An Off-the-Shelf Multivalent Vaccine Containing Cancer's Dark Matter, DPV-001, Combined with PD-1 +/- GITR in Head & Neck Cancer: Safety, Efficacy, and Immunodynamics from the Phase 1 GITRVax Trial

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Background: Immune responses to shared cancer antigens have correlated with durable clinical responses. DPV-001 is a novel microvesicle vaccine that contains >300 proteins for genes commonly overexpressed by cancer and is enriched for short-lived proteins (SLiPs), and defective ribosomal products (DRiPs), including non-canonical peptides, representing potential shared non-mutated alternative cancer neoantigens (dark matter). Preclinical studies identified increased therapeutic efficacy when this vaccine strategy was combined with anti-PD-1 and anti-GITR, leading to this clinical trial for patients with advanced or metastatic HNSCC.

Methods: Following safety run-in, eligible pts were randomly assigned 1:1 to receive DPV-001 +/- GITR agonist mAb (INCAGN-1949; q2wks). All received sequenced PD-1 mAb (retifanlimab; q4wks) starting D15. Safety was evaluated as the primary endpoint and efficacy was evaluated as a secondary endpoint. Tumor biopsies were taken pre-treatment, Wk 2 and 8, and assessed by CITE-seq, scRNA-seq, BCR-seq, and TCR-seq. Multiplex immunofluoresence (mIF) was performed on biopsies. Blood samples were taken pre-treatment and at multiple timepoints and analyzed by flow cytometry and seromics.

Results: 18 pts received backbone therapy of DPV-001 + sequenced PD-1; of these, 9 pts also received GITR starting D1 (Arm 2). G3 irAE's included: hypothyroidism & mucositis (Arm 1, same pt); pneumonitis and adrenal insufficiency (Arm 2, separate pts). No G4 toxicity was observed. Combining both arms, the response rate (RR) for PD-1 naïve pts was 55% (5/9), and 33% (3/9) for PD-1 experienced pts. Two CR's have been observed in Arm 2. Durability of response (DOR) and progression-free survival (PFS) will be reported at the meeting. DPV-001 induced increased Ki67+ CD4 EM (p<0.042) by D15, prior to first dose of PD-1. Addition of GITR at D1 further increased Ki67+ CD4 (p=0.008) & CD8 EM cells (P=0.0006) by D15. Activated or proliferating CD4 and CD8 EM were significantly increased in both arms by D30 (p<0.025). Across above specified analyses, pts receiving GITR had the greatest increase. Evaluable pts had significantly (all p<0.032) increased numbers of TIL expressing IFN-γ and/or GZMB, LAG-3, and TIM3 in on-treatment biopsies. TCR, BCR, and seromic analyses, as well as studies to identify targets of anti-cancer immunity, are ongoing.

Conclusions: This 18 pt trial of DPV-001 and sequenced PD-1 +/- GITR shows a promising RR and evidence of increased activation and expansion of effector T cells in PBL and tumor. Upregulation of LAG-3 and TIM3 by T cells that infiltrate the tumor and have increased in number, provide a rationale for including inhibitors for both in this treatment strategy. Current efforts include evaluating whether immune responses target shared non-canonical alternative neoantigens, or Dark Matter, contained in DPV-001, and whether synchronized antibody and cellular response is evidenced.

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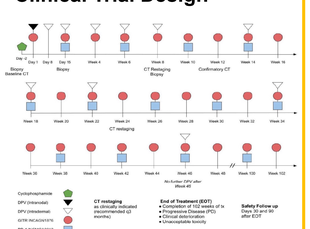
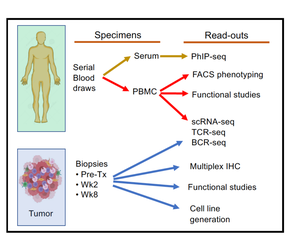
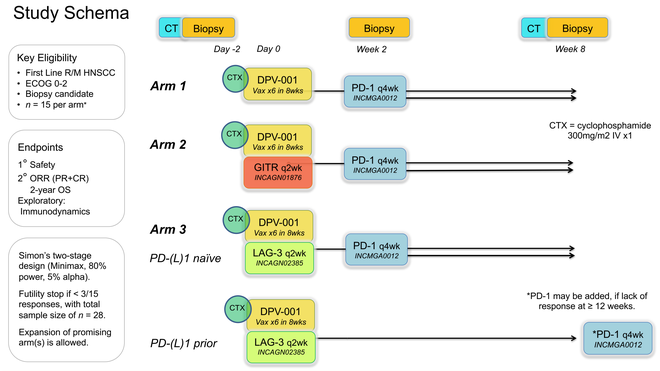
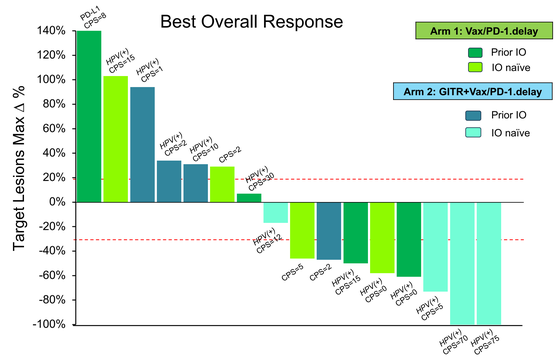
Clinical Trial Design

