Personalized immunization with GNOS-PV02 of patients with hepatocellular carcinoma drives TCR clonal expansion, tumor infiltration, and vaccine-specific reactivity

Background: Tumor-specific neoantigens can be identified from cancer biopsies and used to develop personalized therapeutic cancer vaccines (PTCV) to prime neoantigen-specific T cell responses. Here, we characterized the antitumor neoantigen-specific reactivity of tumor-infiltrating, high-frequency TCR clones in a patient treated with personalized therapeutic DNA cancer vaccine GNOS-PV02 in the ongoing GT-30 advanced hepatocellular carcinoma single-arm open-label multi-center phase Ib/IIa trial (NCT04251117).

Methods: Paired blood and tumor biopsy samples from patient #8 enrolled in the GT-30 study were collected before and after treatment with GNOS-PV02 (1mg) + plasmid-encoded IL-12 (0.3mg) + Pembrolizumab (200mg). GNOS-PV02 and IL-12 were administered at Q3W for the first 4 doses, then, at Q9W. Pembrolizumab was administered Q3W. Neoantigen positivity was evaluated by IFNγ-ELISpot. TCRβ sequencing was performed on all 4 samples and single-cell TCR and transcriptome sequencing was performed from T cells isolated from the post-treatment blood sample. After vaccination, three newly identified TCRs in blood and tumor were inserted into a pMXs-IRES-GFP retroviral plasmid vector and used to generate engineered TCR T cells. Engineered T cells were tested against the neoantigens included in PTCV by flow cytometry.

Results: The treatment resulted in a partial response with a decrease in tumor size of 44% by RECIST1.1. Five vaccine-encoded responding neoantigens were identified. Differential abundance frame network analysis revealed that 27 of 42 (64.28%) significantly expanded peripheral TCR clones were also found enriched in the tumors post-treatment. Importantly, we observed an increase in cumulative frequency (from 0.4 to 7.7%), and absolute numbers (from 3 to 14) of significantly expanded vaccine-specific TCR clones in the tumor. Increased TCR clonality confirmed a focused tumor repertoire response. Single-cell sequencing data analysis revealed that the 6 most expanded clones in blood were activated CD8+CD69+ T cells (81.82%). Three full TCR sequences from T cell clones newly present in the tumor post-vaccination were selected, synthesized and cloned. TCR-engineered patient-specific T cells showed a dose-dependent CD8+ and CD4+ T cell activation (CD69+) upon stimulation with a pool of epitopes covering all the neoantigens in the patient's PTCV.

Conclusions: PTCV treatment resulted in neoantigen-specific T cell responses, clonal expansion in the periphery and primary lesion, and tumor infiltration of T cells with an activated phenotype. TCR-engineered high-frequency T cells found in the tumor are reactive to PTCV-encoded neoantigens post-treatment. These results may account for the observed objective decrease in the primary tumor size.

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