Multi-parametric Assessment of the Immune Response to a Trio Immunotherapy in Patients with Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma

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ABSTRACT / BACKGROUND

Background

Our group developed a strategy for generating an "off-the-shelf" multivalent proteasome-blocked autophagosome vaccine that contains proteins for many genes commonly overexpressed in adenocarcinoma and squamous cell cancers. This strategy exploits in vitro manipulation of the antigen presentation pathway to concentrate the dominant epitopes presented by MHC, including short-lived proteins (SLiPs), defective ribosomal products (DRiPs), and Dark Matter, the short-lived non-canonical peptides that are not expressed in the thymus and represent potential shared alternative cancer neoantigens [1]. In preclinical models this vaccine strategy provides significant protection as a single agent [2], and significantly increased therapeutic efficacy when combined with anti-GITR and anti-PD-1 [3]. Based on these studies we hypothesize that addition of anti-GITR to the human vaccine, DPV001, and anti-PD-1, will augment expansion and limit contraction of the anti-cancer immune response. A phase I clinical trial was initiated, and preliminary immunological monitoring data will be presented.

Methods

Patients received DPV-001, with sequenced checkpoint inhibition (aPD-1 mAb; retifanlimab), with or without aGITR agonist mAb (INCAGN1876), in recurrent or metastatic HNSCC (NCT04470024). Tumor biopsies were taken pre-treatment, week 2 and 8. Blood samples were taken pre-treatment and at multiple timepoints and analyzed by flow cytometry and seromics. Tumor biopsies and blood were assayed by CITE-seq, scRNA-seq, BCR-seq, and TCR-seq. Multiplex immunofluorescence (mIF) was performed on biopsies.

Results

In the first 4 patients evaluated, tumor-infiltrating T cells at week 8 increased from pretreatment levels by an average of 4.3 fold (range 2.9 – 6.7, p=0.032). The density of CD39/CD103 double positive cells, previously shown to identify tumor-reactive T cells [4], also increased in all week 8 biopsies (mean 14.7 fold, range 5-40). All patients showed increased numbers of cells expressing IFNG and GZMB and increased numbers of T cells expressing LAG3+ in week 8 biopsies. Preliminary TCR evaluation of the tumor identified proliferation of clones previously undetected in PBL, including αβ T cells, iNKT and MAIT cells. Expansion of clones that predated treatment was also identified.

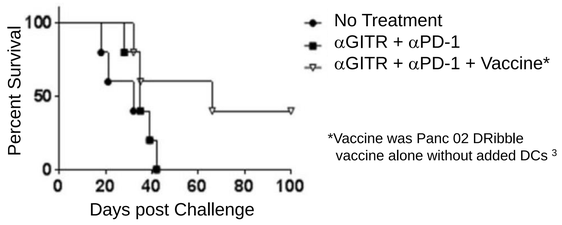
Conclusions

An increase in intra-tumoral T cells expressing activation and effector molecules is encouraging and studies are underway to expand the number of patients analyzed. Increased expression of LAG3 by T cells that infiltrate and have expanded in the tumor, provide a rationale for including anti-LAG-3 in this treatment strategy. Future plans include evaluating whether immune responses target shared non-canonical alternative neoantigens, or Dark Matter, contained in DPV-001 and whether antibody responses identify targets of cellular immunity.

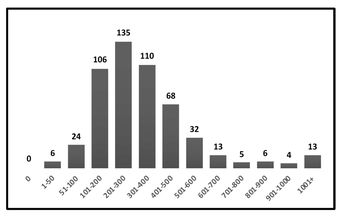
Acknowledgements: Support: Incyte, UbiVac, Providence Medical Foundation, The Chiles Foundation, The Harder Family, Nancy Lematta, Robert W. Franz and Elsie Franz Finley, The Murdoch Memorial Trust. **Trial Registry**: NCT04470024 **Ethics Approval**: PSJH IRB# 2020000480

