Lack of Immunotherapy Efficacy in a Syngeneic Bone Metastasis Model of Triple-negative Breast Cancer

Tiina E. Kähkönen, Mari I. Suominen, Jenni H.E. Mäki-Jouppila, Jussi M. Halleen, Jenni Bernoulli
Pharmatest Services, Turku, Finland

E-mail correspondence to Tiina Kähkönen (tiina.kahkonen@pharmatest.com)

Introduction

Triple-negative breast cancer (TNBC) is an aggressive cancer with treatment options limited to standard-of-care chemotherapy. TNBC patients develop metastases in high incidence, and almost all patients have bone metastases at end-stage disease. Breast cancer bone metastases are mainly osteolytic, causing severe bone loss that can be prevented by bone-targeted agents. Currently, there is no effective cure for bone metastases. Activation of the patient’s own immune system by immunotherapies is widely studied in various cancers, but their efficacy on bone metastases is not well established.

In this study, we aimed to evaluate the efficacy of immunotherapies in comparison to standard-of-care compounds in a preclinical TNBC bone metastasis model.

Materials and Methods

Mouse 4T1-GFP (ATCC) TNBC cells were inoculated intracardially to immunocompetent female Balb/c mice (Envigo) to model bone metastasis. Treatment of single agents of chemotherapy (cyclophosphamide, Baxter, 100 mg/kg Q7D), bone-targeting agent (zoledronic acid, Hamein Pharmaceuticals, 0.1 mg/kg Q7D), and immunotherapies programmed cell death 1 (PD-1) antibody (mouse anti-PD-1, BioXcell, 10 mg/kg Q5D) and indoleamine-pyroline 2,3-dioxogenase (IDO) inhibitor (epacodastat, Selleck Chemicals, 100 mg/kg BID), and the combination of anti-PD-1 and IDO were started on the day following the cancer cell inoculations (n = 9-12 in each group). The study was terminated 12-13 days after the inoculations. Tumor burden was evaluated by GFP imaging (Lightools Research) and tumor-induced bone loss by X-ray imaging (Faxitron) at sacrifice. Tumor histology was evaluated by hematoxylin and eosin (HE) staining, and tumor infiltrating lymphocytes (TILs) were evaluated by immunohistochemical stainings of CD3+, CD4+, and CD8+ T cells of the tumors growing in bone in vehicle treated mice.

Study timeline

FIGURE 1. Timeline for the study. 4T1-GFP cells were inoculated intracardially at study day 0, and treatments were started at day 1. During the study, mouse well-being was followed by body weight recording and following clinical signs. The study was terminated at day 12-13, GFP imaging for tumor burden and X-ray imaging for tumor induced bone changes were performed, and tissue samples were collected for histological analysis.

Tumor burden

![Tumor burden](image)

Histology and Immunohistochemistry

![Histology and Immunohistochemistry](image)

Summary

- Maximum study length was 13 days
- The mice developed metastases to multiple organs, but predominantly into the skeleton
- Bone metastases induced osteolytic lesions
- Cyclophosphamide decreased tumor burden and the area of tumor-induced osteolytic bone lesions
- Zoledronic acid decreased the area of osteolytic lesions but had no effect on skeletal tumor burden
- Anti-PD-1, IDO inhibitor epacodastat and their combination had no effect on tumor burden or the development of osteolytic lesions
- Histological analysis showed large tumor growth in bone marrow space of bothibia and femur in vehicle treated mice
- Low number or no TILs were observed in the tumors growing in bone of vehicle-treated mice, indicating immunologically ‘cold’ tumors

Conclusions

Cyclophosphamide and zoledronic acid were effective in the model. Immunotherapies did not prevent tumor growth in bone or tumor-induced bone changes in this syngeneic TNBC bone metastasis model. This is probably caused by the low number of TILs in the tumors growing in bone. Further studies are needed to evaluate the effects of treatment combinations and to increase the responsiveness to immunotherapies especially in bone metastases.

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