PERSONALIZED NEOANTIGEN DNA VACCINE (GNOS-PV02) AND PEMBROLIZUMAB AS SECOND-LINE TREATMENT FOR ADVANCED HEPATOCELLULAR CARCINOMA

Mark Yarchoan[1], Edward J. Gane[2], Thomas U. Marron[3], Renzo Perales-Linares[4], Jian Yan[4], Neil Cooch[4], Daniel H. Shu[1], Elana J. Fertig[1,5,6], Luciane T. Kagohara[1], Gabor Bartha[7], Josette Northcott[7], John Lyle[7], Sarah Rochestie[4], Joann Peters[4], Jason T. Connor[8,9], Elizabeth M. Jaffee[1], Ildiko Csiki[4], David B. Weiner[10], Alfredo Perales-Puchalt[4], Niranjan Y. Sardesai[4]

1) Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA. 2) New Zealand Liver Transplant Unit, University of Auckland, Auckland, New Zealand. 3) Early Phase Trials Unit, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. 4) Geneos Therapeutics, Philadelphia, PA, USA. 5) Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA. 6) Department of Applied Mathematics and Statistics, Johns Hopkins University Whiting School of Engineering, Baltimore, MD, USA. 7) Personalis, Inc., Fremont, CA, USA. 8) Confluence Stat, Cooper City, FL, USA. 9) University of Central Florida College of Medicine, Orlando, FL, USA. 10) Vaccine and Immunotherapy Center, The Wistar Institute, Philadelphia, PA, USA

geneos THERAPEUTICS

ABSTRACT #1191

ABSTRACT

Background: PD-1 inhibitors have modest efficacy as monotherapy in hepatocellular carcinoma (HCC). A personalized therapeutic cancer vaccine (PTCV) tailored against neoantigens identified in an individual's tumor may enhance responses to PD-1 inhibitors through the induction of tumor-specific immunity. Here, we present results from a single-arm Phase Ib/2a trial evaluating a DNA plasmid (GNOS-PV02) encoding up to 40 neoantigens co-administered with plasmid-encoded IL-12 (pIL12) in combination with pembrolizumab (PEMBRO) in patients (pts) with advanced HCC.

Methods: Patients were eligible for study therapy upon progression or intolerance with a 1L tyrosine kinase inhibitor (TKI). The PTCV [GNOS-PV02 (1mg) and pIL12 (0.34mg)] was administered intradermally via in vivo electroporation Q3w x 4 doses, and Q9w thereafter. PEMBRO was administered at 200mg IV Q3w. The primary endpoints were safety and immunogenicity. To evaluate the secondary endpoint of ORR per RECIST 1.1 by investigator review with a null hypothesis of an ORR of 16.9% (KN-240, Finn et al ASCO 2019), 36 pts were included to provide 80% power to reject the null hypothesis at the one-sided 0.10 level, assuming the true ORR rate of 33.1%. The data cut date was August 18, 2023.

Results: Among the 36 enrolled pts who received at least one dose of treatment, there were no DLTs or treatment-related grade ≥3 events. The most common treatment-related adverse events were injection site reactions, observed in 41.6% of pts. ORR (mITT) per RECIST 1.1 was 30.6% (11/36; 9 confirmed and 2 unconfirmed) with 8.3% (3/36) of pts achieving a CR. This achieved statistical significance with a one-sided p-value = 0.031 (1-sided 90% CI 20.4%-100%) The mOS was 19.9 months. ctDNA changes correlated with radiographic responses and preceded them. A complete molecular response (100% ctDNA clearance) was detected in 7 pts including the 3 radiographic CRs, and 4 additional pts who continue to show durable tumor control (3PR, 1 SD). Immunological analyses confirmed the induction of neoantigen-specific T cell responses by IFNγ-ELISpot in 19/22 (86.4%) evaluable pts, and pts with a larger ELISpot response showed a trend towards longer OS. Multi-parametric cellular profiling and single-cell analysis revealed active, proliferative, and cytolytic vaccine-specific CD4+ and CD8+ effector T cells in the blood of immunized pts. In 14/14 (100%) of pts with paired pre- and on-treatment blood and tumor biopsies, we identified by TCRβ bulk sequencing expanded T cell clones in the peripheral blood that also trafficked into the tumor.

