

#4915. Evaluation of the Immune Response Following Treatment with Anti-CTLA-4 Antibody, Radiation Therapy or the Combination in a Murine Model of Breast Cancer

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Introduction and Background

- Breast cancers are considered poorly immunogenic tumors.
- However, several approaches utilizing immunotherapies are being undertaken in the clinic to evaluate their potential for improving outcomes.
- Radiation therapy (RT) is a highly utilized clinical treatment modality in breast cancer.
- Radiation is known to modify the tumor microenvironment, induce cytokines and chemokines and has been shown to potentially synergize with immunotherapies.
- The aim of this work was to evaluate the possible synergy between RT and anti-CTLA-4 therapy in a murine model of breast cancer.

Materials and Methods

- Female Balb/C mice (Envigo) were implanted with 5×10^5 cells in the #4 mammary fat pad (mfp). Five days post implant mice were staged into treatment groups with a mean tumor volume of 63-75 mm³.
- On days 5, 6 & 7 localized radiation of 8 Gy at a rate of 1.50 Gy/min was delivered to mfp #4 with an RS2000 Biological X-ray Irradiator (Rad Source Technologies, Alpharetta, GA). Starting on day 6 anti-CTLA-4 antibody (clone 9H10; Bio X Cell, West Lebanon, NH) or an isotype control antibody was given IP. Antibody dosing was 10 mg/kg every 3 days for a total of 4 doses.
- One subset of mice was monitored for anti-tumor activity and metastatic tumor burden and another was terminated for immunophenotypic analysis of tumor-infiltrating cells by flow cytometry. Tumors were processed into single cell suspensions using gentleMACS™ Dissociators (Miltenyi Biotec). Samples were acquired on an Attune Flow Cytometer (Life Technologies) and data was analyzed using FlowJo software (Tree Star).
- In vivo bioluminescence imaging was performed with an IVIS 50 optical imaging system (Xenogen, Alameda, CA) to quantify metastatic tumor burden by shielding the primary tumor. Total axillary lymph nodes and lung metastasis tumor burden was calculated using Living Image 4.3.1 (Xenogen, Alameda, CA) software from a fixed volume ROI covering the non-shielded scan area.

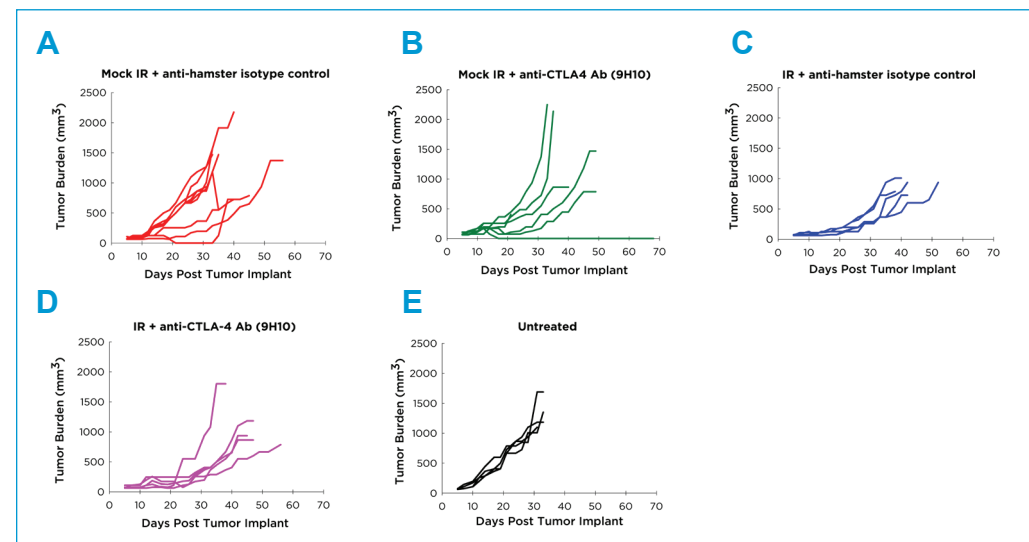
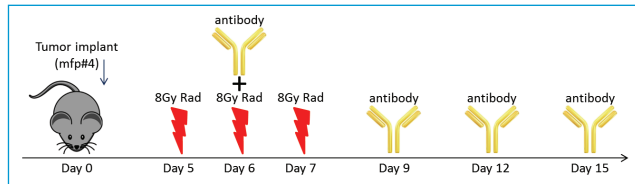


Figure 1. Individual Growth Curves. Treatment effects of anti-CTLA4 antibody, radiation or the combination in the 4T1-luc mouse mammary carcinoma model. Individual growth over time following treatment with isotype control (A), anti-CTLA-4 antibody (B), radiation (C), or the combination (D).

