Establishment of a Novel Prostate Cancer Bone Metastasis Model in Humanized Mice and Early Efficacy Results of Pembrolizumab

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Introduction

Programmed cell death 1 (PD-1) and programmed death ligand 1 (PD-L1) expression is typically low in primary prostate cancer but increases at advanced stages. However, targeting PD-1 or PD-L1 has not proven efficacy in prostate cancer patients, and recent clinical trials are directed to combination therapies. About 85% of advanced prostate cancer patients develop skeletal metastases, and the focus in this context should be addressed to the treatment of these metastases. These efforts have been hindered by the lack of relevant preclinical bone metastasis models in immunocompetent mice.

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 CIEA NOG® mice engrafted with human CD34+ hematopoietic stem cells to generate humanized mice (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 (anti-PD-1, Keytruda®, MSD Finland) or human IgG4 isotype control (Sino Biological) 5 mg/kg, Q5D for 6 weeks (n = 12 in study groups). Tumor growth was monitored 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, IDS Systems), and by X-ray imaging of tibia (Faxitron). Changes in quantity of circulating T cells were monitored by flow cytometry (BD LSRFortessa™, BD Biosciences) performed at Turku Bioscience, Finland. At study termination, tissue samples were collected for histological analysis by hematoxylin and eosin (HE) OrangeG staining of tumor sections.

Timeline of the study

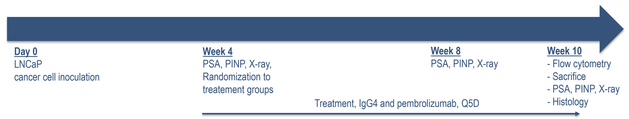

FIGURE 1. Timeline of the study. LNCaP human prostate cancer cells were inoculated intratibially at study day 0. Four weeks later, serum PSA levels were measured and the mice were randomized to receive either pembrolizumab or isotype control treatment. Tumor growth was monitored by serum PSA measurements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

Tumor analysis

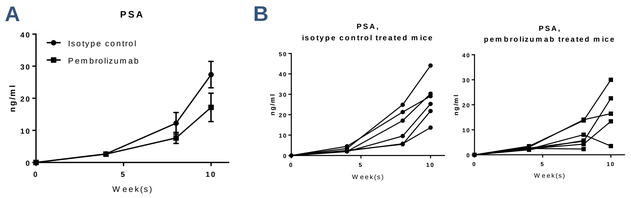

FIGURE 2. A) Serum PSA levels were measured during the study and mean PSA values (ng/ml, mean ± SEM) are presented for each study group. Pembrolizumab had no effect on serum PSA levels ($p > 0.05$). B) Individual values for mice treated with isotype control and pembrolizumab.

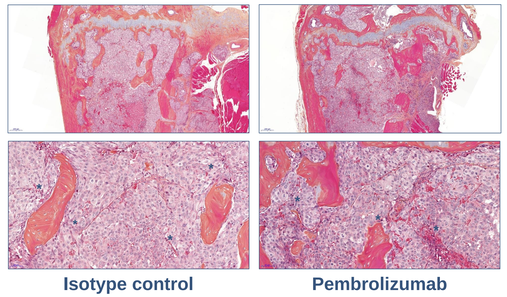

FIGURE 3. HE-OrangeG stained tibia cross sections showing tumor growth in bone marrow. In the lower images, lymphocytes are pointed out by asterisks. Upper images were taken with 5x magnification and lower images with 20x magnification.

Bone analysis

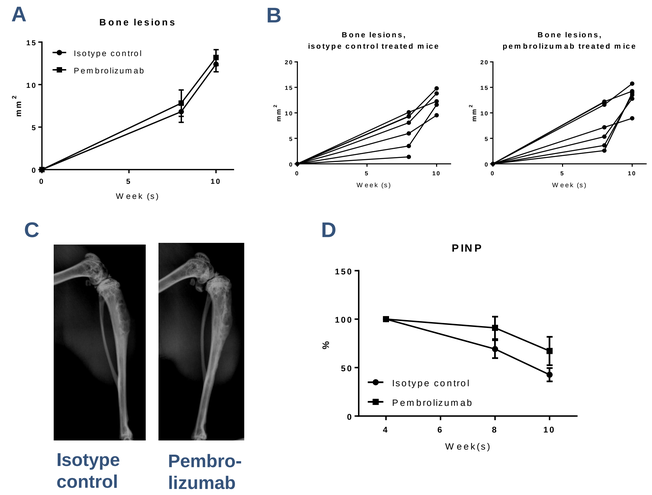

FIGURE 4. A) The area of cancer-induced bone changes (bone lesions) determined by X-ray imaging is presented each study group (mm², mean ± SEM). Pembrolizumab had no effect on bone lesions ($p > 0.05$). B) Individual values for mice treated with isotype control and pembrolizumab are presented. C) Example X-ray images of the tibias of isotype control and pembrolizumab treated mice at sacrifice, showing osteoblastic-mixed lesions. D) Serum PINP levels relative to values at 4 weeks are presented for each study group (%, mean ± SEM). Pembrolizumab had no effect on serum PINP levels ($p > 0.05$).

Flow cytometry

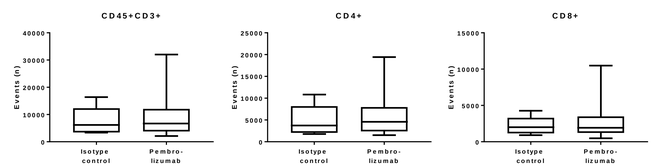

FIGURE 5. The number of CD45+CD3+ T cells, CD4+ helper T cells, and CD8+ cytotoxic T cells was assessed by flow cytometry before sacrifice. The number of events (median, 5-95% percentile) is presented for each study group. Pembrolizumab had no effect on the number of CD45+CD3+, CD4+ or CD8+ cells ($p > 0.05$).

Summary

- Four weeks after inoculation of LNCaP human prostate cancer cells, the humanized mice had well established tumors and the treatments could be initiated. The maximum study length was 10 weeks in this model.
- A tumor take of 90% was observed in the humanized mice as evaluated by serum PSA levels at endpoint
- Pembrolizumab treatment had no effect on serum PSA levels
- Histology confirmed tumor growth in bone marrow of humanized mice, and the presence of low number of lymphocytes in the tumors
- 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 had no effect on circulating levels of CD45+CD3+, CD4+ or CD8+ cells

Conclusions

A novel preclinical model of prostate cancer bone metastasis in humanized mice was established. Intratibial prostate cancer tumors induced osteoblastic-mixed bone lesions and increased serum PSA levels, mimicking the clinical situation in patients. Resembling recent clinical findings, no responses with pembrolizumab as monotherapy were observed.

Acknowledgements

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