#916. Combined Focal Radiation and Immune Checkpoint Inhibition Enhances Anti-Tumor Responses over Single Agent Treatment in a Murine Mammary Carcinoma Model

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Introduction and Background

Tumor models can be generally divided into immunologically 'cold' and 'warm' categories based on their immune cell infiltrates and their ability to respond to checkpoint inhibition. 'Warm' tumors respond well to immuno-therapies while 'cold' tumors do not. Researchers are searching for ways to make 'cold' tumors more responsive. In this study, the 'cold' breast tumor model 4T1-luc was used. Typically insensitive to checkpoint inhibition, the combination treatment of focal radiation with anti-CTLA-4 blockade was tested.

Materials and Methods

- 4T1-luc cells were implanted into a lower mammary fat pad of Balb/c mice (Envigo). Animal care and use was performed in conformance with the Guide for the Care and Use of Laboratory Animals in an AAALAC-accredited facility.
- After tumors were established, the mice were randomized into four treatment groups. Isotype control (clone MPC-11), and anti-CTLA-4 antibody (clone 9D9) were acquired from Bio X Cell and administered IP. Focal radiation was administered with the Small Animal Radiation Research Platform (SARRP) from Xstrahl (Suwanee, GA). Tumor volumes were determined by caliper measurement.
- Flow cytometry analysis was performed on tumors harvested and dissociated into a single cell suspension using the Miltenyi gentleMACs™ mouse tumor kit. The cells were stained with a 19-color CompLeukocyte™ panel and run on a CytoFLEX LX Flow Cytometer (Beckman Coulter). All data analysis was performed using FlowJo™ software (BD).
- Subsets of tumor immune cells were identified using a modified version of the CompLeukocyte™ package using traditional gating methods. The t-Distributed Stochastic Neighbor Embedding (tSNE) analysis was performed using the tSNE FlowJo plugin. For this analysis, we down sampled from the CD45+ CD11b+ population to focus on myeloid cells. Next, we concatenated all samples from all treatment groups to generate a dimensionally reduced map of all myeloid cells.

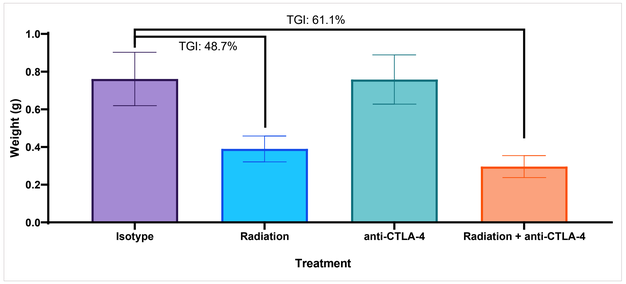

Figure 1. 4T1-luc tumor burden.

Tumor burden following treatment with isotype, anti-CTLA-4, focal radiation or anti-CTLA-4 with radiation is shown. The greatest reduction in tumor volume was observed in the combination treatment group.

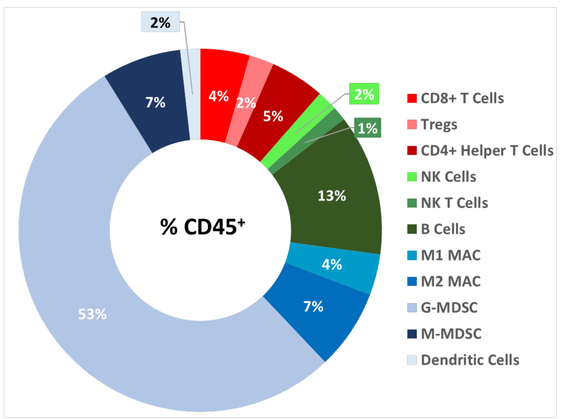

Figure 2. 4T1-luc immune cell subset distribution.

Flow cytometric analysis of 4T1-luc tumors show that myeloid cells make up the majority of immune cells, with G-MDSCs making up more than half of all immune cells while T cells only contributed to ~10% of the immune population.

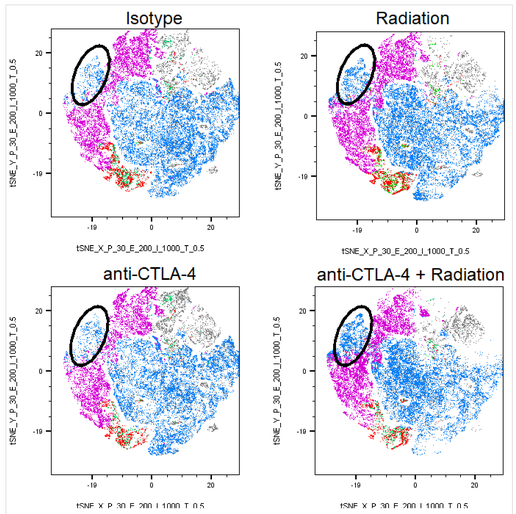

Figure 3. 4T1-luc tSNE analysis.

Plotting the traditional gating method subsets over the tSNE map allows visualization of population shifts between treatment groups. This method also showed which subsets shifted within the heat maps that share the tSNE map. All of the blue areas indicate the G-MDSC population; the circled area corresponds to the same circled area in the heat maps in Figure 4A and B.

