facilitated the infiltration of novel clones in the TME. Importantly, serial ISIM further reshaped the TCR repertoires in the TME which had been destined to become resistant to anti-PD-L1 therapy, and rendered tumors continuously responsive to anti-PD-L1 therapy, resulting in durable complete responses and establishment of tumor-specific immunological memory. Conclusions Taken together, ISIM not only increased CD8+ T-cell infiltration but also reshaped the intratumoral TCR repertoires. These findings provide insights into the utility of an in situ combinatorial immunotherapeutic regimen for overcoming resistance to anti-PD-L1 therapy due to tumor-mediated mechanisms of immune cell exclusion.

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RADIOThERAPY AND CTLA-4 BLOCKADE EXPAND ANTI-TUMOR T CELLS DIFFERENTIATION STATES AND COOPERATE WITH CD40 AGONIST TO INDUCE TUMOR REJECTION

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Background Radiotherapy (RT) in combination with CTLA-4 inhibition (CTLA4i) can expand and activate T-cells to reject tumors in both mice and some patients with tumors unresponsive to CTLA4i alone.1 2 However, only a subset of patients achieves long-term control of metastatic disease. Similar responses to RT+CTLA4i are seen in the 4T1 mouse model of triple negative breast cancer (TNBC), making it an ideal model to interrogate the interaction between RT and CTLA4i, and identify barriers to its effectiveness. Methods Mice were inoculated in one or both flanks with 4T1 cells. In some experiments one tumor was removed for analysis before start of treatment with RT (3 x 8 Gy) and/or anti-CTLA-4 antibody (9H10, 3 x 200 µg i.p.). The intratumoral T cell response was assessed using bulk and single cell RNA/TCR sequencing. The METABRIC dataset3 was used to associate gene expression signatures with patient survival. In some experiments, RT+CTLA4i was combined with PD-1, LAG-3, or CD40 Abs. Results RT, alone and with CTLA4i, increased the TCR repertoire clonality and density of activated T cells in the tumors. (figure 1A-G). In untreated tumors, Gzmb+Prf1+Lag3+Pdl1+Cd8+ T cells (cluster 0) were most common. CTLA4i ‘unlocked’ Ifng+Cd40lg+Cd4+ T cells (cluster 2) while RT favored expansion/persistence of Cd8+ T cell clusters. In tumors of mice treated with RT+CTLA4i activated Treg cells (cluster 1) were decreased and Ifng+Cd40lg+Cd4+ T cells (cluster 2) increased. Relatively among CD8+ T cells, Ifng+Tnf+Cd8+ (cluster 4) was expanded at the expense of cluster 0 (figure 2A-F). Gene signatures defining clusters 0, 2, and 4 were associated with improved survival in the METABRIC TNBC patient cohort using a multivariate model (figure 2G-H). In mice, AH1-tumor antigen-specific CD8+ T cells occupied different transcriptional states, with a shift to cluster 4 in mice treated with RT+CTLA4i (figure 2I), suggesting that multiple functional T cell states are required for tumor rejection. Based on the T cell phenotypes expanded by RT+CTLA4i, antibodies to PD-1, LAG-3, and CD40 were tested for the ability to enhance RT+CTLA4i therapy. Only CD40-agonist improved significantly tumor control (figure 3A-B).

Conclusions Altogether, these results revealed that RT and CTLA4i have complementary effects and besides driving T cells into tumors shape CD4 and CD8 T cell functional differentiation towards subsets that are associated with improved

Abstract 465 Figure 1 RT, alone and with CTLA4i, increased the TCR repertoire clonality and density of activated T cells in the tumors. (A) Design of the experiment enabling collection of pre- and post-treatment (pre-tx and post-tx) 4T1 tumor tissue that was analyzed using RNA- and TCR-sequencing. (B) Tumor growth curves. Statistical significance in tumor volume growth between groups was determined using 2-way repeated measures ANOVA between day 15–21 and t-test at day 21. (C) Shannon clonality of paired pre- and post-tx TCR repertoires. Pairwise and paired t-tests were used to evaluate statistical significance of differences between and within groups, respectively. (D) RNA-seq based gene expression heatmap of selected canonical T cell markers in post-tx tumors. (E) Linear regression between Cd3e and Cd4 or Cd8 gene expression in post-tx tumors. R2 and p indicate R-square and p-value for the models, respectively (F) Ingenuity Pathway Analysis Canonical Pathway and (G) Upstream Regulation analysis. Z-scores indicate predicted activation (> 2) or inhibition (< -2) of pathways and upstream regulators. (all panels), **, and ***?, and #, ## and ### indicate p-values of pairwise and paired statistical tests, respectively. Tukey’s and Holm’s method for adjusting p-values corrected for multiple comparison was used for the ANOVA and t-tests, respectively. (Abbreviations) tx, treatment; RT, radiation therapy; CTLA4, CTLA-4 Ab therapy; TCR, T cell receptor


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Abstract

465 ANTI-EGFR ANTIBODY ADDED TO ONGOING ANTI-PD-1 ANTIBODY TREATMENT FOR METASTATIC CUTANEOUS SQUAMOUS CELL CARCINOMA OF THE FACE: TWO CASE REPORTS

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Background Recurring cutaneous squamous cell carcinoma (SCC) remains an area of high unmet medical need. While anti-PD-1 antibodies are now approved for this diagnosis, more than half the patients will need more effective treatments, supporting the development of new or combination regimens. Agonistic CD40 treatment improves RT+CTLA-4 immunotherapy. Cetuximab is dosed every two weeks rather than weekly and has a relatively favorable toxicity profile.

Methods Two consecutive elderly patients with significant comorbidities presented with a performance status of ECOG 3 and rapidly progressive recurrent cutaneous SCC of the face. The patients were presented treatment with an anti-PD-1 antibody, with an option - were there an inadequate palliative response - to include an EGFR antibody provided tolerance was acceptable. The first cycle of pembrolizumab 2 mg/kg or nivolumab 3 mg/kg, respectively, escalating in both cases to flat dosing once it was apparent that tolerance was acceptable. The first cycle of panitumumab (6 mg/kg), when needed to be invoked, was restricted to H2-Ld restriction in 4T1 tumors; SPSYVYHQF peptide derived from gp70 and complementary determining region 3; UMAP, Uniform Manifold Approximation and Projection for dimension reduction; AH1, tumor antigen in 4T1 tumors; SPSYVYHQF peptide derived from gp70 and restricted to H2-Ld

survival in patients. Unexpectedly, inhibition of checkpoint receptors expressed by a large CD8 T cells cluster did not further improve responses to RT+CTLA4i, whereas agonistic CD40 therapy did, suggesting new therapeutic strategies.

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