Differential Efficacy of PD-1 Targeted Immunomodulation in Preclinical Models of Primary and Bone Metastatic Triple-Negative Breast Cancer

Tina E. Kähkönen1, Mari I. Suominen1, Jussi M. Halleen1, Teppo Haapaniemi2, Azusa Tanaka2, Michael Seiler3, Jenni Bernoulli1
1Pharmatek Services, Turku, Finland; 2BioSiteHisto Ltd., Tampere, Finland; 3Taconic Biosciences, Rensselaer, NY, USA

E-mail correspondence to Tina Kähkönen (tina.kahkonen@pharmatek.com)

Immu-oncology (IO) has provided groundbreaking results in cancer treatment. Triple-negative breast cancer (TNBC) tumors attract immune cells, and the presence of tumor-infiltrating lymphocytes (TILs) is linked to improved survival. Programmed cell death protein 1 (PD-1) is expressed by TILs and its ligand (PD-L1) by TNBC cells. Targeting PD-1 has shown promising results in treatment of patients with primary TNBC. High frequency of bone metastases is typical for TNBC patients. As immuno regulation is different in bone than in other organs it is essential to study the efficacy of IO therapies in the bone metastatic microenvironment to obtain predictive efficacy data from preclinical studies before entering clinical trials.

The aim of the study was to assess the efficacy of anti-PD-1 therapy (pembrolizumab, Keytruda®) in the growth of primary and bone metastatic TNBC in preclinical models.

Materials and Methods

MDA-MB-231(SA)-hc human TNBC cells were inoculated into the mammary fat pad (orthopic model) or 104 bone marrow (bone metastasis model) of female NOG mice engrafted with CD44+ hematopoietic stem cells (huNOG, Taconic Biosciences) from two different donors. Treatments with pembrolizumab (5 mg/kg, i.p., QD, Keytruda, MSD Finland) or human IgG4 (5 mg/kg, i.p., QS, CrownBio) were started 3 days after TNBC cell inoculation (8 mice per group). Tumor growth was followed by caliper measurements in the orthopic model (tumor volume, m3=Width*Length*Height), and tumor induced changes in bone (bone lesions) were followed by X-ray imaging (Faxitron) in bone metastasis model. The study was terminated at 21-24 days after the cancer cell inoculations. Tumors were processed to immunohistochemical (IHC) stainings of TILs (CD45 B6S4, hCD8 B6S5, PD-1 B6S1, and PD-L1 B6S1, all from Nordic BioSite). The number of TILs was assessed by a 4-scale immunoscorening system (0-3: 0 negative, 1-2 moderate and 3 high) and PD-L1 expression was determined by Tumor Proportion Scoring (TPS: <1%, negative, 1-49% low to moderate and >50% high expression).

### References