Traditional flow cytometric analysis did not reveal any differences within myeloid populations between treatment groups. As myeloid cells were the majority of the immune cells in this tumor type we focused on this subset to investigate tSNE analysis for an unbiased analysis of all markers in the panel. This allowed us to visualize where expression of any markers within the panel shift between treatment groups, and to plot traditional gating populations onto the map. Using this analysis, results were plotted with traditional gating populations onto the map (Figure 3).

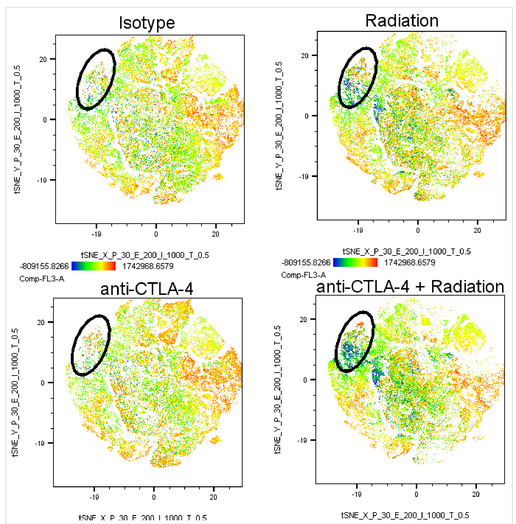

Figure 4A. 4T1-luc tSNE heat map PD-1.

A heat map of PD-1 expression on the tSNE map across the different treatment groups. A portion of the G-MDSC population shows a decrease in PD-1 expression with both the radiation and combination treatment groups. The circled area indicates the area with the largest change.

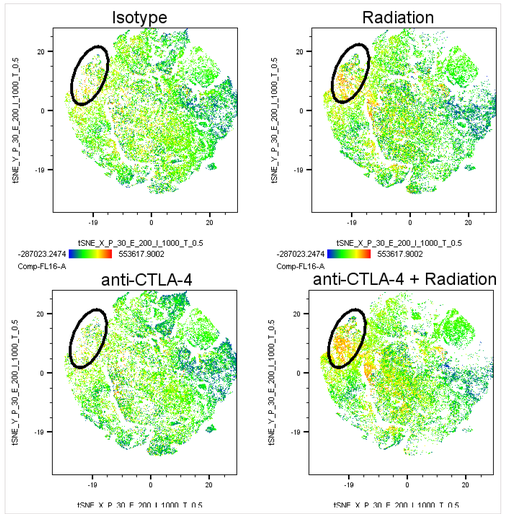

Figure 4B. 4T1-luc tSNE heat map Ki-67.

A heat map of Ki-67 expression on the tSNE map across the different treatment groups. A portion of the G-MDSC population shows an increase in Ki-67 expression with both the radiation and combination treatment groups. The circled area indicates the area with the largest change.

Examining the heat maps produced by all markers within the CompLeukocyte™ panel on top of the tSNE maps allowed for identification of markers that changed within treatment groups that the traditional gating method ignored.

Two atypical markers were specifically examined on myeloid cells, PD-1 (T cell exhaustion) and Ki-67 (cell proliferation).

These two markers showed inverse effects within the treatment groups. PD-1 expression showed a moderate decrease in G-MDSCs in the radiation-treated group, and a more pronounced decrease in the combination-treatment group. Conversely, Ki-67 expression increased slightly in the radiation-alone group and increased even further in the double treatment group. Neither marker was affected by the isotype control antibody or anti-CTLA-4 single agent treatments. These data suggest that a PD-1 negative subset of G-MDSCs is proliferating in response to radiation treatment, and this is enhanced by combination with anti-CTLA-4. The same area of the tSNE map showed increased Ki-67 expression in the radiation treated group and an even further increase in the double treatment group while the isotype and anti-CTLA-4 groups showed little change.

Results and Conclusions

The data demonstrate that tSNE analysis of multiparameter flow cytometric data can be used to detect changes to immune populations that may be missed by traditional gating methods.

The role of PD-1 expression in myeloid cells is not well understood. However, a recent study (Strauss, Jan 2020[1]) may help clarify the role of PD-1. In that study, PD-1 was selectively ablated in either T cells or myeloid cells in a B16-F10 murine melanoma model. T cell-specific deletion of PD-1 did not result in decreased tumor volume. However, deleting PD-1 in myeloid cells did reduce tumor size, even when T cells expressed normal levels of PD-1.

This newly discovered importance of the expression of PD-1 on myeloid subsets reflects the results of the 4T1-luc model here, demonstrating marked proliferation of PD-1 negative G-MDSCs with radiation treatment which is increased with the combination treatment. The switch in the G-MDSC population to a more PD-1 negative phenotype may allow the T cells within the model to more effectively reduce the tumor size.

Reference

1. Targeted deletion of PD-1 in myeloid cells induces antitumor immunity. Laura Strauss, Mohamed A. A. Mahmoud, Jessica D. Weaver, Natalia M. Tijaro-Ovalle, Anthos Christofides, Qi Wang, Rinku Pal, Min Yuan, John Asara, Nikolaos Patsoukis, Vassiliki A. Boussiotis. *Science Immunology* 03 Jan 2020.

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