Introduction

Bone metastases count 30-80% of metastases in the most common cancers including breast, lung and bladder cancer, and multiple myeloma. Despite recent progress in cancer treatment bone metastases remain incurable. However, novel therapies including immunotherapies have the potential to cure bone metastatic disease.

The aim of this study was to establish syngeneic models with a focus on bone metastasis that could be used in preclinical efficacy studies.

Materials and Methods

Syngeneic models were established for breast (4T1-GFP, ATCC), bladder (MBT-2, Riken) and lung cancer (KLN-205, Riken), and multiple myeloma (STG1, licensed from University of Texas Health Science Center at San Antonio) in Balb/c, C3H, DBA and KaluRij mice, respectively (all mice from Envigo). The cells were inoculated into systemic circulation (4T1 intratibially or STG1 into the tail vein) or into the bone marrow (MBT-2 and KLN-205). In the 4T1 model, tumor burden was imaged ex vivo by GFP (Lightools Research) and in the STG1 model, by measuring serum IgG2b paraprotein levels (Bethyl Laboratories Inc.). Tumor-induced bone changes were followed by X-ray imaging (Faxitron) in all models. At sacrifice, hind limbs were collected and analyzed by histology. Tumor-infiltrating lymphocytes (TILs; CD3: Springer Bioscience, CD4: Sino Biological, CD8: Bioz) were assessed by immunohistochemistry. The effects of standard-of-care (SOC) compounds were assessed in the 4T1 (cyclophosphamide, 100 mg/kg, Baxor; or zoledronic acid, 0.1 mg/kg, O1W, Hameln Pharmaceuticals) and STG1 (bortezomib, 1 mg/kg, Q3D, LC Laboratories) models. The effect of anti-PD-1 therapy (200 µg/dose, Q3D, BioXcell) was evaluated in the MBT-2 model.

Breast cancer - 4T1 intracardiac model

![Figure 1](image1.png)

**FIGURE 1.** A) Tumor burden by GFP analysis at endpoint (mm², mean ± SEM): Cyclophosphamide (Cyclo) treatment decreased the tumor burden (p<0.05) compared to vehicle-treated mice. B) Example GFP image of a vehicle-treated mouse showing metastasis. The arrow points to metastasis in the adipose gland. C) Total bone lesion area analyzed by X-ray images at endpoint (mm², mean ± SEM). Cyclo (p=0.001 ***) and zoledronic acid (ZOL; p=0.01 **) decreased the bone lesion area. D) Example image of osteolytic lesions (indicated by arrows) in tail and femur of a vehicle-treated mouse. E) IHC staining of CD3+, CD4+ and CD8+ T cells in tumors growing in bone, showing low to negative staining.

Lung cancer - KLN-205 intratibial model

![Figure 2](image2.png)

**FIGURE 2.** A) Total bone lesion area analyzed from X-ray images at indicated time points (mm², mean ± SEM). Anti-PD-1 treatment decreased bone lesion area (p=0.01 **). B) Representative endpoint X-ray images of isotype control and anti-PD-1 treated mice (osteolytic lesion pointed out by arrows). C) Tumor area (mm², mean ± SEM) quantitated from HE stained sections. D) Example IHC stainings showing high number of CD3+ and CD8+ cells and low number of CD4+ cells.

Conclusions

A high incidence of bone metastases was achieved in all models. The use of systemic models allows studying the effects of test compounds in preclinical treatment or treatment of metastases. Intraaxial models can be used when the primary interest is in cancer-induced changes or tumor growth in bone. Mimicking the clinical situation, none of the SOC compounds could prevent tumor growth completely, and therefore combination therapies are warranted for better overall efficacy.