Characterization of tumor-infiltrating immune cells and the efficacy of pembrolizumab in preclinical models of bone metastatic triple-negative breast cancer

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Introduction
High number of tumor-infiltrating lymphocytes (TILs) is associated with improved survival, and targeting of programmed cell death protein 1 (PD-1) has shown promising results in treatment of triple-negative breast cancer (TNBC). TNBC patients typically have a high incidence of bone metastasis. As immune regulation in bone is different compared to other organs, it is essential to understand immune cell infiltration also to the metastatic location in bone.

The aim of this study was to assess the efficacy of anti-PD-1 therapy (pembrolizumab, Keytruda®) on growth of primary and bone metastatic TNBC in preclinical models, and to characterize immune cell infiltration into these tumors to support immuno-oncology drug discovery.

Materials and Methods
MDA-MB-231(SA)-lac human TNBC cells were inoculated orthotopically into the mammary fat pad (primary tumor model) or tibia bone marrow (bone metastasis model) of female huNOG mice (Taconic Biosciences). Treatments with pembrolizumab or IgG4 isotype control (5 mg/kg, i.p., Q5D, n=8) were started at day 3. Tumor growth was monitored by caliper measurements or X-ray imaging of tumor-induced bone lesions for 24-21 days in the primary tumor and bone metastasis models, respectively. Immunohistochemical stainings were performed at endpoint (Table 1). PD-L1 staining was assessed by tumor proportion score (TPS), and the other stainings by 4-scale immunoscore system.

Summary
- A response rate of 38% was observed in the primary tumor model, but no effects were observed in the bone metastasis model by pembrolizumab treatment
- Generally, intratumoral and periluminal variation of expression and localization of TILs was observed, and it was more profound in the bone tumors
- PD-1 expression was low in the control-treated orthotopic tumors and absent in the pembrolizumab treated mice due to antibody blocking of the epitope
- Moderate number of CD4+ and CD8+ TILs was observed and granzyme B expression correlated with CD8 positivity
- Lower number of CD8+ cytotoxic T cells was observed in the bone metastasis model, potentially explaining the lack of efficacy of pembrolizumab in these tumors

Conclusions
Orthotopic tumors responded to pembrolizumab treatment but bone metastatic growth was not inhibited. Bone marrow has a unique immune cell microenvironment that is different from primary tumor. The lack of efficacy of pembrolizumab could be explained by low number of CD8+ and PD-1 positive cells in the tumor growing in bone. These results highlight the importance of validation of immunotherapeutic potential in preclinical models before entering clinical trials.

TABLE 1. Antibodies used in immunohistochemical stainings.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1</td>
<td>BR1</td>
<td>Nordic Biosite</td>
</tr>
<tr>
<td>PD-L1</td>
<td>ZR3</td>
<td>Nordic Biosite</td>
</tr>
<tr>
<td>CD8</td>
<td>SS1</td>
<td>Nordic Biosite</td>
</tr>
<tr>
<td>CD3</td>
<td>SS2</td>
<td>Nordic Biosite</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>SSI2</td>
<td>Nordic Biosite</td>
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</tbody>
</table>

FIGURE 1. Orthotopic tumor volume (mm3, mean ± SEM) at each time point. Individual values are presented on the right panel. Pembrolizumab treatment decreased tumor growth compared to control (p<0.007, *). 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 a complete rejection of the tumor.

FIGURE 2. Bone lesion area (mm2, mean ± SEM) measured from the X-ray images is presented for both study groups together with individual values on the right panel. No statistically significant differences were observed (p=0.73, NS).

FIGURE 3. HE-staining shows overall tumor morphology. PD-L1 expression was high in orthotopic tumors (TPS > 50%), varied from low to high in intratibial tumors (TPS 1% to >50%), and was unaffected by pembrolizumab (pemb) treatment. The number of CD4+ TILs varied from low to high in both tumors (score 2-3), and the number of CD4+ cells was decreased by pembrolizumab treatment in orthotopic tumors. The number of CD8+ TILs was moderate in orthotopic tumors (score 2) and low in intratibial tumors (score 1). Pembrolizumab treatment decreased the number of CD8+ cells in both models. PD-1 staining was low in orthotopic tumors (score 0-1), highly variable in intratibial tumors, and negative in all pembrolizumab treated tumors. Granzyme B staining showed active T cells in tumors and it was lower in the pembrolizumab treated mice.

References