

DISTINCT TUMOR INFILTRATING TREG LINEAGES ARE ASSOCIATED WITH RESPONSE TO ANTI-PD1 CHECKPOINT BLOCKADE IN NON-SMALL CELL LUNG CANCER

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Background Immune-checkpoint blockade (ICB) has proved a major success, especially in highly mutated tumors such as lung cancer. Nevertheless, not all patients respond to ICB.¹ It is possible that regulatory T cells (Tregs) play a role in this lack of response by suppressing tumor-reactive cytotoxic T cells,² however the specific mechanisms that lead to this suppression remain elusive. Additionally, Tregs are necessary for protection against autoimmune disease³ and broadly depleting them could induce severe immune adverse events. It is therefore necessary to understand the functional programming and suppressive nature of Treg subsets in the tumor microenvironment to define targetable molecules for future biomarker-driven therapeutics.

Methods In this study we performed single cell RNA-sequencing on T cells isolated from resected tissue and peripheral blood from 15 neoadjuvant nivolumab (anti-PD1)-treated and 10 untreated non-small cell lung cancer (NSCLC) patients. We identified and analyzed 71,251 CD4+ FoxP3+ Tregs. Refined clustering was performed, and we used pseudotime and differential gene analyses to understand the transcriptional relationship between clusters and patient groups. We plan to relate our Treg subcluster compositions and enriched gene sets with previously defined mouse models of ICB response as well as human head and neck squamous cell carcinoma (HNSCC).

Results With our highly refined clustering approach, we identified 7 distinct Treg clusters that could reflect differing functionalities within the tumor microenvironment. We demonstrate two separate Treg subsets that diverge towards either an activated state, expressing members of the tumor necrosis factor receptor (TNFR) superfamily: OX40, 41BB, GITR, or a resting state. These lineages separate ICB responders from non-responders, whose tumors are enriched in activated Tregs. We plan to stimulate receptors associated with non-response using agonist ligands or antibodies and hypothesize that their induced signaling will result in transcriptional program changes leading to highly suppressive Tregs.

Conclusions Together, this study provides an in-depth look at the Treg-derived suppressive mechanisms governing their function in the TME of anti-PD-1-treated vs. untreated tumors. Using biospecimens obtained from the neoadjuvant setting, we were also able to study the impact of PD-1 blockade on Treg intra-tumoral function. This in-depth analysis of tumor Tregs has identified specific targetable biomarkers which could be used to improve ICB response while mitigating off-target immune adverse events by specifically inhibiting a small subset of Tregs without disturbing systemic immune homeostasis.

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Ethics Approval This study was approved by the Institutional Review Boards (IRB) at Johns Hopkins University (JHU) and Memorial Sloan Kettering Cancer Center (NA_00092076; NCT02259621) and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The patients described in this study provided written informed consent.

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