# Preclinical Bone Metastasis Technology Platform – Predictive Evaluation of Experimental Therapies on Bone Metastasis

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## Introduction

Most cancer deaths are due to metastases, and bone metastases are a considerable problem especially in breast and prostate cancer, being developed in 70-90% of advanced-stage patients. Despite major investments in oncology drug development, bone metastases are currently incurable and a high unmet medical need with only 5% of patients being alive 5 years after the diagnosis. Development of therapies for bone metastasis has been challenging due to lack of appropriate preclinical models, especially in the context of immuno-oncology, available to support decision making in next phases of drug development. The current strategy is to rely on preclinical efficacy data obtained with subcutaneous models that lack the clinically relevant local tissue microenvironment, which has a major impact on tumor growth in the bone. The use of clinically non-relevant models in preclinical-stage development is one important reason for the current >95% failure rate of oncology drugs in clinical trials. To support predictive evaluation of therapies for bone metastatic cancers, we describe a preclinical Bone Metastasis Technology Platform for evaluating efficacy of novel therapies on bone metastases.

## Materials and Methods

Syngeneic or humanized mouse models with tumor and immune cells of same species are needed for supporting development of immunotherapies. Such models can be used for studying interactions of tumor and immune cells in bone metastatic microenvironment, which is a requirement for developing effective therapies for bone metastases according to a novel osteoimmuno-oncology (OIO) concept (Kähkönen et al., 2021). The preclinical Bone Metastasis Technology Platform utilizes tumors growing in bone microenvironment, mimicking growth of bone metastases in patients. The Platform provides a predictive tool for studying unique biological features associated with different types of bone metastases and immune cell infiltration in cancer type -specific manner.

## Results

### SUPPORTING MATERIAL
### Preclinical modeling of metastatic disease

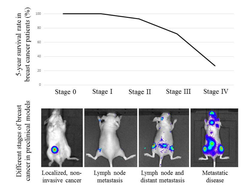


**FIG 1: Preclinical modeling of different stages of cancer.** Image from Kähkönen et al., 2022.

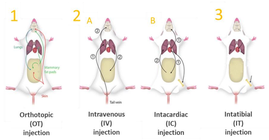


**FIG 2: Selection of model system based on the stage of cancer to be modelled.** Image modified from Daphu et al., 2013.

### Overview of the Preclinical Bone Metastasis Technology Platform

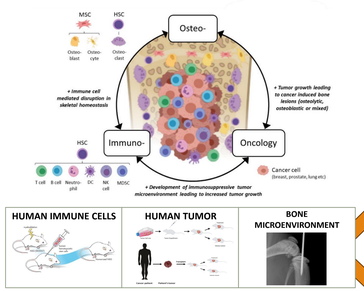


**FIG 3: Preclinical Bone Metastasis Technology Platform combines three important cellular components that affects growth of bone metastases: human immune cells from Human Immune System (HIS) mouse models, human tumor cells from cell line -derived xenografts (CDX) or patient-derived xenografts (PDX), and most importantly bone microenvironment where the cancer cells are inoculated to allow tissue-spesific interactions in the bone metastasis specific microenvironment.** Images from Kähkönen et al., 2021.

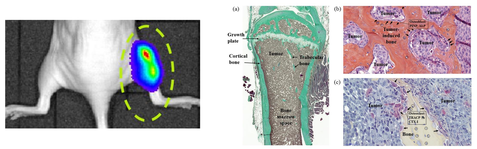


**FIG 4: Analysis of bone metastasis growth.** Bioluminescence imaging (BLI) can be used to assess tumor growth in vivo. Histological analysis reveals that bone marrow is filled with tumor cells that interact with bone-forming osteoblasts to form osteoblastic bone metastases, or with osteoclasts to induce rapid bone reportion and formation of osteolytic bone metastases. Images from Kähkönen et al., 2022.

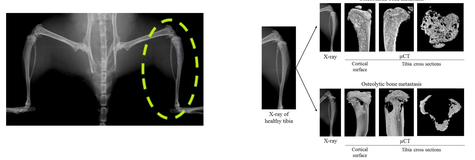


**FIG 5: Analysis of cancer-induced bone changes.** Imaging techniques such as X-ray and μ-computed tomography (μCT) can be used to assess tumor-induced bone growth in vivo. Cancer type -specific effects are observed in preclinical models and patients. In prostate cancer, osteoblastic bone metastases are typically observed, where bone marrow becomes filled with rapidly forming pathologic new bone causing bone pain. In breast cancer, osteolytic bone metastases with rapid resorption of bone are typically observed, leading to increased risk of fractures. Images from Kähkönen et al., 2022.

### SUPPORTING MATERIAL
### Use on HIS mice in assessing efficacy on metastases and safety of immunotherapies

**TABLE 1: Summary of effects of immunotherapies in HIS models of metastasis.** Table from Kähkönen et al., 2020.

| Cancer type | Preclinical oncology model | | Treatment | Anti-tumor effect on metastasis |
|---|---|---|---|---|
| | Model type | HIS model | | |
| TNBC | PDX model (sc) | CD34-NSG mice | Nivolumab and ipilimumab | Nivolumab anti-tumor effect, ipilimumab no effect |
| | PDX model (ot) | Autologous CD4 and CD8 T cells | BKM120 | Decreased number of lung metastasis |
| | CDX model (ot and it) | CD34-NOG mice | Pembrolizumab | Decreased primary tumor growth but no effect in bone metastases |
| Melanoma | CDX model (sc) | CD34-NOG mice | Pembrolizumab and ONCOS-102 | Anti-tumor effects in primary tumors, not effect on liver metastases |
| | CDX model (iv) | NK cell reconstituted NSG mice | MICA α3 antibody | Decreased number of lung and liver metastases |
| | PDX model (sc) | Autologous TILs in NOG and NSG mice | IL-2 | Eradication of metastasis growth |
| Bladder cancer | PDX model (sc) | Lymphocyte transplanted mice | Durvalumab | Decreased growth of metastasis |
| ACC | PDX model (sc) | CD34-BRGS mice | Pembrolizumab | Tumor growth inhibition |
| Osteo-sarcoma | CDX model (sc) | PBMC model | Nivolumab | No effect on primary tumor growth but lowered the number of lung metastases |
| Prostate cancer | CDX model (it) | CD34-NOG mice | Pembrolizumab | No effect in bone metastases |