BASELINE PATIENT DEMOGRAPHIC, CLINICAL CHARACTERISTICS, AND SAFETY

A

Demographic Information (n=36)	Number (%)
Median age, years (range)	66.5 (40-83)
Sex	
Female	11 (30.6%)
Male	25 (69.4%)
Race	
White	21 (58.4%)
Asian	8 (22.2%)
Other (Black and Pacific Islander)	7 (19.4%)
ECOG Performance Status	
0	25 (69.4%)
1	11 (30.6%)
Child-Pugh score A	36 (100%)
BCLC Stage	
B	18 (50.0%)
C	18 (50.0%)
Etiology	
HBV	8 (22.2%)
HCV	12 (33.3%)
HBV + HCV	1 (2.8%)
Non-viral	15 (41.7%)
Prior treatment	
Sorafenib	2 (5.6%)
Sorafenib + Lenvatinib	1 (2.8%)
Lenvatinib	33 (91.6%)
PVI	7 (19.4%)
WNT / Beta Catenin mutation	10 (27.8%)
Baseline AFP, ng/mL	
>/=400	8 (22.2%)
<400	28 (77.8%)
Targetable Neoantigens*	
≤ 20	10 (27.8%)
21-40	16 (44.4%)
41-67	10 (27.8%)

B

Treatment-Related Adverse Events** (n=27/36)	Grade 1-2
Injection Site Reactions	
Pain, erythema, pruritus, or swelling	15 (41.6%)
Gastrointestinal	
Gastroesophageal reflux	2 (5.6%)
Diarrhoea	1 (2.8%)
Abdominal pain upper	1 (2.8%)
Nausea	1 (2.8%)
Dry Mouth	1 (2.8%)
Skin	
Rash	4 (11.1%)
Pruritus	2 (5.6%)
Dermatitis	1 (2.8%)
Alopecia	1 (2.8%)
Skin disorder	1 (2.8%)
Dry Skin	1 (2.8%)
Endocrine	
Hypothyroidism	2 (5.6%)
Musculoskeletal	
Pain in extremity, musculoskeletal pain or stiffness	3 (8.3%)
Arthralgia	1 (2.8%)
Metabolism	
Decreased appetite	2 (5.6%)
Hypophosphatemia	1 (2.8%)
Polydipsia	1 (2.8%)
General	
Fatigue	4 (11.1%)
Pyrexia	1 (2.8%)
Chills	1 (2.8%)
Anemia	1 (2.8%)
Dysaesthesia	1 (2.8%)
Lethargy	1 (2.8%)
Somnolence	1 (2.8%)
Infusion related reaction	1 (2.8%)
Chest discomfort	1 (2.8%)
Hepatitis	1 (2.8%)
Elevated liver function test	1 (2.8%)
Immune-related	
Immune-mediated nephritis	1 (2.8%)
Pneumonitis	1 (2.8%)
Immune checkpoint Inhibitor hepatitis	1 (2.8%)
Use of systemic steroids	7 (19.4%)

Note: Data cut: 18.AUG.2023
Abbreviations: AFP: alpha-fetoprotein, BCLC: Barcelona Clinic Liver Cancer, ECOG: Eastern Cooperative Oncology Group HBV: hepatitis B virus, HCV: hepatitis C virus, PVI: Portal vein invasion
*Individual patient plasmids are up to 40 neoantigens

Fig. 1. A, GT-30 Baseline patient demographic and clinical characteristics. **B,** Overall summary of GT-30 treatment-related adverse events. Treatment-Related AEs are determined by the investigator for those adverse events deemed as possibly, probably, or definitely-related to PTCV, IL-12, EP and/or CPI.

