Establishment of a Novel ex vivo Bone Metastasis Culture Platform for Triple-negative Breast Cancer

Tiina E Kähkönen[1], Saif Wahid[2], Sangita Kushary[2], Debabani Roy Chowdhury[2], Arnab Roy Chowdhury[2], Jussi M Halleen[1]

1) OncoBone Ltd, Finland; 2) Mestastop Solutions Pvt Ltd, India; *Contact tiina.kahkonen@oncobone.com for further information*

AACR ANNUAL MEETING 2026
April 17 - 22, 2026
San Diego Convention Center
San Diego, California

Introduction

Drug development for bone metastasis has been hindered by the lack of appropriate in vitro models in early testing. Typical in vitro models contain only cancer cells or are co-cultures of cancer cells and another cell type. The problem with such cultures is that they lack the crucial bone microenvironment, which may lead to wrong conclusions about efficacy of experimental therapies on bone metastases in vivo.

In this study, we established a novel bone metastasis ex vivo culture platform including bone discs from tibiae of mice with 4T1 triple-negative breast cancer (TNBC) tumors.

Materials and Methods

Female Balb/c mice were injected intratibially with 10,000 (10k) or 20,000 (20k) 4T1 mouse TNBC cells or PBS. Tumor-induced bone changes were monitored by X-ray imaging for up to 14 days.

At designated timepoints, 4T1 tumor-containing bones (4T1 tumors) and bones injected with PBS (control) were collected and cut into 2-3 mm thick discs, rinsed with antibiotic-containing medium, and cultured in basal medium (RPMI-1640 medium supplemented with 10% FBS) in a humidified incubator.

Different cell culture conditions were tested to optimize the growth conditions. Osteoblastic medium included ascorbic acid and beta-glycerophosphate, and osteoclastic medium included M-CSF and RANKL supplements. The culture medium was partially replenished every 72 hours. Culture lengths from 7-21 days were tested.

The cultures were analyzed for cell viability (trypan blue), proliferation (CCK-8 assay), bone resorption (TRACP5b ELISA) and bone formation (PINP EIA and Alizarin S Red staining).

Results

Overview of the ex vivo Bone Metastasis Culture Platform

Figure 1. Overview of the ex vivo Bone Metastasis Culture Platform. 1) 4T1 mouse breast cancer cells are inoculate to bone marrow of immunocompetent female Balb/c mice; 2) Tumor-induced bone changes are monitored by X-ray imaging; 3) Bones are extracted and cut to 2-3 mm thick discs; 4) The bone discs are placed to cell culture with growth medium; 5) Different cell culture conditions are explored, including osteoblastic and osteoclastic culture mediums; 6) At endpoint, the ex vivo cultures are analyzed with different methods, including microscopy.

Step 1: Bone metastasis material

Key findings: Intratibial inoculation of 10k and 20k 4T1 cells induced progressive osteolytic lesions that were monitored for 14 days by X-ray imaging. Based on the extent of osteolytic lesions, days 3-5 were selected to model early bone metastasis growth with visible tumor-induced effects on bone, and 20k 4T1 cells were selected for future studies due to more uniform tumor growth.

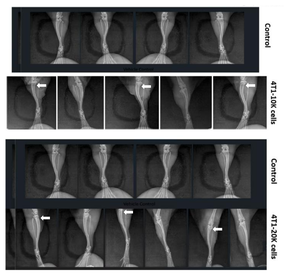

Figure 2: X-ray images from control bone injected with PBS and 10k (upper panel) or 20k (lower panel) 4T1 tumor cells. The X-ray images at day 5 show small osteolytic lesions that are more consistent in mice injected with 20k 4T1 cells. Therefore, 20k 4T1 cells were selected for subsequent studies. Some osteolytic lesions are marked with white arrows in the images.

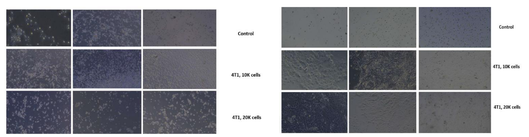

Figure 3: Microscopy images of control cultures and cultures originating from mice inoculated with 10k and 20k 4T1 tumor cells. Images on the left show cultures after 3 days and images on the right after 5 days culture period in vitro. The images were taken from areas where some of the tumor cells had migrated out from the bone discs, which allowed them to be imaged by standard microscopy techniques.

Step 2: Cell viability

Key findings: In ex vivo cultures of 4T1 tumor and control bones in basic growth medium, cell viability remained high at days 7 and 10 (88 and 91%, respectively) but dropped to 47% at day 14. Therefore, 10 days was considered maximum culture length. In discs with 4T1 tumors, cell doubling time was lower than in control discs.

