#535. Preclinical Use of Focal Radiation and Immune Checkpoint Blockade to Improve Therapeutic Response in an Immunologically Cold Tumor

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Introduction and Background

- Radiation therapy (RT) is a highly utilized clinical treatment modality with more than 50% of all cancer patients receiving some type of radiotherapy during the course of their illness. Appropriate systems and models to test preclinical radiation combinations are needed.
- In mouse models, radiation treatment has been shown to increase the level of tumor antigen presentation and the variety of peptides available for cross-presentation. Current work in the field focuses on using radiation as a tool to bridge the gap from tumor equilibrium to tumor elimination, which could improve the response rate of immuno-oncology agents.
- 4T1 is a murine breast cancer model known to have a large percentage of myeloid derived suppressor cells (MDSC) making the model resistant to many immunotherapies and is considered an immunologically cold tumor.
- We hypothesized that treatment with focal radiation would sensitize 4T1 tumors to anti-mCTLA-4 treatment.

Materials and Methods

- 4T1-Luc2 cells were implanted into a lower mammary fat pad of female Balb/c mice (Envigo). Mice were staged into treatment groups when the mean tumor volume was ~100mm^3. Tumor growth changes were tracked by caliper measurements. Anti-mCTLA-4 antibody (9D9 clone) was acquired from Bio X Cell (West Lebanon, NH).
- Image-guided irradiation was performed under 1-2% isoflurane anesthesia on the Small Animal Radiation Research Platform (SARRP; Xstrahl Inc., Suwanee, GA). Treatment was delivered at 220 kV and 13.0 mA using an appropriately sized collimator to the total indicated dose (in Gray; Gy) in 2 equally weighted beams. Antibody treatment was given intraperitoneally just after focal radiation.
- Metastatic tumor burden was tracked by bioluminescence imaging (BLI) using an IVIS Spectrum (PerkinElmer, Waltham, MA).
- For immune profiling, tumors were collected and dissociated into single cell suspensions (gentleMACS™, Miltenyi), samples were labeled with a comprehensive leukocyte panel (MI-CompLeukocyte™) and analyzed by an Attune™ NxT flow cytometer (Thermo Fisher Scientific). Immune subsets were delineated using FlowJo (FlowJo, LLC, Ashland, OR). The number of cells/gram of tumor was quantified using Precision Count Beads™ (Biolegend). For phospho-flow, cell samples were fixed and permeabilized after antibody labeling for surface markers, and then stained with a fluorescently labeled anti-p-STAT3 antibody (S727).
- Statistical analysis was performed using a Student's t-test.
- For immunohistochemistry, tissues were fixed in 10% NBF and embedded in paraffin. Sections were processed and labeled using direct methods with chromogen substrate on the BOND RXm (Leica Biosystems). Images were obtained on the Aperio VERSA (Leica Biosystems). Immunopositive cell counts were done under 100X objective fields. Six fields/sample were counted from each tumor core and periphery.
- Animal care and use was conducted in alignment with animal welfare regulatory requirements in an AAALAC-accredited facility.

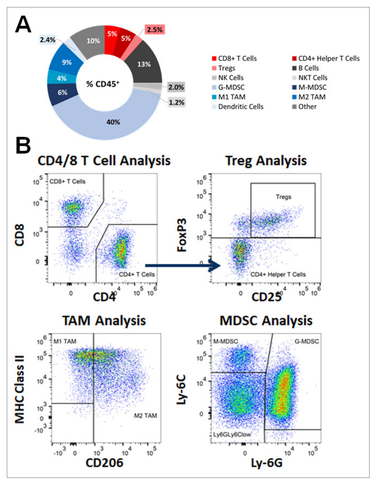

Figure 1. TILs from 4T1-Luc2 tumors are MDSC Prevalent. 4T1-Luc2 baseline TIL immunophenotype. (A) Mean percentages of subsets among total CD45+ cells (n=6 untreated mice). (B) Pseudocolor plots are representative data from a single mouse that is used to illustrate the gating strategy of select immune cell subsets.

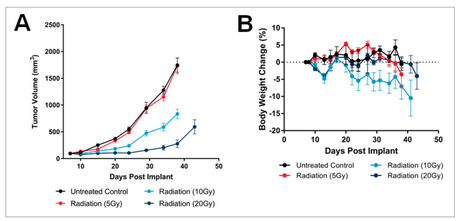

Figure 2. Radiation dose response in 4T1-Luc model. Mice with 4T1-Luc2 mammary fat pad tumors were treated as described with a single dose of focal RT. Tumor volumes (A) and changes in body weight (B) were tracked over time.

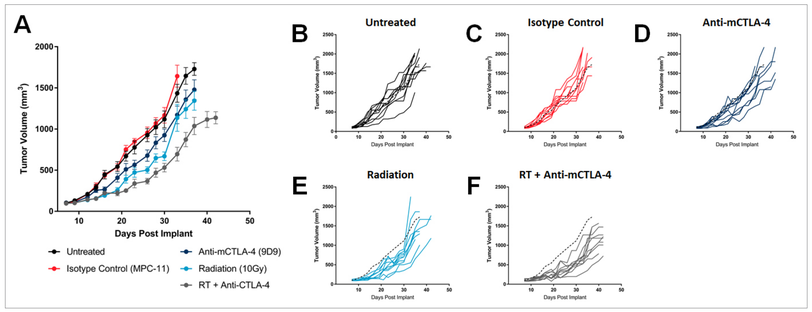

Figure 3. Anti-mCTLA-4 and focal RT reduce primary tumor burden. Mice with 4T1-Luc2 tumors were treated as described with anti-mCTLA-4 (9D9), 10 Gy focal RT or the combination. Mean (A) and individual (B-F) tumor volumes were determined over time. Dotted lines represent untreated mean volume.

