5691. Evaluation of Immunomodulatory Agents in Classically Immunologically “Cold” Cancers Using Syngeneic Mouse Models of Breast and Ovarian Cancer

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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 caliper measurements.
- For tumor immune profiling, mammary fat pad (mfp) tumors from 4T1-Luc, E0771 and EMT6 implanted mice were collected between 300-600 mm^3 and digested to a cell suspension for flow cytometry (Miltenyi, Germany). MI-CompT™ panel (CD8+ T cells, CD4+ T helper cells and Tregs) and MI-TAM™ panel (M-MDSC,
G-MDSC, M1 TAM and M2 TAM) were used on an Attune NxT Flow Cytometer (Thermo Fisher Scientific) and analyzed with FlowJo software (Tree Star, Inc., Ashland, OR).
- Image-guided irradiation was performed under 1-2% isoflurane anesthesia on the Small Animal Radiation Research Platform (SARRP; Xstrahl Inc., Suwanee, GA). Treatment (220kV, 13.0mA) was applied using a 10x10mm collimator and delivered to a total dose of 5Gy, 10Gy or 20Gy in 2 equally weighted beams.
- For unstaged studies, treatment was initiated three days post implant. For staged studies, mice were randomized into treatment groups following establishment of tumors. Checkpoint inhibitor antibodies and T cell costimulatory agonist antibodies were acquired from Bio X Cell and dosed intraperitoneally.

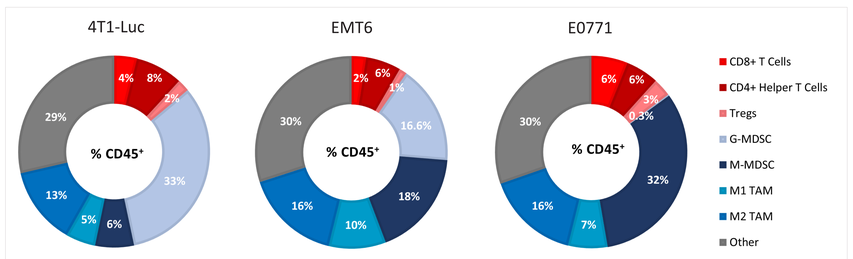

Figure 1. Immune profiling of syngeneic breast cancer models.

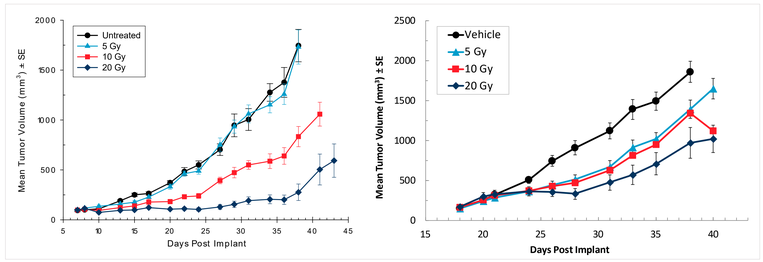

Figure 2. Focal beam radiation inhibits breast tumor growth. Established orthotopic 4T1-Luc or E0771 tumors were treated with a single dose of focal beam radiation and monitored for tumor growth.

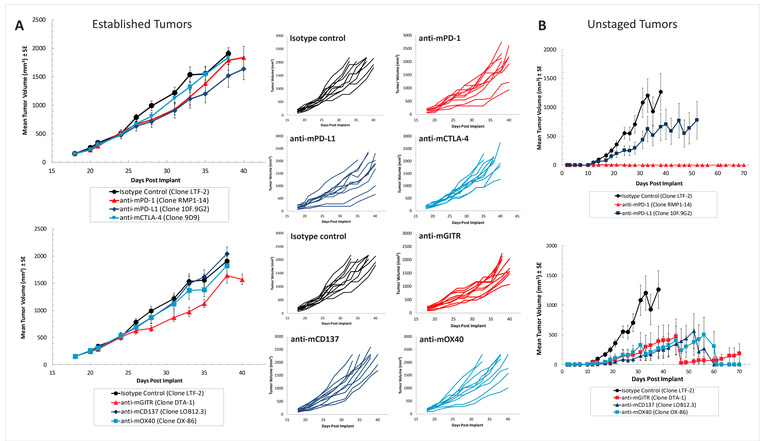

Figure 3. Response of orthotopic E0771 breast tumors to checkpoint blockade and costimulatory agonists. Established (A) and unstaged (B) orthotopic E0771 breast tumors were treated with a series of checkpoint inhibitors or costimulatory agonist antibodies and monitored for growth. Mean tumor volume and individual tumor volumes are shown.

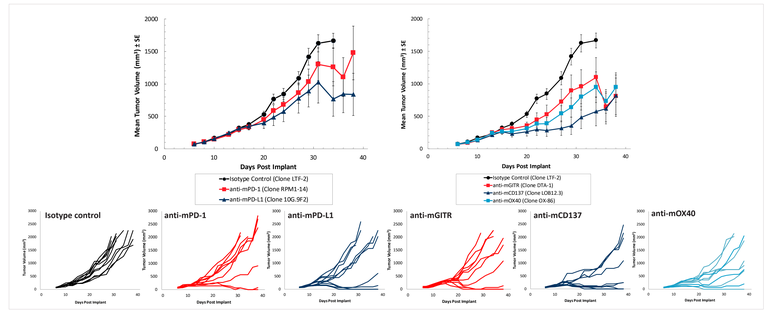

Figure 4. Response of established orthotopic EMT6 breast tumors to checkpoint blockade and costimulatory agonists. Established orthotopic EMT6 breast tumors were treated with a series of checkpoint inhibitors or costimulatory agonist antibodies and monitored for growth. Mean tumor volume and individual tumor volumes are shown.

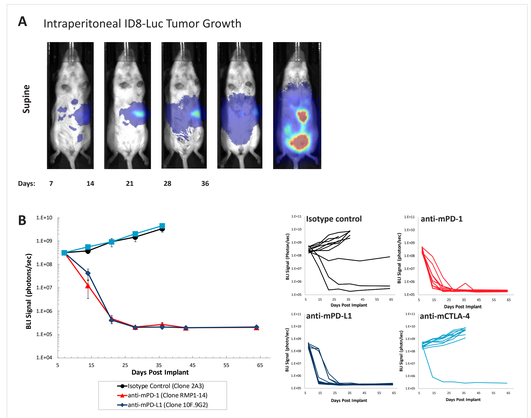

Figure 5. ID8 ovarian cancer response to checkpoint blockade. Representative images of ID8-Luc intraperitoneal tumor growth (A). Established ID8-Luc tumors were treated with a series of checkpoint inhibitors and monitored for growth by bioluminescence imaging (B).

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.

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