**TABLE 2: Summary of adverse effects observed with different immunotherapies in HIS mice.** Table from Kähkönen et al., 2020.

| Model | Treatment | Adverse effects |
|---|---|---|
| BTL-NOG, BTL-NOG-EXL | Nivolumab | Pneumonitis, hepatitis, nephritis, dermatitis, adrenalitis |
| BTL-NSG, BTL-NRG BTL-NOG | Muromomab | Cytokine release syndrome |
| Transgenic CTLA-4 mice | Ipilimumab | Anemia, severe dilated cardiomyopathy, inflammation |
| CD34-BRGS | IL-2 | Body weight loss, ruffled fur, loose stool, splenomegaly, nephrotoxicity, pulmonary edema |
| CD34-NOG | Estradiol supplement and pembrolizumab | Anemia, increased mortality |

### Validations in prostate cancer

**Case Example: Radium-223 dichloride (Positive effect)**

**Aim:** To study effects of radium-223 dichloride on prostate cancer bone metastases

**Model:** CDX model using human prostate cancer cells inoculated into mouse bone marrow

**Results:** Decreased tumor growth, as evaluated by decreased serum PSA values and osteoblastic bone growth (Suominen et al., 2017)

**Clinical relevance:** Radium-223 dichloride has been approved for bone metastatic mCRPC, and it increases life expectancy of patients but does not cure the cancer.

**Case Example: Pembrolizumab (No effect)**

**Aim:** To study effects of anti-PD-1 antibody pembrolizumab on prostate cancer bone metastases

**Model:** CDX model using human prostate cancer cells inoculated into mouse bone marrow

**Results:** No effects on tumor growth, as evaluated by decreased serum PSA values and osteoblastic bone growth (Kähkönen et al., 2019a)

**Clinical relevance:** Pembrolizumab failed to demonstrate efficacy on patients with prostate cancer bone metastasis in 4 separate Phase III trials and the development for bone metastasis has been halted.

### Validations in breast cancer

**Case Example: Zoledronic acid (Positive effect)**

**Aim:** To study effects of the bisphosphonate zoledronic acid on breast cancer bone metastasis

**Model:** CDX model using mouse breast cancer cells inoculated into mouse bone marrow

**Results:** Prevented cancer-induced bone loss but had no effect on tumor growth (Kähkönen et al., 2019b)

**Clinical relevance:** Zoledronic acid is used in bone metastatic breast cancer patients to prevent cancer-induced bone loss.

**Case Examples: Pembrolizumab, IDO-inhibitor (No effects)**

**Aim:** To study effects of anti-PD-1 antibody pembrolizumab and an IDO-inhibitor on primary and bone metastatic triple-negative breast cancer (TNBC)

**Models:** Syngeneic model using mouse TNBC cells inoculated to bone marrow, and CDX model using human TNBC cells inoculated to breast (primary tumor) or bone marrow of humanized mice

**Results:** IDO inhibitor had no effect on bone metastasis growth in the syngeneic model (Kähkönen et al., 2019b). Pembrolizumab inhibited primary tumor growth in the humanized model, but had no effect on growth of bone metastases, which was due to lack of tumor-infiltrating lymphocytes in the bone microenvironment (Kähkönen et al., 2018).

**Clinical relevance:** Pembrolizumab has been approved for cancers including melanoma but failed in metastatic TNBC.

## Conclusions

We describe a preclinical Bone Metastasis Technology Platform and summarize case examples where results align with clinical findings of different therapies approved or evaluated for bone metastasis.

Radium-223 dichloride, an approved treatment for bone metastatic castration-resistant prostate cancer in patients, showed reduced prostate cancer growth and decreased tumor-induced bone changes.

Zoledronic acid, an anti-resorptive bisphosphonate currently used in breast cancer patients with bone metastases to prevent cancer-induced bone loss, showed improved bone health but no effects on tumor growth.

As for immunotherapies, an IDO1 inhibitor had no effects on breast cancer bone metastases and the anti-PD-1 antibody pembrolizumab had no effects on breast or prostate cancer bone metastases, predicting observed lack of efficacy in clinical breast and prostate cancer trials.

We conclude that the Bone Metastasis Technology Platform is a biologically relevant tool for preclinical evaluation of the efficacy of experimental therapies on bone metastasis. The technology has been validated with positive and negative case examples, demonstrating its clinically predictive power.

## Future directions

The preclinical Bone Metastasis Technology Platform will be used to assess the effects of our pipeline therapies. Our current pipeline includes three preclinical-stage immuno-modulating agents with proof-of concept data supporting efficacy on metastases.

***About OncoBone Ltd:***
OncoBone Ltd a Virtual Contract Research Organization (CRO) with over 20 year of experience in preclinical CRO business

***About OncoBone Ventures:***
OncoBone Ventures is a biotech startup company developing novel therapies for cancer patients with bone metastases.

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