CONCLUSIONS

- GT-EPIC™ personalized vaccines containing up to 40 neoantigens can be designed, manufactured, and administered successfully in as short as 6-8 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 **30.6% (11/36)** with **8.3% (3/36)** of patients achieving a **complete response** (CR) and **22.2% (8/36)** of patients achieving a **partial response** (PR). The disease control rate (DCR) was 55.6% (20/36). The therapy presents an unremarkable safety profile with no treatment-related SAEs.
- Treated patients presented new and expanded (0.1% to 2% of circulating T cells) vaccine-specific T cell clones in the blood that trafficked to the tumor. Poly-functional, vaccine-specific CD4+ and CD8+ T cells show activated phenotype and are present in a CD8:CD4 high ratio (>85% are CD8 T effector memory cells). Neoantigen-specific T cell responses were reported in 19/22 (86.4%) evaluable patients by IFNg ELISpot analysis.
- Our results support the PTCV mechanism of action based on the induction of anti-tumor T cells and show that a PTCV plus pembrolizumab has clinical activity in advanced HCC.

PTCV DEVELOPMENT AND CLINICAL RESPONSE

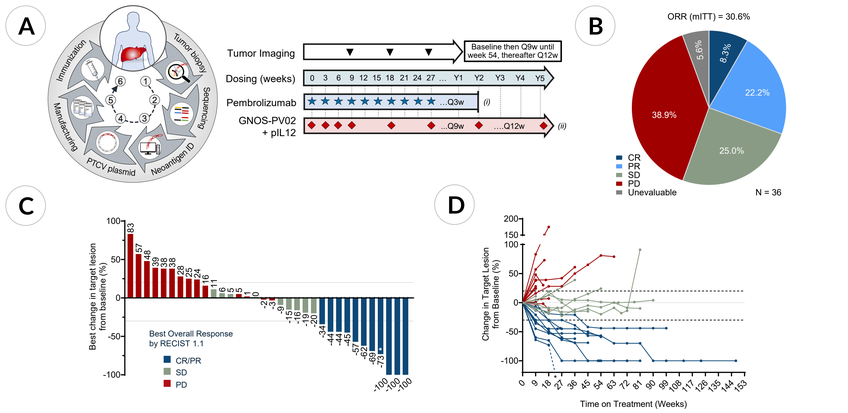

Fig. 2. A, Manufacturing process for GNOS-PV02 and clinical trial design. In subjects without disease progression, treatment with (*i*) pembrolizumab may continue Q3w for 2 years per label; (*ii*) GNOS-PV02 + pIL12 may continue Q3w x 4; Q9w for 2 years; and Q12w beyond 2 years. **B,** Pie chart with percent ORR, CR, PR, SD, and PD according to RECIST 1.1 (n=36; mITT). **C,** Waterfall plot showing the best overall response achieved by the 34 evaluable subjects of the GT-30 trial at the time of the data cut (18AUG2023). *, denotes the PR patient with primary liver lesion and two lung metastases achieved secondary resectability due to tumor shrinkage and remained tumor-free for 18.2 months post the first dose of treatment. **D,** Spider plot showing change in target lesion from baseline for the 34 evaluable patients of the GT-30 trial at the time of the data cut (18AUG2023).

