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 & Methods
Female Balb/C mice (Envax) were implanted with 5 x 10^6 cells in the 4th mammary fat pad (4T1). Five days post implant mice were staged into treatment groups with a mean tumor volume of 63.75mm3. On days 5, 6, 7 & 8, 2.7 x 10^6 luciferase-expressing 4T1 cells were injected IP. A subset of mice was treated with radiation therapy (40 Gy) 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 13120; Bio X Cell), anti-CTLA-4 (clone 9H10) 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 immunohistochemical analysis of tumor-infiltrating cells by flow cytometry. Tumors were processed into single cell suspensions using gentleMACSTM Dissociators (Miltenyi Biotec). Samples were acquired on an Attune Flow Cytometer (Life Technologies) and data was analyzed using FlowJo software (Treestar).

In vivo bioluminescence imaging was performed with an IVIS TD optical imaging system (Xenogen, Alameda, CA) to quantify metastatic tumor burden by viewing the primary tumor. Total axillary lymph nodes and lung metastasis were acquired using Living Image 4.3 (Xenogen, Alameda, CA) software from a fixed volume ROI covering the non-shielded area.

Tumor burden was calculated using a formula: Image 4.3 (Xenogen, Alameda, CA) software from a fixed volume ROI covering the non-shielded area.

Results & 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 agent 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.

Immunofluorescence Microscopy

Fig. 2 – Metastatic Tumor Burden

A. Mock IR + Anti-hamster Isotype Control

B. IR + Anti-CTLA-4 Ab (9H10)

C. Untreated

Fig. 3 – Quantitation of Total Metastatic Tumor Burden

Fig. 4 – Gating Strategy for Flow Cytometry

Live Cell Gate

Hematopoietic Cell Gate

MSDC Gate

Fig. 5 – Immune Response by Flow Cytometry

A. % CD4+ B. % CD8+ C. % MDCs (CD11b+F4/80) D. % CTLA-4+ E. % PO-1

Fig. 6 – Use of Focal Radiation

- This work was performed with localized radiation from a cobalt 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.
- Radiates clinical practice.
- Treatment Planning System allows for more control over dose delivery.
- Collaborative approach to customize treatment plans to meet research needs.