**Introduction**

Immunotherapy has provided promising results in the treatment of breast cancer but there is a high variability in response between different subtypes. Triple-negative breast tumors typically have a high number of tumor infiltrating lymphocytes (TILs) and high expression of programmed death ligand 1 (PD-L1), and these tumors have good response to immunotherapy. On the contrary, hormone receptor positive breast tumors attract less immune cells and have low PD-L1 expression, and are less sensitive to immunotherapy. Based on the immune attractive phenotype, these tumors are referred as “hot” and “cold” tumors.

Independently from the breast cancer subtype, the patients typically develop bone metastases in high frequency. Bone metastases are incurable and novel immunotherapies hold the potential to treat patients with bone metastatic disease. Up to recent years, preclinical validation of efficacy of immunomodulators has been limited to the use of syngeneic models. To shorten the gap in clinical translation, humanized mouse models with functional human immune system have been developed.

### BT-474 model

**Figure 1.** Timeline for BT-474 intratibial model. Cancer cells were inoculated to tibial bone marrow at day 0. Tumor-induced bone changes were followed by X-ray imaging at 4, 6 and 8 weeks. The study was completed at 8 weeks and samples were collected for ex vivo analysis. PD-L1 staining was low to negative in vitro.

**Figure 2.** Cancer-induced bone lesions. Cancer-induced bone changes (bone lesions) were quantified from X-ray images and bone lesion area (mm², means±SEM) is presented at indicated time points. Example images of osteolytic bone reaction visualized by X-ray and µCT reconstruction of tibial cross sections.

**Figure 3.** Immunohistochemical characterization of Intratibial BT-474 tumors and TILs. Representative images with 20x magnification are presented.

### Summary on BT-474 model

- BT-474 cancer cells induced osteolytic new bone growth in mice. This was visualized by X-ray and µCT imaging.
- After 8 weeks in study, the bones resembled a late-stage bone metastatic disease in humans.
- The model resembled a “cold” tumor and was weakly immune attractive. A low number of CD4+ and CD8+ TILs and PD-1 and PD-L1 positive cells were observed.

### Conclusions

Two models for bone metastatic breast cancer in humanized mice were established. The use of humanized mouse models allows testing of immunotherapies as monotherapy and in combination with other therapies in a variety of human cell line derived tumors.

**Materials and Methods**

Female CIEA NOG mice engrafted with human CD34+ hematopoietic stem cells (huNOG, Taconic Biosciences) were inoculated with human BT-474 (ER+, PR+, HER2+, PD-L1 high) or MDA-MB-231(SA) (ER-, PR-, HER2-, PD-L1 low) cells into bone marrow of tibia. Tumor-induced changes in bone were monitored by X-ray imaging (Faxitron) during the study. At endpoint tumor-induced bone gain/loss was imaged by micro-computed tomography (µCT, Bruker). Histological analyses were performed for quantitation and localization of TILs (hCD4 = human T helper cells, BSR4 and hCD8 = human cytotoxic T cells, BSR5) and PD-1/PD-L1 positive cells (hPD-1 = human programmed cell death protein 1, BSR1 and hPD-L1 = human programmed death ligand 1, ZR3, all antibodies from Nordic BioSite).

### MDA-MB-231(SA) model

**Figure 4.** Timeline for MDA-MB-231(SA) intratibial model. Cancer cells were inoculated to tibial bone marrow at day 0. Tumor-induced bone changes were followed by X-ray imaging at 1, 2 and 3 weeks. The study was completed at 3 weeks and samples were collected for ex vivo analysis. PD-L1 staining was moderate to high in vitro.

**Figure 5.** Cancer-induced bone lesions. Bone lesion area (mm², means±SEM) quantified from X-ray images is presented at indicated time points. Example images of osteolytic bone reaction visualized by X-ray and µCT reconstruction of tibial cross sections.

**Figure 6.** Immunohistochemical characterization of Intratibial MDA-MB-231(SA) tumors and TILs. Representative images with 20x magnification are presented.

### Summary on MDA-MB-231(SA) model

- MDA-MB-231(SA) cancer cells induced osteolytic bone loss in mice. In a 3-week study, the osteolytic lesions were moderate in size and some loss of trabecular bone was observed.
- The mice exhibited pre-cachectic features including decreased body weight, curved spine, and loss of muscle mass especially in the hind limbs.
- The model resembled a “hot” tumor with CD4+ and CD8+ TILs and variable PD-1 expression. PD-L1 showed moderate to high expression in the tumor cells.

### References

- Baschuk, N et al., Bone specific immunity and its impact on metastasis. BoneKEY Reports. 2015.