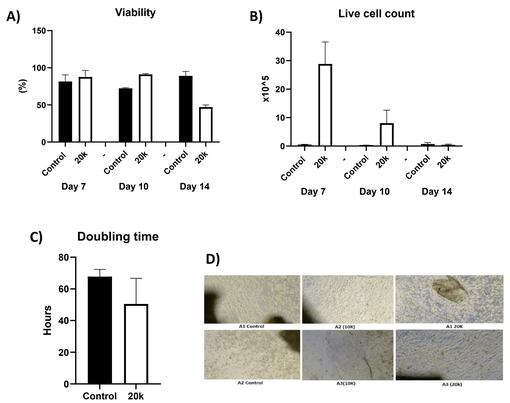

Figure 4: A) Cell viability and B) live cell count in control and 4T1 tumors at 7, 10 and 14 days in culture. C) Cell doubling time in control and 4T1 tumor-bearing bone discs after 48h in culture. D) Representative microscopy images. The images were taken from areas where some of the tumor cells had migrated out from the bone discs, which allowed them to be imaged by standard microscopy techniques.

Step 3: Bone cell activity

Key findings: Addition of osteoblastic supplements increased PINP levels in control discs and in discs including 4T1 tumors at day 10, after which the values decreased. Increased Alizarin Red staining was observed at day 10. Addition of osteoclastic supplements increased TRACP5b levels at days 10 and 14, after which the values decreased. Similar increase was not observed when adding both osteoblastic and osteoclastic supplements. Discs with 4T1 tumors had higher baseline TRACP5b levels than control discs, and the values were further increased with osteoclastic and both osteoclastic and osteoblastic supplements.

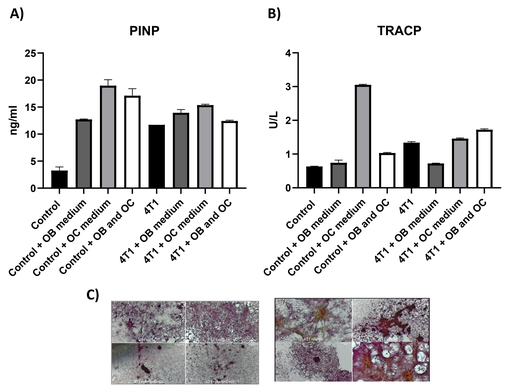

Figure 5: A) Bone formation evaluated by measuring PINP and B) number of osteoclasts evaluated by measuring TRACP5b levels released into the culture medium after 10 days in culture. C) The cultures presented areas of induced bone formation, which was visualized by Alizarin Red staining after 2 weeks in culture in control discs (left) and 4T1 tumors (right).

Conclusions

We have established a novel ex vivo bone metastasis culture platform where cancer cell, osteoclast and osteoblast activities were maintained after in vivo extraction of bone discs.

The ex vivo cultures were able to maintain in vivo properties of the tumor and its bone microenvironment.

We conclude that this platform provides a miniaturized ex vivo culture system for studying bone metastases.

Further validation, including testing known therapies in the platform, are currently being conducted.

References

Molla MS, Katti DR, Katti KS. In vitro design of mesenchymal to epithelial transition of prostate cancer metastasis using 3D nanoclay bone-mimetic scaffolds. J Tissue Eng Regen Med. 2018 Mar;12(3):727-737. doi: 10.1002/term.2492. Epub 2017 Oct 4. PMID: 28603879.

Salamanna F, Borsari V, Brogini S, Giavaresi G, Parrilli A, Cepollaro S, Cadossi M, Martini L, Mazzotti A, Fini M. An in vitro 3D bone metastasis model by using a human bone tissue culture and human sex-related cancer cells. Oncotarget. 2016 Nov 22;7(47):76966-76983. doi: 10.18632/oncotarget.12763. PMID: 27765913; PMCID: PMC5363563.

Kar S, Molla MS, Katti DR, Katti KS. Tissue-engineered nanoclay-based 3D in vitro breast cancer model for studying breast cancer metastasis to bone. J Tissue Eng Regen Med. 2019 Feb;13(2):119-130. doi: 10.1002/term.2773. Epub 2019 Jan 7. PMID: 30466156.

Han W, El Botty R, Montaudon E, Malaquin L, Deschaseaux F, Espagnolle N, Marangoni E, Cottu P, Zalcman G, Parrini MC, Assayag F, Sensebe L, Silberzan P, Vincent-Salomon A, Dutertre G, Roman-Roman S, Descroix S, Camonis J. In vitro bone metastasis dwelling in a 3D bioengineered niche. Biomaterials. 2021 Feb;269:120624. doi: 10.1016/j.biomaterials.2020.120624. Epub 2020 Dec 24. PMID: 33421710.

Kähkönen TE, Halleen JM, Bernoulli J 2022 Preclinical Osteoimmuno-Oncology Models to Study Effects of Immunotherapies on Bone Metastasis. Chapter in the book Animal Models for the Development of Cancer Immunotherapy, pages 167-209. John Wiley & Sons, Inc

Kähkönen TE, Halleen JM, Bernoulli J 2021 Osteoimmuno-Oncology: Therapeutic Opportunities for Targeting Immune Cells in Bone Metastasis. Cells 10(6):1529