

COMBINATION THERAPY WITH ANTI-PD1 AND ANTI-LAG3 LEADS TO UNIQUE PERIPHERAL AND INTRATUMORAL CD8+ T CELLS SIGNATURES IN PATIENTS WITH METASTATIC MELANOMA

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Background CD8+ T cell exhaustion, characterized by co-expression of multiple inhibitory receptors, limits antitumor immunity. Combination therapy with anti-PD1 and anti-LAG3 synergistically improves CD8+ T cell function in murine models of cancer and has recently demonstrated improved progression-free survival versus anti-PD1 alone in metastatic melanoma. However, the mechanisms underlying improved efficacy of this combination therapy have yet to be fully elucidated.

Methods A phase 2 clinical study was designed to assess the antitumor efficacy of anti-PD1 or anti-LAG3 alone versus anti-PD1 with anti-LAG3 in combination in patients with advanced melanoma naïve to prior immunotherapy. Peripheral blood mononuclear cells (PBMC) and tumor biopsies were obtained prior to and at four weeks following therapy. Single-cell RNA-seq (scRNAseq) was performed using 5'-based chemistry (10X Genomics) on live FACS-sorted immune cells from blood and tumors. scRNAseq data was normalized across patients using scVI, and CD8+ T cells from PBMC and tumors were bioinformatically isolated for detailed analyses. miloR was used to identify CD8+ T cell transcriptional signatures that would distinguish effects associated with each treatment arm.

Results We identified a total of 33,646 CD8+ T cells from PBMC and tumor infiltrating leukocytes (TIL) from 22 patients. Using miloR to define subpopulations of CD8+ TIL, we found that each treatment arm led to a distinct CD8+ T cell transcriptional signature at just four weeks post-treatment. Using a gene set derived from genes upregulated in CD8+ TIL following combination therapy, we found that melanoma patients from The Cancer Genome Atlas with high enrichment of this anti-PD1 plus anti-LAG3 combination signature had improved overall survival (HR=0.39, p<0.001), indicating that this signature is associated with antitumor immunity. We also found that CD8+ T cells from PBMC of patients receiving combination therapy had higher levels of T cell receptor (TCR) and interferon gamma (IFN γ) signaling, suggestive of peripheral signatures of pharmacodynamic response to combination therapy. Notably, TCR and IFN γ signaling were correlated between CD8+ T cells in PBMC and TIL at week 4 (rho=0.67, p=0.020 for TCR; rho=0.59, p=0.046 for IFN γ), demonstrating that peripheral CD8+ T cells may serve as a surrogate for intra-tumoral responses.

Conclusions The anti-PD1, anti-LAG3, and anti-PD1 plus anti-LAG3 treatment arms each led to unique CD8+ TIL signatures. A transcriptional signature derived from combination therapy was associated with favorable prognosis in patients from an independent cohort. Functional signatures expressed by CD8+ T cells in PBMC were reflective of signatures occurring within tumors during therapy.

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