GNOS-PV02 DRIVES POLYFUNCTIONAL ANTITUMOR NEOANTIGEN-SPECIFIC T CELL IMMUNITY

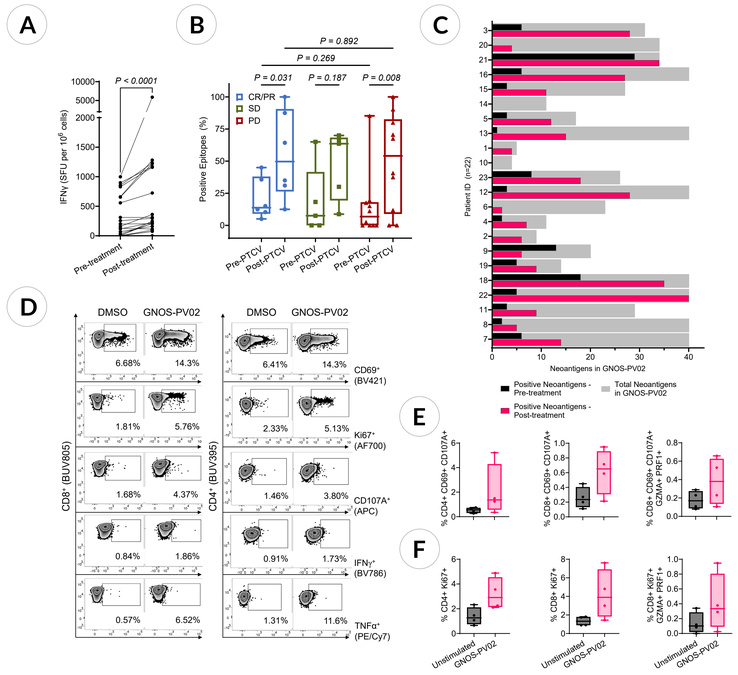

Fig. 3. A, Vaccine-induced responses by IFNγ ELISpot without cytokine stimulation (n=22). Cumulative magnitudes were collected from positive epitopes at pre- and post-treatment. Post-vaccination response is the 'best' (highest magnitude) response for each patient across timepoints. Significance between groups was evaluated by a two-tailed Mann-Whitney test. **B**, Percentage of positive responding epitopes by groups. The definition of a neoantigen-specific ELISpot response can be found in Methods. **C**, Total and positive neoantigens pre- and post-vaccination (grey, black, and red bars, respectively) in each patient's PTCV by IFNγ ELISpot. **D**, Representative density plots (Pt# 22) of T cell markers CD69+, Ki67+, CD107a+, IFNγ+, and TNFα+ upon stimulation with patient-specific PTCV epitope pools. **E-F**, Polyfunctionality assessed via Boolean gating of CD4+ or CD8+cytokine+ populations. T cell activation (CD69 and CD107A) and proliferation (Ki67) were assessed together with the double positive expression of granzyme A (GZMA) and perforin (PRF1) to evaluate the cytolytic potential of neoantigen-reactive T cells. Four pts (#7, 11, 18, and 22) were analyzed in **D-F**.