Science Supporting Clinical Trial Design

Immunotherapy Trio Improved Survival in Panc 02 Model



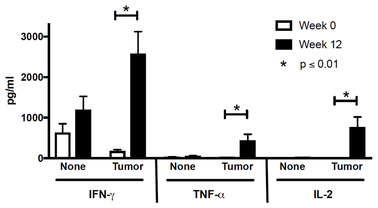
DPV-001 Vaccine Contains HNSCC Antigens



The average patient tumor sample in the Head and Neck Squamous Cell Carcinoma data set over-expressed 335.3 genes for which the protein is present in the DPV-001 vaccine (LC MS/MS data), with a range from 21 to 1464 (TCGA, Provisional, Tumor Samples with mRNA data (RNASeq V2) (n = 522), RNA- Seq data z-score > 2

# of genes overexpressed (z-score > 2) in patient tumor (n=279) and also represented in DPV-001 Vaccine

DPV-001 Vaccine Induces Immunity to Patient's Autologous NSCLC

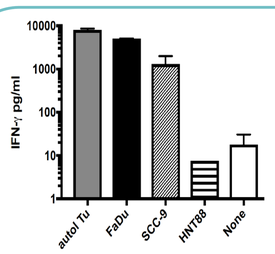


Baseline and week 12 PBMC from DPV-001-vaccinated NSCLC patient were stimulated ex-vivo with DPV-001 for 24 hours prior to expansion for 11 days on IL2 and IL15. Cells were harvested and restimulated with autologous tumor (LT101) or no stimulus. 24 hour cytokine secretion assessed with cytokine bead array. Mean and SEM of experimental nonuplets shown.

DPV-001 Vaccine Induces Immunity to HNSCC Antigens

Vaccination with DPV-001 induces IgG responses to proteins in DPV-001 that are over-expressed in HNSCC.

Gene	# Serum Responses (16 pts)	Fold Incr	Gene	# Serum Responses (16 pts)	Fold Incr
SENP2	1	7	PPIA	1	44
VPS8	2	11/5	AURKA	2	4/25
PIK3CA	3	8/5/6	ACP1	2	8/5
STK3	2	10/5	GPI	2	41/4
NCBP2	1	8	PCYT1B	2	9/6
EIF2B5	1	6	MERTK	2	5/228
RFC4	1	7	AKT2	2	7/8
CCND1	1	16	POLDIP2	3	10/5/5
UBXN7	1	6	NUDT21	2	5/12
DBR1	2	5/16	PAK4	3	90/5/5
EBAG9	1	9	PXN	2	11/4
MTDH	1	12	ABL1	3	4/6/5
ATP2C1	1	6	FBXO32	3	51/4/4
RNF13	2	4/14	MET	2	4/21
OSBPL11	1	5	SPHK1	2	27/5
EIF4A2	1	4	BDNF	2	10/6
PCCB	1	5	SYMPK	2	9/14
MAPRE1	3	6/4/4	DDX39A	2	20/6
ZFAND1	1	24	PRKCZ	2	6/28
FSCN1	2	2/41	RPAIN	2	39/5
SRC	1	17	HCLS1	3	11/19/16
SNX16	1	32	ANKRD49	2	15/38
ATXN3	1	31	NGFRAP1	2	21/5
AKT1	3	15/10/5	PSMA6	2	11/5
RCP9	1	841	CDK5/CDK5	9	6-to-91 fold
GABPB2	1	21	CDH1	3	5/5/5
LDHB	1	23	DHX8	3	7/14/6
IRAK1	7	16/4/6/7/54/5/13	WTAP	2	16/4
CBX3	1	18	MAP3K7	2	13/4
RBM4	1	40	SIX1	2	22/9
C9orf78	2	9/5	PLK1	2	4/4
CDC42BPB	8	6-to-279 fold	RPS6KA5	2	55/4
LGALS8	4	11/4/5/5	EIF2AK2	2	68/6
SCRN3	1	22	CDK1	3	89/4/6
PPM1A	2	21/6	HMG20A	1	13

