differentiation and the activity of osteoclasts, while mature osteoblasts produce bone matrix (collagen synthesis and mineralization).
Human bone marrow-derived mesenchymal stem cells (MSC) are pluripotent stem cells that can be differentiated into many mesodermal lineages, including chondroblasts, adipocytes or osteoblast lineages (osteoprogenitor, osteoblast and osteocyte) (Figure 1), by appropriate stimulations. The effect of cancer cells on osteoblast differentiation is unknown.

Material and Methods:
MSC- Fn from 9 donors were isolated from fresh healthy bone marrow specimens and identified by expression of SH2 and SH3 domains. Undifferentiated MSC were expanded in alpha MEM supplemented with 10% FCS without any change in their differentiation potential. Briefly, MSC were seeded under subconfluence and then trypsinized and replated at low density for all subsequent passages (P1, P2, P3).

Results:
Five days after seeding of mononuclear cells, monocyte and round hematopoietic cells are present and spindle-shaped fibroblast like cells appear adherent (Figure 2, A). The differential attachment to plastic allows the selection of MSCs (strongly attached to the plastic dishes). After 2 weeks, homogeneous fibroblast-like MSCs are present in the cultures (Figure 2, B). The treatment of MSCs for 21 days with DAG medium induces osteogenic differentiation, as revealed by calcium deposits (Figure 2, C) and the von Kossa reaction or alcian red staining. The alkaline phosphatase (ALP) activity was measured using p-Nitrophenyl phosphate as substrate (LabAssay™ALP Wako) and semi-quantitative RT-PCR for osteoblast-typical markers (osteopontin (OPN), ALP and the PTH receptor) (Qiagen Multiplex) were also performed.

In summary, describes an in vitro model of DAG-induced osteogenic differentiation of bone marrow mesenchymal stem cells, using various osteoblastic markers. Our data indicate the need to use MSC during the second and third cell passages. The more passaged cells may lose their ability of differentiation into osteoblast. Human MSC should constitute a useful tool to study the effects of osteotropic cancer cells on osteoblast differentiation and to better understand the inhibition of normal bone formation during the process of tumor-induced osteolysis.