A clear increase in TILs and modest tumor growth inhibition by pembrolizumab in prostate cancer tumors growing in bone of CD34+ engrafted NOG mice

Mari I. Suominen1, Justyna Zdrojewska1, Jenni H.E. Mäki-Jouppila1, Philip Dube2, Ivan Gladyn-Ng2, Paul Volden2, Jukka Rissanen1
1 Pharmatest Services, Turku, Finland. 2 Taconic Biosciences, Rensselaer, NY, USA
E-mail correspondence to Mari Suominen (mari.suominen@pharmatest.com)

Introduction
The recent KEYNOTE-199 trial raises hope for new treatment options for prostate cancer patients with the encouraging results of checkpoint inhibitor activity in a subset of prostate cancer patients, also including patients with bone-predominant disease. However, the patient subset that benefited from the treatment was small, indicating a need for identification of predictive biomarkers [1]. Preclinical models can help in the biomarker quest as well as in the search and selection of the best possible combination partners for future clinical trials.

In this study, we aimed to establish a prostate cancer bone metastasis model in humanized mice and to assess pembrolizumab efficacy in the established model.

Materials and Methods
Two million LNCaP human prostate cancer cells (ATCC) were inoculated into tibia bone marrow of male C57B16 NOG mice engrafted with human CD34+ hematopoietic stem cells (huNOG model, Taconic Biosciences). Serum prostate-specific antigen (PSA, R
d Systems) levels were measured at 4 weeks, and the mice were allocated to receive either pembrolizumab (10 mg/kg, Keytruda®, MSD Finland) or human IgG4 isotype control (Bio Logic) 5 mg/kg, Q5D for 6 weeks (n = 12 in study groups). Tumor growth was measured by measuring serum PSA levels. Tumor induced bone changes were monitored by measuring serum levels of the bone formation marker N-terminal propeptide of type I procollagen (PINP, R
d Systems), and by X-ray imaging of tibia (Faxitron). Changes in circulating T cells were monitored by flow cytometry (BD LSRFortessa™, BD Biosciences) performed at Turku Bioscience, Finland. Midgut sections were obtained from fixed and decalcified tumor-biased tibia and stained with HE+OrangeG. Three random samples from both groups were stained for CD4, CD8, Granzymell and PD-L1 (BRU, BRS, BRS150 and BRS90, Nordic BioSis).

Tumor analysis
FIGURE 2. A) Mean serum PSA concentrations during the study (ng/ml, mean ± SEM) per group. Pembrolizumab had no effect on serum PSA levels (p > 0.05). B) Individual values for mice in both groups.

Blood flow cytometry
FIGURE 5. The number and percentage of CD4+ and CD8+ T cells, CD4+ helper T cells, and CD8+ cytotoxic T cells in peripheral blood was assessed by flow cytometry before sacrifice. Pembrolizumab increased the percentage of CD4+ and CD8+ double positive cells (p < 0.01 for both) but had no effect on the percentage of CD4+ or CD8+ cells (median±Q25%±Q75%in,max).

Timeline of the study
FIGURE 1. Timeline of the study. LNCaP human prostate cancer cells were inoculated intratibially at study day 0. Four weeks later, serum PSA levels were measured and the mice were randomized to receive either pembrolizumab or isotype control treatment groups. Tumor growth was monitored by serum PSA measurements and tumor-induced bone changes by serum PINP measurements and X-ray. The study was terminated at 10 weeks. Flow cytometry analysis was performed before sacrifice.

Summary
A tumor take of 90% was observed in the humanized mice as evaluated by serum PSA levels at endpoint.
Pembrolizumab treatment had no significant effect on serum PSA levels.
Histology revealed lower tumor area in the pembrolizumab group.
The number of TILs was low in the control group and clearly higher in the pembrolizumab group.
Tumor-induced osteoblastic-mixed lesions were observed by X-ray imaging.
Pembrolizumab treatment had no effect on bone lesion area or serum PINP levels.
Pembrolizumab treatment increased the percentage of CD3+ and CD4+CD8+ double positive cells in peripheral blood.

Conclusions
The model successfully mimicked the prevalent clinical situation, where clear responses in PSA or target lesions are not observed. However, a dramatic increase of cytotoxic T-cells in the tumor was observed, revealing the effects of pembrolizumab in a model of prostate cancer growth in bone of huNOG mice. The model presents a suitable platform for studying combinations with partners that would boost or unlock the anti-tumor activity of the increased TILs.

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References