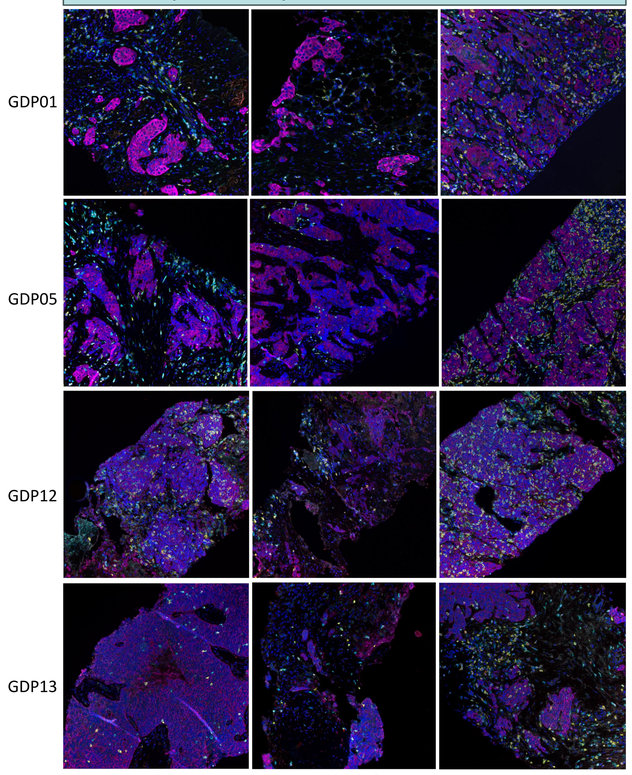


Tu-reactive CD8 T cells were selected by 4-1BB expression, expanded and assayed for reactivity to autol. tumor and 2 HNSCC cell lines (FaDu, SCC-9) sharing HLA with patient. HNT88 and T cells alone (None) stimulated little IFN-g.

NSCLC patients vaccinated with DPV-001 make 147 IgG responses to at least 70 proteins for genes overexpressed by HNSCC (TCGA). Gene name listed with the number of patients making serum response and the fold increase in IgG response for each patient.

METHODS AND RESULTS

scRNAseq and CITEseq can benefit from the context of mIF

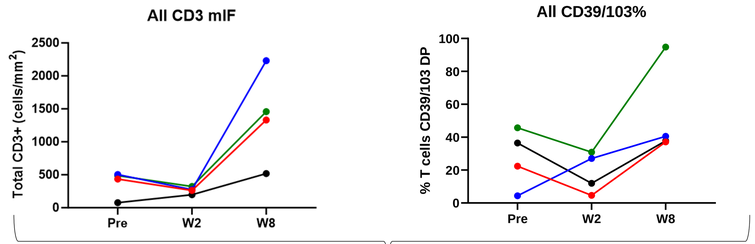


mIF Summary

Patient ID	Week	Total CD3+
GDP01	Pre	435.0
	Week 2	260.7
	Week 8	1329.5
	Fold Increase (WK8/Pre)	3.1
GDP05	Pre	504.1
	Week 2	274.8
	Week 8	2230.0
	Fold Increase (WK8/Pre)	4.4
GDP12	Pre	487.1
	Week 2	322.5
	Week 8	1456.8
	Fold Increase (WK8/Pre)	3.0
GDP13	Pre	76.9
	Week 2	197.1
	Week 8	518.2
	Fold Increase (WK8/Pre)	6.7

Figure 1. The density of T cells increased in all week 8 biopsies tested. Tumor biopsies were sampled pre-treatment, at week 2 and week 8. A fraction of each biopsy was analyzed by mIF for CD3+ cells/mm2. Representative ROI from pre-treatment and week 8 biopsies shown. Total values and week 8/Pre fold change presented in the table to the right.

CD39/CD103 Double Positive (DP) is a Marker of Tumor-Reactive TIL[4] and Increases in all Patients



$$\frac{\text{Cells/mm}^2 \times \text{Percent scRNAseq}}{100} = \text{Proportion Adjusted Density (PAD)}$$

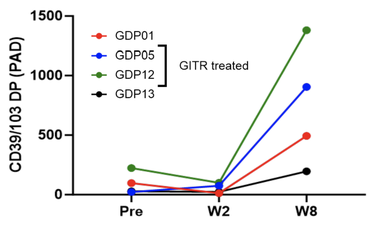


Figure 2. The density of CD39/CD103 DP cells increased in all week 8 biopsies tested. Tumor biopsies were sampled pre-treatment, at week 2 and week 8. A fraction of each biopsy was analyzed by mIF for CD3+ cells/mm^2. More in depth genotyping/phenotyping of TCR+ T cells was performed on paired biopsy fractions by scRNAseq and CITEseq. This allowed for extrapolation of inferred densities of T cells expressing genes associated with immune responses. **This was termed proportion adjusted density (PAD).** CD39 (CITEseq) > 2 and CD103 (CITEseq) > 1 for all patients.

