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 metastases 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 C57BL/NOG® mice engrafted with human CD34+ hematopoietic stem cells to generate humanized mice (huNUG 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, QSD for 6 weeks (n = 12 in each study group). 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.

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.

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