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

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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]. Proper preclinical models can help in the biomarker quest as well as in the search and selection of the best possible combination partners for further 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 CIEA 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 (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 circulating T cells were monitored by flow cytometry (BD LSRFortessa™, BD Biosciences) performed at Turku Bioscience, Finland. Midsagittal sections were obtained from fixed and decalcified tumor-bearing tibias and stained with H&E+OrangeG. Three random samples from both groups were stained for CD4, CD8, GranzymeB and PD-L1 (BSR4, BSR5, BSR150 and BSR90, Nordic BioSite).

Timeline of the study

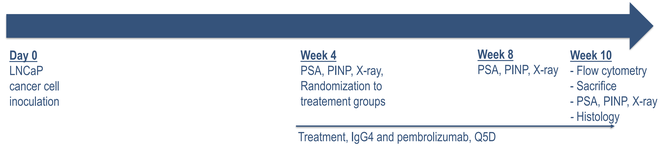

FIGURE 1. Timeline of the study. LNCaP human prostate cancer cells were inoculated intratibially at study day 0. Four weeks later, serum PSA levels were measured and the mice were randomized to receive either pembrolizumab or isotype control treatment. Tumor growth was monitored by serum PSA measurements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

Tumor analysis

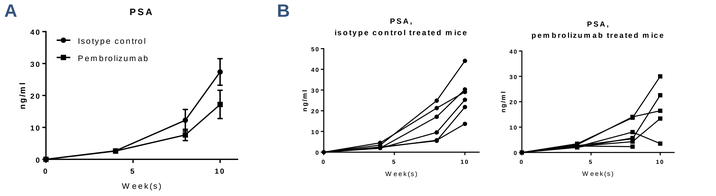

FIGURE 2. A) Mean serum PSA concentrations during the study (ng/ml, mean ± SEM) per group. Pembrolizumab had no effect on serum PSA levels ($p > 0.05$). B) Individual values for mice in both groups.

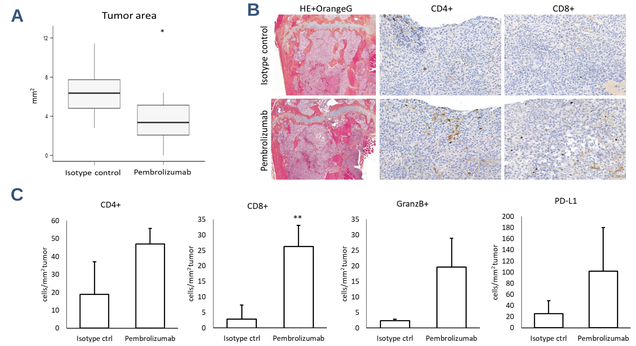

FIGURE 3. A) Tumor area in histological sections was decreased in the pembrolizumab group (median±IQR25%±min/max, $p < 0.05$). B) Representative images. C) Amount of CD4+, CD8+ and GranzB+ TILs, and PD-L1 expressing tumor cells. The number of cytotoxic T-cells (CD8+) was increased ($p < 0.01$).

Bone analysis

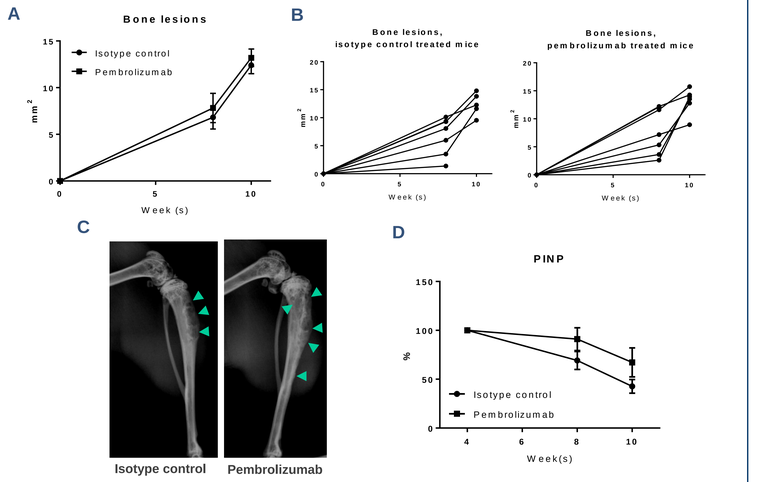

FIGURE 4. A) The areas of cancer-induced bone changes (bone lesions) determined by X-ray imaging are presented for each study group (mm², mean ± SEM). Pembrolizumab had no effect on bone lesions ($p > 0.05$). B) Individual values in mice treated with isotype control and pembrolizumab are presented. C) Example X-ray images of the tibias of isotype control and pembrolizumab treated mice at sacrifice, showing osteoblastic-mixed lesions marked by arrowheads. 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 ($p > 0.05$).

Blood flow cytometry

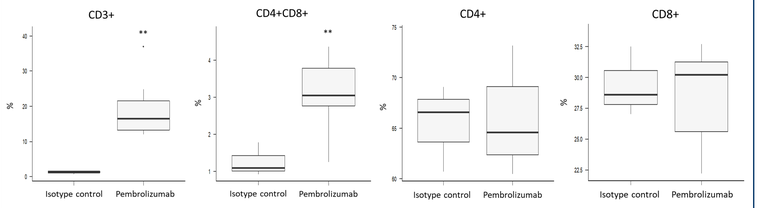

FIGURE 5. The number and percentage of CD45+CD3+ T cells, CD4+ helper T cells, and CD8+ cytotoxic T cells in peripheral blood was assessed by flow cytometry before sacrifice. Pembrolizumab increased the percentage of CD3+ and CD4+CD8+ double positive cells ($p < 0.01$ for both) but had no effect on the percentage of CD4+ or CD8+ cells (median±IQR25%±min/max).

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 combination partners with pembrolizumab, that would boost or unlock the anti-tumor activity of the increased TILs.

Acknowledgements

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References

1. Antonarakis ES, Piulats JM, Gross-Goupil M, et al. Pembrolizumab for Treatment-Refractory Metastatic Castration-Resistant Prostate Cancer: Multicohort, Open-Label Phase II KEYNOTE-199 Study. J Clin Oncol. 2020;38:395-405.