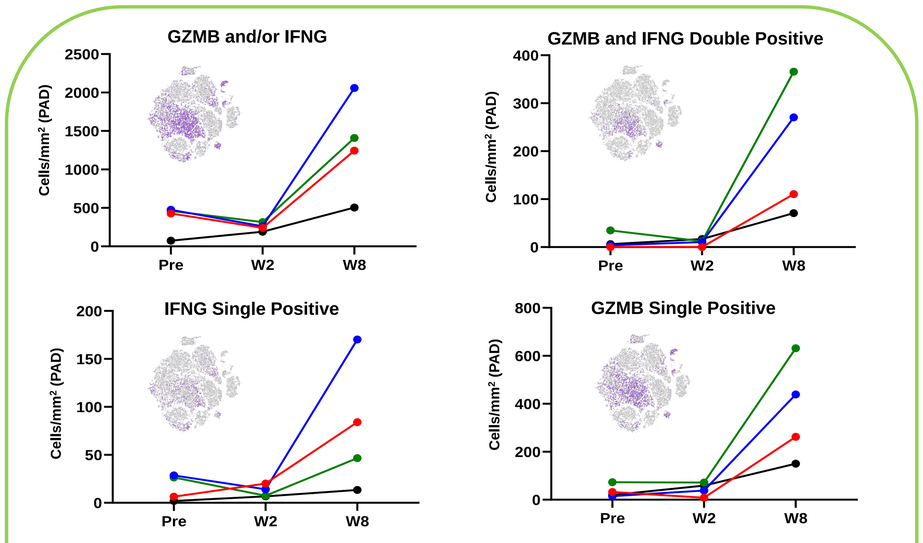


Figure 3. The density of IFNG and GZMB positive T cells increased in all week 8 biopsies tested. Tumor biopsies were sampled pre-treatment, at week 2, and week 8. A fraction of each biopsy was analyzed by mIF for CD3+ cells/mm2. More in depth genotyping of T cells was performed on paired biopsy fractions by scRNAseq. IFNG and GZMB proportion adjusted density (PAD) values are shown. Upper left plot shows all T cells expressing either molecule, while the inset t-SNE illustrates the proportion and distribution of cells for a single patient. Upper right shows all T cells expressing both molecules while the lower left and right plots show T cells expressing only IFNG or GZMB, respectively.

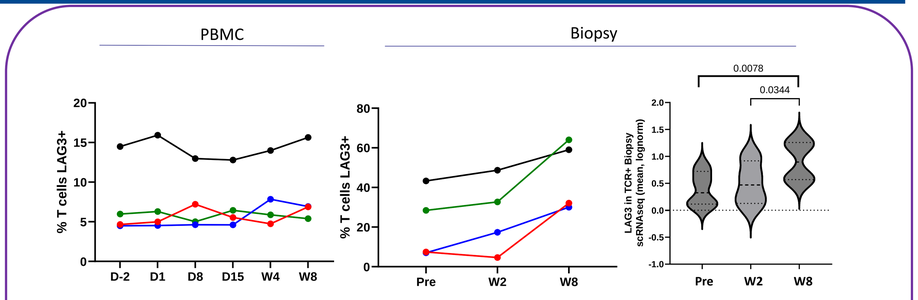


Figure 4. LAG3 is expressed by more T cells and is more highly expressed in the biopsies at W8. LAG3 gene expression of tumor biopsies was determined by scRNAseq. Percentages represent proportion of TCR+ barcodes with LAG3 lognorm expression > 0.5. LAG3 lognorm gene expression for all TCR+ cells in patient biopsies is shown on the right. Significant results of two-tailed paired t test shown.