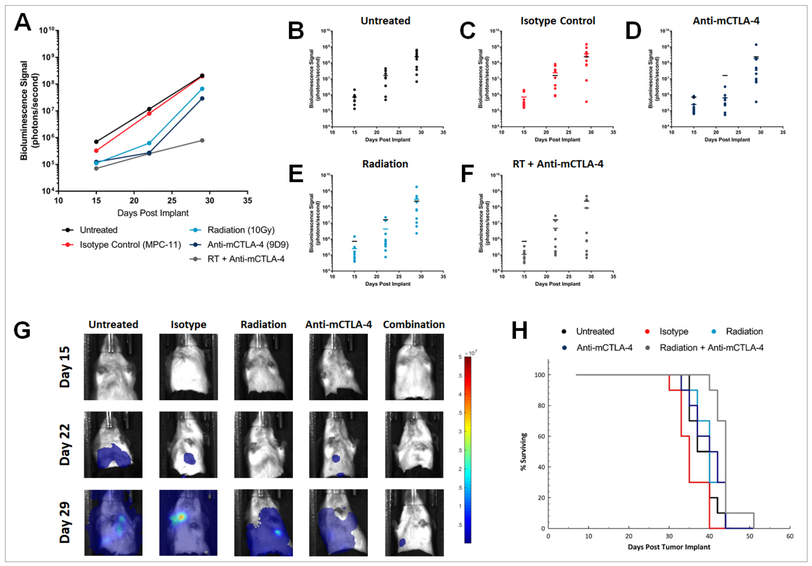

Figure 4. Anti-mCTLA-4 and focal RT delay onset of metastasis. Mice with 4T1-Luc2 tumors were treated as described with anti-mCTLA-4 (9D9), 10 Gy focal RT or the combination. Median (A) or individual (B-F) thoracic area tumor burden tracked by BLI over time. (G) Representative images and (H) Overall survival. In B-F, solid dash is median untreated signal, colored dash is treated median signal.

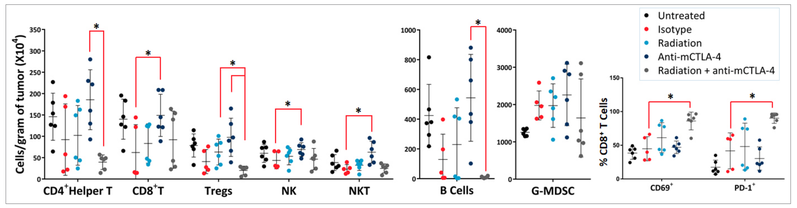

Figure 5. Increased T cell activation and reduced tregs and B cells. Immune subset infiltration into the tumor was measured using the MI-CompLeukocyte™ panel. Changes in response to combined treatment include significant reductions in Tregs, and B cells along with increased CD69 and PD-1 expression on T cells. M-MDSC, M1 TAM, M2 TAM, and DCs did not change in response to treatment (not shown). *p value < 0.05

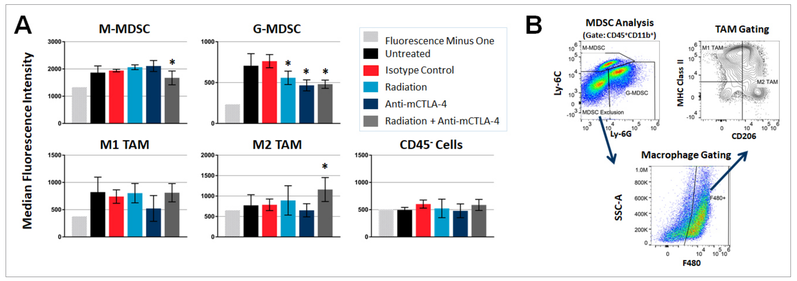

Figure 6. Changes in pSTAT3 following anti-mCTLA-4 treatment. (A) Bar graphs represent pSTAT3 levels in MDSC and TAM subsets. pSTAT3 was undetectable in the tumor-derived CD45- cells. (B) MDSC and TAM gating strategy. *p value < 0.05, compared to isotype control.

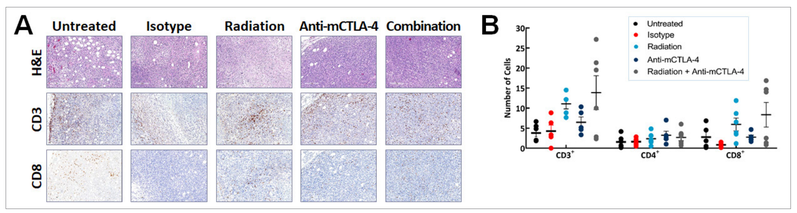

Figure 7. Anti-mCTLA-4 and RT increase T cells and T cell infiltration. (A) Representative H&E and IHC per group. (B) Combined counts of immunopositive cells from 6 core and 6 periphery sections of each tumor. Untreated, isotype and anti-mCTLA-4 pictures taken from periphery; radiation and combination pictures taken from core.

Results and Conclusions

- Focal radiation combined with concurrent anti-mCTLA-4 treatment improved anti-tumor activity when compared to either monotherapy in the 4T1-Luc model suggesting that this approach can be used to modify "cold" tumors into more responsive, or "warm" tumors.
- Substantial depletion of B cells and Tregs was caused by combined therapy, but additional work would be needed to determine what, if any, role this plays in the biology of the model and its response to therapies.
- Changes in TIL populations along with decreased pSTAT3 levels following treatment may be important regulators of combination activity in this model.
- Future work is needed to elucidate the mechanism by which radiation and anti-mCTLA-4 therapy alters myeloid STAT3 phosphorylation.

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