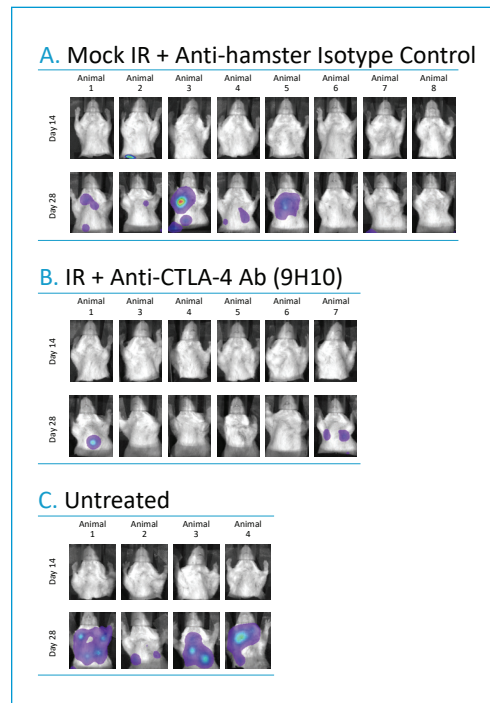


Figure 2. Metastatic Tumor Burden. Evaluation of total metastatic tumor burden over time by bioluminescence imaging.

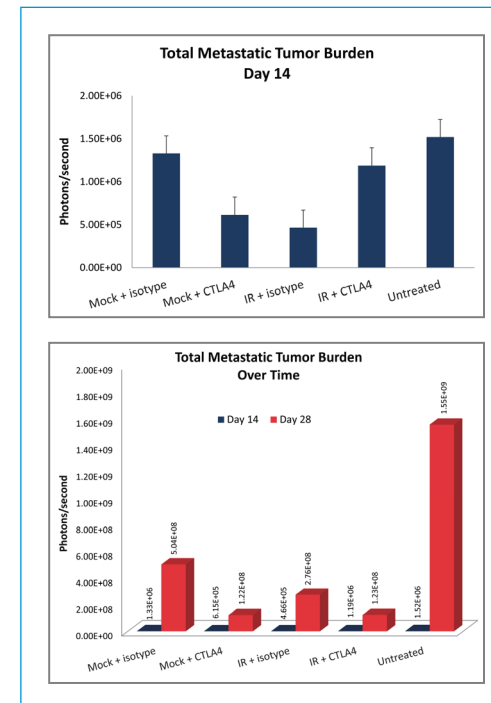


Figure 3. Quantitation of Total Metastatic Tumor Burden.

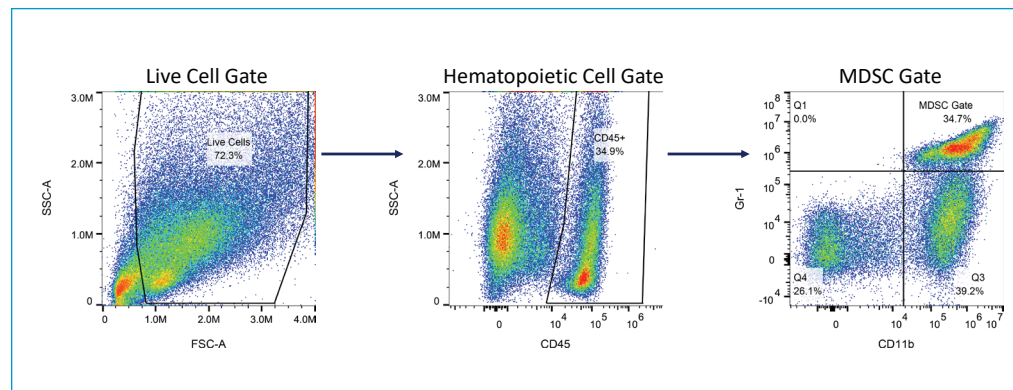


Figure 4. Gating Strategy for Flow Cytometry.

