Introduction and Background

- T cell checkpoint inhibitors have demonstrated significant clinical benefit in immunologically "hot" cancer types like melanoma, kidney, bladder and lung cancers. "Hot" tumors are characterized by a significant CD8+ T cell infiltrate and high neoantigen burden.
- Breast cancer is regarded as an immunologically "cold" cancer, often with minimal CD8+ T cell infiltration and a much lower mutational burden. Preclinical researchers need robust and representative breast cancer models to test immuno-oncology (I-O) combination strategies that may convert these "cold" tumors into "hot" tumors.
- Radiation therapy (RT) is a clinical treatment modality utilized in breast cancer and is known to modify the tumor microenvironment, induce cytokines and chemokines, and has been shown to potentially synergize with immunotherapies.
- The 4T1 cell line is the most prevalent syngeneic breast cancer cell line model used in I-O research because of useful traits that include an immunosuppressed microenvironment with Tregs and G-MDSCs and highly metastatic phenotype.
- Mice with 4T1 tumors can develop a fatal hypersensitivity reaction upon repeated treatment with rat antibodies to PD-1, PD-L1, GITR or OX40.
- As alternative models for the study of immunologically "cold" breast cancers, we have characterized the tumor immune profiles of two breast cancer models, EMT6 and E0771; and the response of EMT6 and E0771 to radiation, costimulatory agonists and checkpoint inhibitors in pharmacology efficacy studies.
- Ovarian cancer is another cancer with low neoantigen burden and immunologically "cold." The response of intraperitoneal ID8 ovarian cancer model to checkpoint inhibitors has been characterized.

Materials and Methods

- Female Balb/c (4T1-Luc, EMT6) or C57BL/6 (E0771) mice were implanted in the lower mammary fat pad. Tumor progression was monitored by palpation.
- For tumor immune profiling, mammary fat pad (mfp) tumors from 4T1-Luc, E0771, and EMT6 implanted mice were collected between 300-600 mm³ and digested to a cell suspension for flow cytometry.
- Tumor immune profiles of two breast cancer models, EMT6 and E0771; and the response of EMT6 and E0771 to radiation, costimulatory agonists and checkpoint inhibitors in pharmacology efficacy studies.
- Established orthotopic EMT6 breast tumors were treated with a single dose of focal beam radiation and monitored for tumor growth.
- Established orthotopic 4T1-Luc or E0771 tumors were treated with a single dose of focal beam radiation and monitored for tumor growth.
- Intraperitoneal ID8-Luc ovarian tumors were responsive to anti-CTLA-4 and anti-PD-L1, but have no response to anti-CTLA-4 which suggests that T cell exhaustion is the primary immune escape mechanism for this ovarian cancer model.

Results and Conclusions

- E0771 tumors have a near complete absence of G-MDSCs compared to 4T1-Luc and EMT6 breast cancers. This could account for the greater magnitude of response to T cell checkpoint inhibition in the E0771 model relative to 4T1-Luc.
- Focal beam radiation inhibits tumor growth in 4T1-Luc, E0771 and EMT6 (not shown) tumors and doses amenable for immunotherapy combination studies have been established.
- Unstaged E0771 tumors are highly sensitive to checkpoint inhibition and costimulatory agonists; however, established tumors are more refractory to both classes of agents with only a subset of mice responding.
- Established EMT6 tumors have partial responses to checkpoint blockade and costimulatory agonists.
- Intraperitoneal ID8-Luc ovarian tumors are responsive to anti-PD-1 and anti-PD-L1, but have no response to anti-CTLA-4 which suggests that T cell exhaustion is the primary immune escape mechanism for this ovarian cancer model.