Establishment of primary tumor models of TNBC and ER+/HER2- breast cancer in humanized mice and validation of pembrolizumab efficacy

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Introduction
Immunotherapies are emerging treatment options for different subtypes of breast cancer. Pembrolizumab (Keytruda®) targets PD-1 expressing T cells in tumors and mediates anti-tumor effects. Targeting PD-1 has shown promising results in advanced breast cancer and is being evaluated in clinical trials in triple-negative breast cancer (TNBC) and in estrogen receptor positive, human epidermal growth factor 2 negative (ER+/HER2-) breast cancer patients. Immunotherapy is not yet an established treatment option for early breast cancer.

Aims of this study were to establish preclinical primary tumor models for TNBC and ER+/HER2- breast cancer in humanized mice, and to evaluate efficacy of pembrolizumab in the models.

Materials and Methods
Immunodeficient female C57BL/6 mice engrafted with human CD34+ hematopoietic stem cells (huNOG mice, Taconic Biosciences) were inoculated orthotopically into mammary fat pad with either 5×10^5 MDA-MB-231(SA) (TNBC) or 5×10^5 MCF-7 (ER+/HER2-) human breast cancer cells (from Dr. Theresa Guise and ATCC, respectively). The mice receiving MCF-7 cells were implanted with estradiol (E2) releasing implants (MedRod, PreclinApps) before cancer cell inoculation to support tumor growth. The mice were treated with pembrolizumab (5 mg/kg, MSD Finland) or isotype control (Crown Bioscience) in a QSD schedule (n=8 for the TNBC groups and 12 in the ER+/HER2- groups). The treatments were started 3 or 14 days after cancer cell inoculation in the TNBC and ER+/HER2- models, respectively. Tumor growth was monitored by caliper measurements, and the study was terminated at 3 (TNBC model) or 7 weeks (ER+/HER2- model). Tumors were collected and analyzed immunohistochemically for TILs, PD-1 and PD-L1 expression, and hematological analysis was performed in the ER+/HER2- model ( VetScan).

FIGURE 1. Orthotopic tumor volume (mm^3, mean ± SEM). Individual values are presented on the right panel. Pembrolizumab treatment decreased tumor growth compared to control (** p < 0.01). Three mice treated with pembrolizumab (3/8, 37.5%) responded to the treatment, which was seen as reduced tumor growth. One mouse (1/8, 12.5%) had complete rejection of the tumor. Donor’ 1 and 2 refers to the donor of hematopoietic stem cells used in the humanization of the mice.

FIGURE 2. HE-staining shows overall tumor morphology. PD-L1 expression was high in the model. PD-1 staining was low and negative in all pembrolizumab (pembro) treated tumors. The number of CD4+ TILs varied from low to high and the number of CD8+ TILs was moderate in the tumors. Pembrolizumab treatment decreased the number of CD4+ and CD8+ cells. Granzyme B staining showed active T cells in the tumors and it was lower in the pembrolizumab treated mice.

FIGURE 3. Orthotopic tumor volume (mm^3, mean ± SEM). Orthotopic tumors did not grow without E2 supplementation (** p < 0.01). Pembrolizumab treatment had no effect on tumor growth (NS p > 0.05). E2 supplementation decreased the survival of huNOG mice (* p < 0.05). E2 supplemented huNOG mice showed decreased white (2×10^9 e/l to 0.2×10^9 e/l) and red blood cell count (7×10^12 e/l to 5×10^12 e/l), hemoglobin (120 g/l to 97 g/l) and hematocrit (36% to 30%) values in hematological analysis.

FIGURE 4. HE-staining shows overall tumor morphology. PD-L1 and PD-1 expression were low in the model. The number of CD4+ TILs was low to moderate and the number of CD8+ and granzyme B+ TILs was low in tumors. Pembrolizumab (pembro) treatment did not induce major changes in the number of TILs.

Summary
In the TNBC model:
- Tumors responded to pembrolizumab treatment.
- Three out of eight mice responded to the treatment and one of them had tumor rejection.
- Pembrolizumab treatment was associated with a decrease in CD8+ and granzyme B+ TILs.

In the ER+/HER2- model:
- E2 supplementation was essential for tumor growth.
- However, E2 caused a decrease in the clinical condition, including decrease in white and red blood cells, hemoglobin and hematocrit, leading to early sacrifices.
- Only modest responses for pembrolizumab were observed in a few mice and the results did not reach statistical significance.
- No major changes were observed in TILs due to pembrolizumab treatment.

Conclusions
In the established breast cancer models in humanized mice, pembrolizumab decreased tumor growth in the TNBC model but induced only minor tumor responses in the ER+/HER2- model. Estrogen was essential for ER+/HER2- tumor growth but induced severe anemia and had immunomodulatory effects potentially influencing the lack of pembrolizumab efficacy in the model.

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