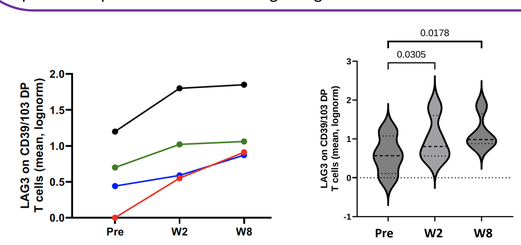


Figure 5. Among CD39/103 DP T cells in the biopsies, LAG3 intensity increases significantly by W2. LAG3 gene expression of tumor biopsies was determined by scRNAseq. CD39 and CD103 double positive cells were selected among the TCR+ T cells. LAG3 lognorm gene expression for all CD39 and CD103 double positive cells in patient biopsies is shown. CD39 (CITEseq) > 2 and CD103 (CITEseq) > 1 for all patients. Significant results of two-tailed paired t test shown.

Clonal proliferation assessed by 2 methods:

1. Primed/Potentially New Clones
Clones with no representatives prior to vaccination
Clone size 10+

2. Boosted/Pre-existing Clones
Clones Enriched > 4-fold after vaccination
Clones Enriched in Biopsy > 2-fold
Clone size 20+

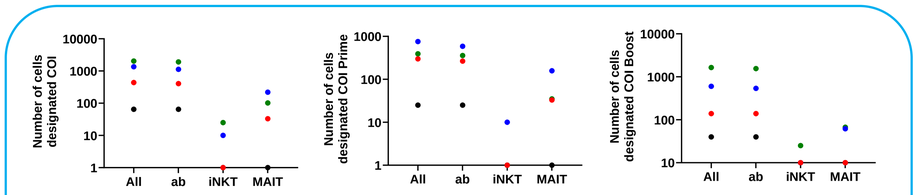


Figure 6. TCR evaluation of the tumor identified proliferation of clones previously undetected in PBL, including αβ T cells, iNKT and MAIT cells. Clones of Interest (COI) were chosen for expansion and enrichment in post vaccine samples (details in inset above right). Total COI are shown in the figure to the left, with subset breakdown by ab T cells, iNKT, and MAIT cells. The middle figure shows only those COI deemed to be potentially new clones (Primed) while the right figure shows pre-existing COI (Boosted).

CONCLUSIONS AND FUTURE DIRECTIONS

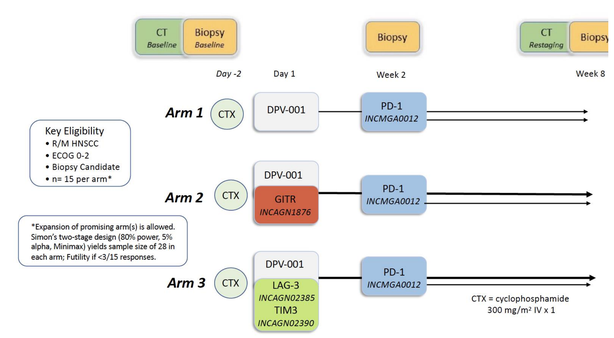
Conclusions:

- Vaccination with delayed anti-PD-1 +/- anti-GITR significantly (p=0.032) increased TIL in all 4 patients analyzed. As higher number of TIL is a positive biomarker of response to immunotherapy, we consider this a positive impact of this treatment.
- Treatment was also associated with an increase in CD39/CD103 DP cells – A marker of tumor-reactivity [4], as well as an increase in the density of IFNG+ and GZMB+ T cells. This was most prominent in patients receiving the immunotherapy Trio.
- LAG-3 expression was significantly (p=0.0078) upregulated by week 8 TIL. Among the CD39/CD103DP "tumor-reactive" TIL, LAG-3 was significantly (p=0.0305) upregulated by week 2. We also noted consistent upregulation of TIM3 by week 8 TIL in all patients (data not shown).
- By week 8 of treatment TCR evaluation of TIL identified proliferation of clones previously undetected in PBL, including αβ T cells, iNKT and MAIT cells. Expansion of clones that predated treatment was also identified.

Future Plans:

- Based on data showing increased expression of LAG-3 on CD39/CD103 tumor-reactive T cells by week 2, as well as increased expression of TIM3, we plan to add a third arm to the trial that will include treatment with DPV-001, anti-LAG-3 (INCAGN02385) + anti-TIM3 (INCAGN02390) + anti-PD-1 (INCMGA0012).
- Preliminary studies to characterize canonical and non-canonical (dark matter) peptides contained in the vaccine and expressed by cancer is presented at #SITC2023 poster #148 on Nov. 3rd

Revised Trial Design



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