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**INDUCTION OF CLONAL EXPANSION OF TUMOR-REACTIVE T CELLS AND EFFECTIVE TUMOR SUPPRESSION BY NEOANTIGENS PREDICTED FROM THE 'VACINUS' PLATFORM**

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**Background** The efficacy of therapeutic cancer vaccines mainly depends on the selection of neoantigen that can induce strong cytotoxic CD8 T-cell response required to reject tumors. Previously, we developed a neoantigen prediction platform called 'VACINUS,' which predicts neoantigens based on the binding between tumor-reactive tumor-infiltrating lymphocytes (TILs) TCR and peptide-MHC(pMHC). In this study, we investigated anti-tumor immune responses mediated by neoantigens predicted by VACINUS using a mouse tumor model.

**Methods** Seven-week-old male mice were implanted subcutaneously on the flank with B16F10 cells (C57BL/6). After two rounds of vaccination with tier 1 neoantigens, we compared tumor growth and obtained splenocytes to examine the tier 1 neoantigen-specific T cell response. We performed single-cell analysis on tumor tissue to analyze TILs. Splenocytes were sorted for tier 1 neoantigen-specific T cells using pMHC-dextramer and subjected to single-cell sequencing for comparative analysis.

**Results** To assess the anti-tumor efficacy, tumor size and survival rate were measured. The vaccinated groups showed tumor control and longer survival than unvaccinated groups. To investigate mechanisms regarding response to our vaccine, we compared the extent of TCR repertoire overlap in CD8 T cells between TIL and splenic T cells using single-cell TCR sequencing. In the vaccinated group, around 20% of T cells in the spleen and tumor overlap. These clones were more prevalent in tumors than in spleens, and more abundant in the vaccinated group than in the unvaccinated group. An analysis of a total of 10,000 single cells for distributions of phenotypes showed 13 subtypes of T cells based on gene expression markers. The overlapping clones were found in effector/exhausted CD8 types. Most of the enriched cell types in tumor cells were exhausted cells. Terminally exhausted T cells were dominant in the vaccinated group, whereas pre-exhausted T cells were dominant in the combination treatment group.

**Conclusions** Neoantigens derived from VACINUS showed better anti-tumor effects than anti-PD-1 alone. An increase in overlapping TCR clones of spleen and tumors in vaccinated group suggested that our vaccines induce infiltration of T cells into tumor. The overlapping T cell clonotypes were enriched in effector and/or exhausted types which recognize and respond to antigens. A neoantigen prediction platform 'VACINUS' with high hit rate shed light on this field.

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