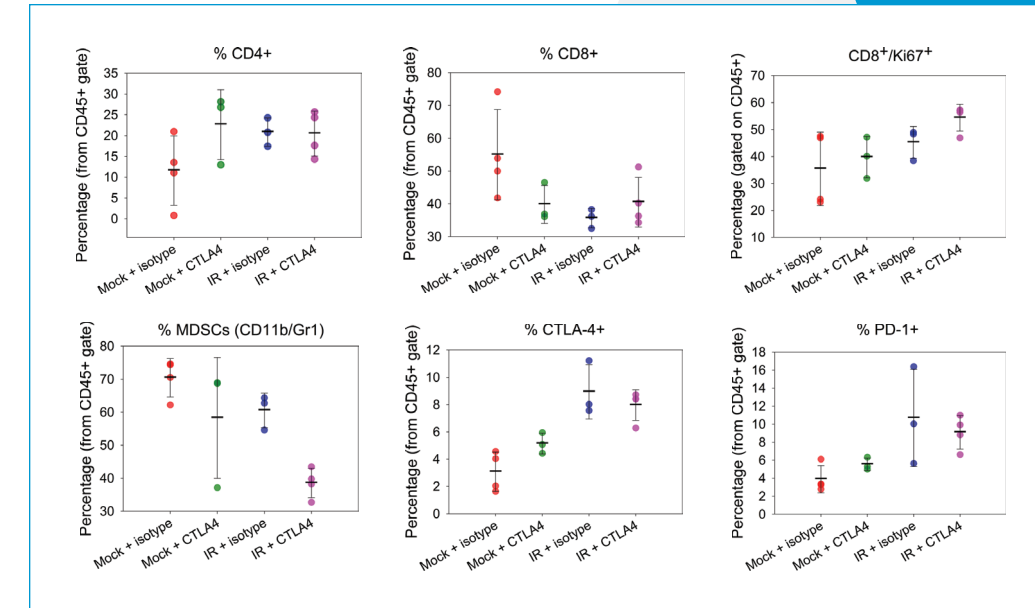


Figure 5. Immune Response by Flow Cytometry.

- This work was performed with localized radiation from a cabinet irradiator.
- We saw some morbidity/mortality in the radiation treatment arms likely due to radiation-induced toxicity.
- Future work would be done utilizing the small animal radiation research platform (SARRP) by Xstrahl:
 - Replicates clinical practice.
 - Treatment Planning System allows for more control over dose delivery.
 - Collaborative approach to customize treatment plans to meet research needs.

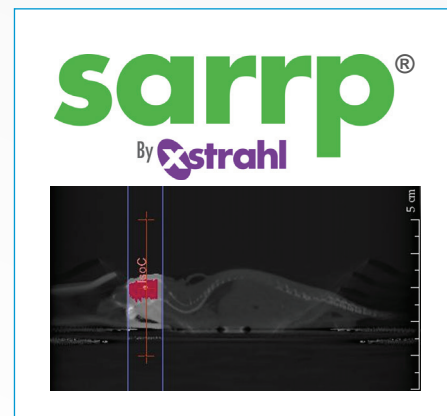


Figure 6. Use of Focal Radiation.

Results and Conclusions

- Both radiation and anti-CTLA-4 antibody treatments have some anti-tumor activity in the 4T1-luc model.
- Use of the appropriate isotype control is critical to fully define the specific extent of single-agent treatments.
- Radiation therapy (RT) in combination with anti-CTLA-4 antibody treatment reduces overall metastatic tumor burden in the lung and axillary lymph nodes.
- Combination treatment triggers both pro- and anti-tumor signaling pathways thus providing a possible explanation for the marginal anti-tumor responses we observed in this model.
- Use of precise focal radiation with SARRP could provide improvements in either single-agent RT or RT combined with checkpoint inhibitors.