PERSONALIZED IMMUNIZATION WITH GNOS-PV02 OF PATIENTS WITH HEPATOCELLULAR CARCINOMA DRIVES TCR CLONAL EXPANSION, TUMOR INFILTRATION, AND VACCINE-SPECIFIC REACTIVITY

Renzo Perales [1], Neil Cooch[1], Mark Yarchoan[2], Niranjan Y. Sardesai[1], Jian Yan[1]

[1] Geneos Therapeutics; [2] Johns Hopkins Sidney Kimmel Comprehensive Cancer Center; Correspondence: rperales@geneostx.com

ABSTRACT #2629

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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.

PERSONALIZED VACCINES CAN BE MANUFACTURED IN 6-8 WEEKS, ALLOWING CONCURRENT START WITH ANTI-PD1

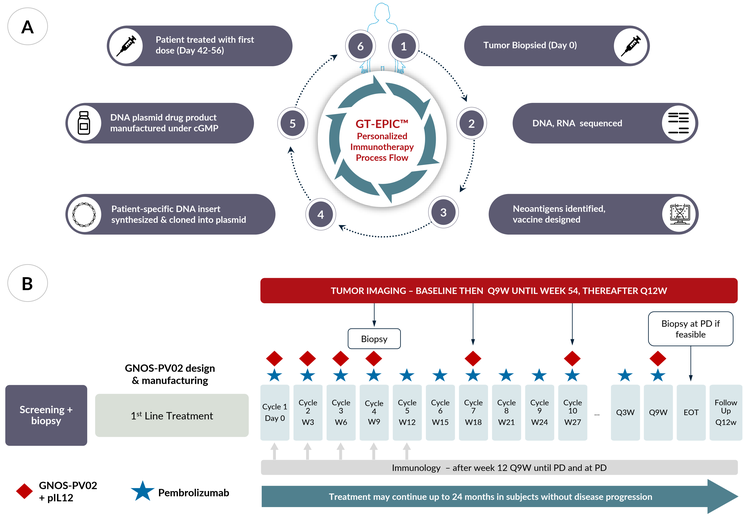


Figure 1: A) Manufacturing process for personalized DNA vaccines. Needle-to-needle has been achieved in as low as 6 weeks and can be regularly achieved in 6-8 weeks. **B)** Clinical Trial design.

PTCV DRIVES NEOANTIGEN-SPECIFIC RESPONSES THAT ARE DETECTED IN BLOOD

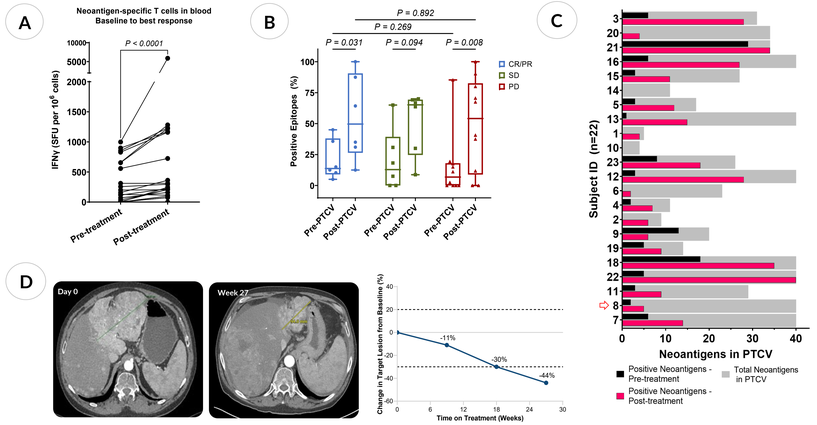


Figure 2: A) Patient's PBMC samples were evaluated for the presence of vaccine-induced neoantigen-specific responses prior to and post-PTCV vaccination as measured by IFNγ ELISpot assay (n=22), without cytokine stimulation. Cumulative magnitudes were collected from positive epitopes at pre- and post-treatment. **B)** Percentage of positive responding epitopes by groups and timepoint. The definition of a neoantigen-specific ELISpot response can be found in the Methods section. **C)** Positive neoantigens pre- and post-vaccination (black and red bars, respectively) relative to the total number (grey bars) included in each patient's PTCV as defined by IFNγ ELISpot assay. **D)** Sequential tumor imaging scans evaluated by RECIST1.1 over time, and change in target lesion (%) from baseline. Significance between groups was evaluated by a two-tailed Mann-Whitney test; significance within groups was evaluated by a two-tailed Wilcoxon rank test.