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

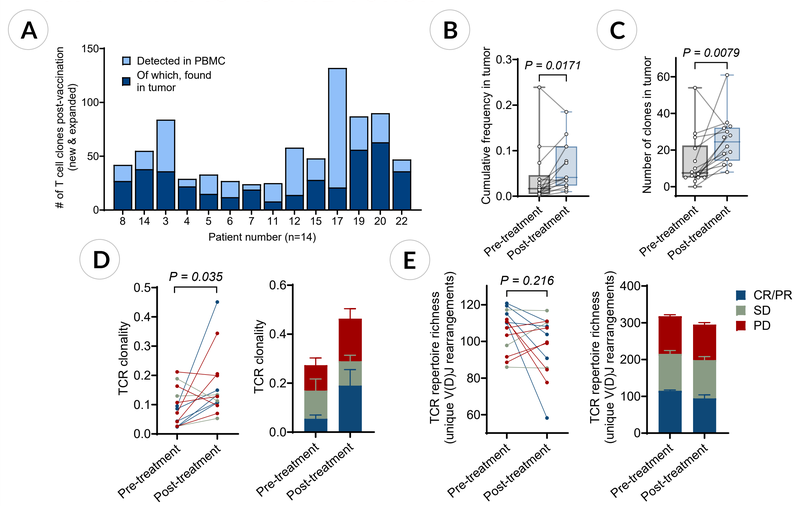

Fig. 4. A, T cell clones expanded in the periphery and the new or expanded clones enriched in the matched tumor sample for each patient (n=14). Total PBMC and tumor-associated T cell expansion is calculated by comparing post-treatment over pre-treatment PBMC or tumor samples (differential abundance statistical analysis). **B**, Cumulative frequencies of peripherally expanded TCR rearrangements in tumor biopsies. **C**, Expanded clone numbers in tumor biopsies. **D-E**, TCR clonality and repertoire richness in tumor biopsies (n=14). Error bars correspond to the upper SEM of each group. Simpson clonality reports the distribution of TCR rearrangements in a sample, where 0 indicates an even distribution of frequencies and 1 indicates an asymmetric distribution. TCR repertoire richness reports the mean of unique rearrangements. Lower numbers indicate focused TCR diversity. Significance within groups was evaluated by a two-tailed Wilcoxon rank test (**D-E**).

POST-VACCINATION EXPANDED TCR CLONES IDENTIFIED IN THE TUMOR ARE REACTIVE TO PTCV-ENCODED ANTIGENS

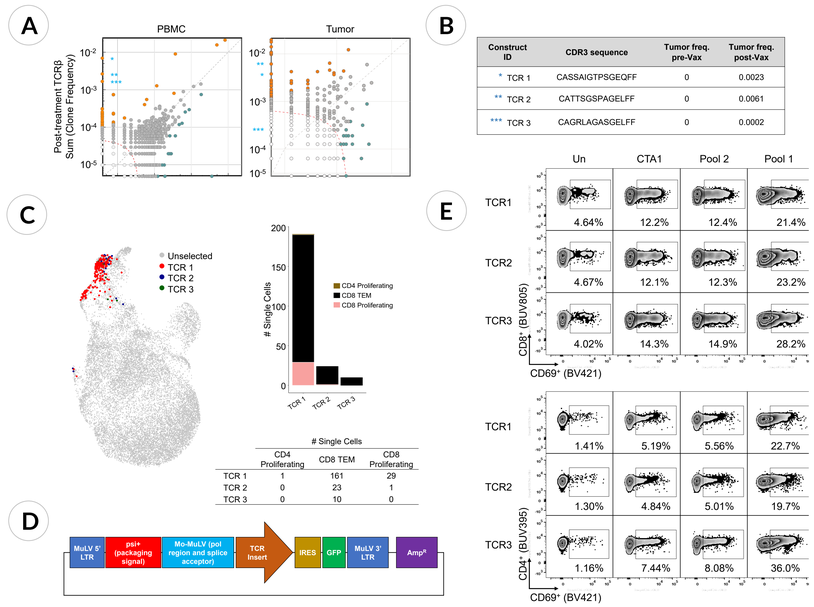

Fig. 5. A, Most frequent TCRs identified by TCRβ and RNA sequencing in a patient. Pre-vaccination versus week 9 post-vaccination (Pair-wise scatter plot). Blue asterisks show selected high-frequency new T cell clones detected in the PBMC post-vaccination and their abundance in the tumor. Orange, green, and grey circles represent expanded, contracted, and not significantly changed T cell clones, respectively. **B**, CDR3 sequence of the 3 TCRs (Pt#8 TCR1, TCR2, and TCR3) selected for cloning, and their frequency in tumor pre and post- vaccination. Selected cloned TCRs were only present in high frequency in peripheral blood and tracked into the tumor at post-treatment. **C**, UMAP and stacked barplot indicating the single cell cluster identities and number of cells for each of the three TCRs selected for cloning. **D**, 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. **E**, TCR-engineered T cells (GFP positive) from unvaccinated PBMC were stimulated for 6 hours with epitope pools or the unspecific epitope CTA1 (10 μg/mL), and the expression of CD69 was evaluated by flow cytometry. Peptide pool #1 included the most reactive epitopes measured by ELISpot, whereas pool #2 (consisting of peptides corresponding to neoantigens 21-40) served as an internal negative control.