DEVELOPMENT AND VALIDATION OF A NEOANTIGEN-SPECIFIC T CELL GENE SIGNATURE TO IDENTIFY ANTITUMOR T CELLS IN LUNG CANCER AND MELANOMA

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Background Mutation-associated neoantigen (MANA)-specific T cells play a key role in tumor control and response to immune checkpoint inhibition (ICI).^{1,2} However, the majority of tumor-infiltrating lymphocytes (TIL) are not specific for the tumor.³ Herein, we developed and validated MANAscore, a bioinformatic scoring algorithm based on the transcriptional programs of MANA-specific T cells to isolate antitumor T cells from bystander T cells in lung cancer and melanoma.

Methods Combined single-cell (sc) RNA-seq/TCR-seq was performed on TIL obtained from 15 resectable non-small cell lung cancer (NSCLC) patients receiving neoadjuvant anti-PD-1 (NCT02259621). MANA-specific clonotypes were identified by coculturing autologous T cells with predicted MANA, and were validated by cloning the full TCR alpha and beta chain as previously described.¹ Using the TCRβ CDR3 as a barcode, antigen-specific T cells were linked with their intratumoral sc expression profile. Using the first two patients enrolled in the clinical trial as a discovery cohort, MANAscore was developed to identify gene programs that best distinguish MANA-specific vs viral-specific T cells from NSCLC. Prediction performance was assessed in independent patients from the NSCLC and melanoma cohort.² Seven MANAscore > hi clones TCRs were cloned and queried for reactivity to peptide libraries of putative MANA derived from whole-exome sequencing of the respective tumor. Association of MANAscore hi clones with response to ICIs among all patients was assessed.

Results A total of 890 MANA- and 542 viral-specific T cells were identified in sc TIL from six NSCLC patients. MANA- and viral-specific TIL presented with unique transcriptional profiles. Particularly, MANA-specific CD8 TIL expressed a partially activated cytolytic program with co-expression of multiple immune checkpoints and upregulated transcriptional regulators of T cell dysfunction. MANAscore showed high prediction accuracy and outperformed CD39 in identifying tumor-reactive T cells in independent NSCLC patients, as well as in an external validation cohort of melanoma patients (3936 MANA-specific T cells and 626 viral-specific T cells from 4 patients). Of seven MANAscore > hi clones tested for reactivity, three were confirmed as MANA-specific. The pseudobulk expression profile of MANAscore > hi clones showed a significant correlation with response to ICI, which is not observed in total CD8+ TIL.

Conclusions MANA-specific TIL demonstrated a distinct gene signature that enabled us to identify de novo antitumor TIL in NSCLC and melanoma. MANAscore may serve as a useful tool in facilitating mechanistic studies of ICI response and resistance.

Trial Registration NCT01970358, NCT02259621

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Ethics Approval The melanoma clinical trial was approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board (IRB) (NCT01970358). The NSCLC clinical trial was approved by the Institutional Review Boards (IRB) at Johns Hopkins University (JHU) and Memorial Sloan Kettering Cancer Center (NCT02259621)

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal

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