GNOS-PV02 RESULTS IN THE EXPANSION OF NEW T CELLS THAT TRAFFIC TO THE TUMOR

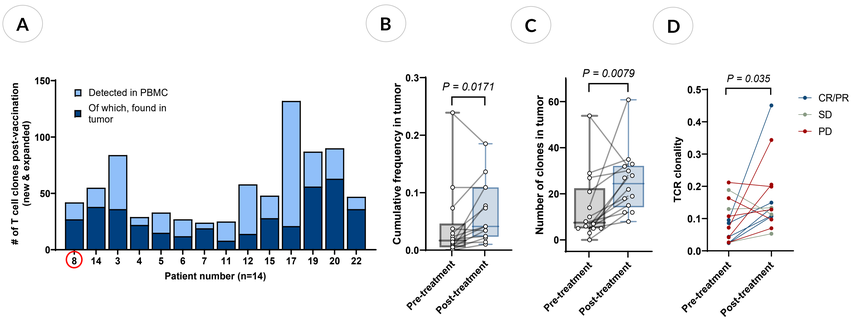
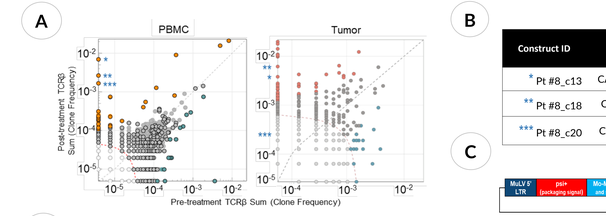


Figure 3: A) Significant T cell clonal expansion in 14/14 (100%) of subjects in both peripheral blood and tumor as evaluated by differential abundance statistical framework (Adaptive Biotechnologies, immunoSEQ®). Overall significant increase in **B)** cumulative frequency and **C)** absolute clone numbers of expanded clones in the tumor pre- vs post-vaccination (week 9) per patient. **D)** TCR clonality reports the distribution of TCR rearrangements in a sample, where 0 indicates an even distribution of frequencies and 1 indicates an asymmetric distribution.

GNOS-PV02 GENERATES NEOANTIGEN-SPECIFIC, CD8+ AND CD4+ ANTI-TUMOR RESPONSES



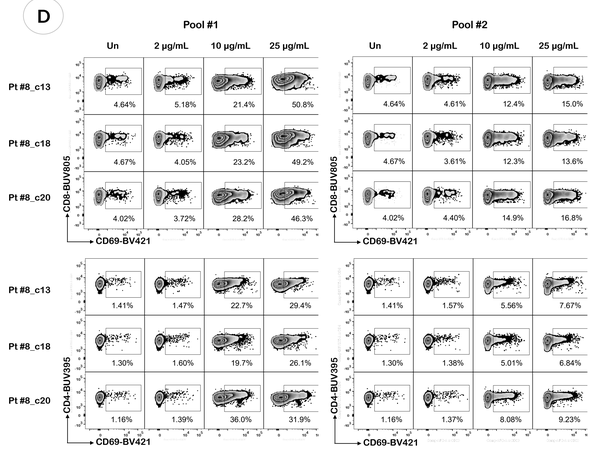


Figure 4: A) TCRβ and RNA sequencing in Pt #8. Pre- vs post-vaccination (Pair-wise scatter plot). Blue asterisks: selected high-frequency new T cell clones. Orange, green, and grey circles represent expanded, contracted, and not significantly changed T cell clones, respectively. **B)** Evaluated TCRs were selected for cloning based on their occurrence in high frequency in the PBMC and trafficking to the tumor post-vaccination. **C)** Patient-specific clonal TCR sequences were gene optimized and inserted into the pMXs-IRES-GFP retroviral plasmid vector containing viral packaging signal, transcriptional and processing elements, and GFP reporter gene. **D)** TCR-engineered T cells (GFP positive) from unvaccinated PBMC were stimulated for 6 hours with increasing concentrations of epitope pools (2, 10, 25 μg/mL), and the expression of CD69 was evaluated by flow cytometry. Peptide pools 1 and 2 comprise the first or second half of the neoantigens included in the PTCV.

CONCLUSIONS

- GT-EPIC™ personalized vaccines containing up to 40 neoantigens can be designed, manufactured, and administered successfully in as short as 6 weeks allowing concurrent start with anti-PD1 in 2nd line HCC.
- GNOS-PV02 + INO-9012 in combination with pembrolizumab achieved an ORR (mITT) per RECIST 1.1 of 29.2% (7/24) in the first 24 patients of the GT-30 trial (2 CR/ 5 PR). The disease control rate (DCR) was 54.2% (13/24).
- Patients treated with GNOS-PV02 + INO-9012 in combination with pembrolizumab had new T cell clones in blood following vaccination, with new clones comprising up to 1% of the peripheral T cell repertoire.
- Most newly detected and expanded T cell clones trafficked into the tumor, representing newly detected TILs following treatment. Neoantigen-specific T cell responses were reported in 19/22 (86.4%) evaluable patients.
- Engineered TCRs identified from high-frequency T cell clones in tumor post-treatment, respond to peptides encoded in the vaccine.
- GNOS-PV02 + INO-9012 presents an unremarkable safety profile with no treatment-related SAEs.