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**STRUCTURE-BASED PREDICTION OF NEOANTIGENS
PAIRED WITH T CELL RECEPTORS ON PHENOTYPE-
SELECTED CD8+ TUMOR-INFILTRATING LYMPHOCYTES**

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Background Vaccination by tumor neoantigens are promising immunotherapy by providing more tumor-reactive T cell pool, which could be boosted by anti-PD-1. Identification of neoantigens cognate to tumor-reactive tumor-infiltrating lymphocytes (TILs) is critical for clinical efficacy of neoantigen vaccines. Here, we developed *in silico* neoantigen prediction platform by structure-based pairing with T cell receptors (TCR) on pre-existing CD8+ TILs selected by single-cell transcriptome profiles. Neoantigens derived by our strategy reflect in vivo immunogenicity and tumor reactivity.

Methods Tumor resections from solid cancer patients are subject to whole exome and transcriptome sequencing. In addition, TILs from the patients are subject to scRNAseq/scTCRseq to stratify TCRs of TILs into target TILs and non-target TILs by single-cell transcriptome profiles. Neoantigen epitopes are filtered with HLA binding/immunogenicity and prioritized by tricomplex (TCR-peptide-HLA) structure-based TCR binding score from our platform Vacinus. To evaluate the immunogenicity of selected neoantigens, the neoantigen peptides are tested for *in vitro* IFN γ ELISPOT assay using peripheral blood mononuclear cells (PBMCs) from the same patients, which sensitively detects antigen-experienced T cells.

Results We have screened the immunogenicity of 286 neoantigens derived from Vacinus platform for 34 solid cancer patients. In particular, we could detect the immunogenic neoantigens in the majority of Hepatocellular carcinoma (HCC) patients (13/14) which had low mutational burden with median 95 (46-373) non-synonymous mutations. Single-cell transcriptome of CD8 TILs from HCC revealed that TILs had primarily exhausted/cytotoxic phenotype (target TIL) and non-exhausted memory phenotypes (non-target TIL). We applied TCRs derived from target or non-target TILs to select cognate neoantigens predicted by Vacinus platform, which are tier1 and tier2 neoantigens, respectively. Interestingly, when those neoantigens were tested for immunogenicity with IFN γ ELISPOT assay, higher T cell responses were detected in tier1 (31%) than tier2 neoantigens (17%), reflecting tier1 neoantigens have more capability to induce in vivo immunogenicity in cancer patients. Using mouse tumor models, we are investigating therapeutic efficacy of tier1 neoantigens.

Conclusions It is feasible to develop cancer neoantigen vaccine in HCC which has low mutational burden. Neoantigens paired with TILs in an activated state were identified to have greater immunogenicity compared to TILs in a memory state, underscoring the selection of target TILs. Neoantigen prediction by structure-based pairing of neoantigens and phenotype-selected TILs showed promising potential to better select therapeutically-relevant cancer vaccines.

Ethics Approval The study with samples from HCC patients was approved by the Asan Medical Center Institutional Review Board (IRB) (2022-0263)

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