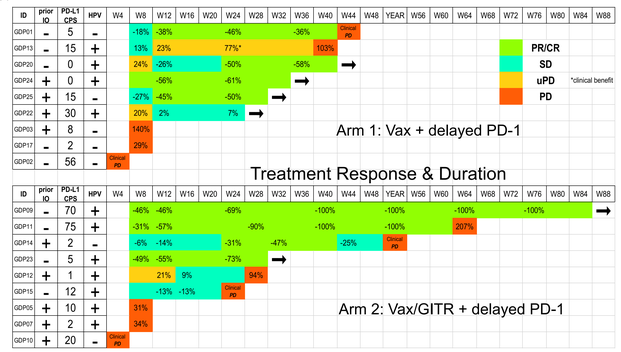


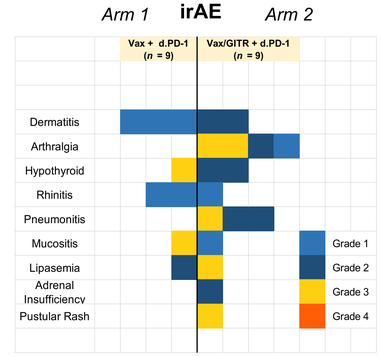


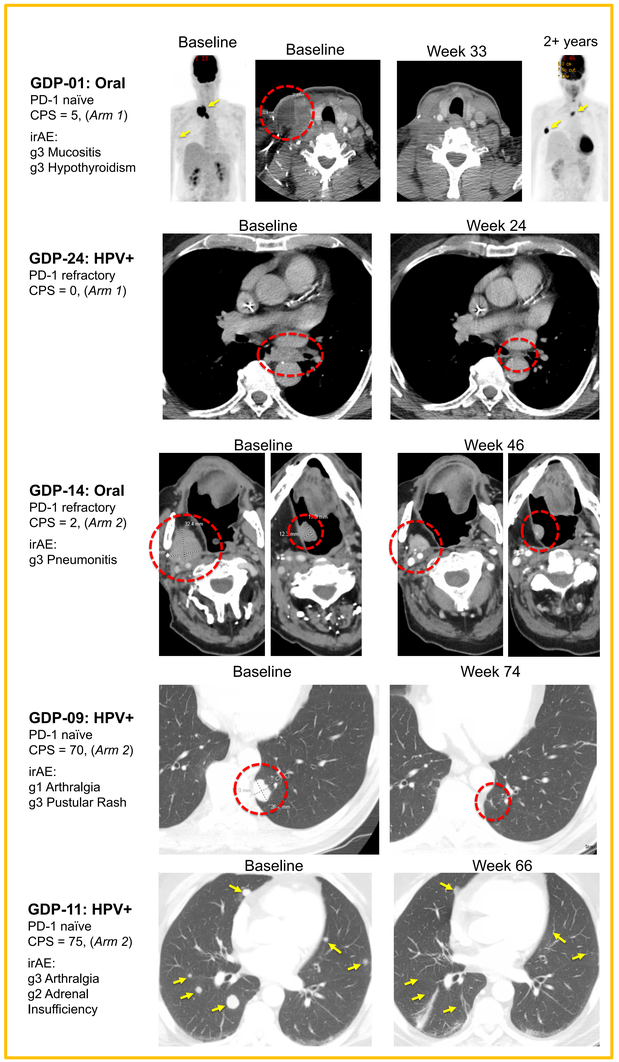
Study Schema



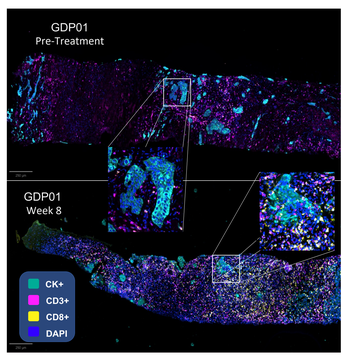






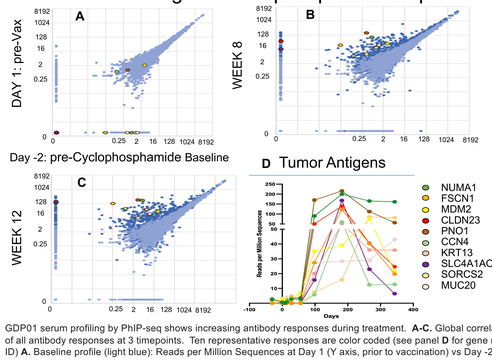


GDP01 Tumor



Biopsies obtained pretreatment or week 8. Increased T cell infiltration observed at week 8 demonstrated by multispectral immunofluorescence for CD3 (magenta), CD8 (yellow), cytokeratin (teal), and DAPI (blue)."

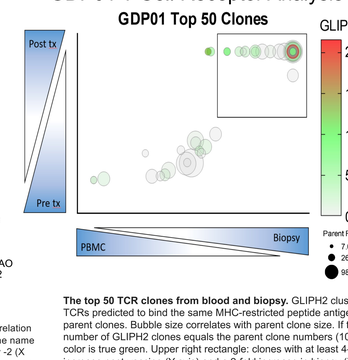
GDP01 Sera: Phage Immunoprecipitation-seq



GDP01 serum profiling by PhIP-seq shows increasing antibody responses during treatment. **A-C.** Global correlation of all antibody responses at 3 timepoints. Ten representative responses are color coded (see panel **D** for gene name ID) **A.** Baseline profile (light blue): Reads per Million Sequences at Day 1 (Y axis, prior to vaccination) vs Day -2 (X axis; prior to cyclophosphamide). The baseline profile is overlaid on subsequent timepoints for comparison. **B.** Week 8 **C.** Week 12 **D.** Profile of ten representative responses showing Reads per Million Sequences over time.

PMID Reference: NUMA1 - 37462735; FSCN1 - 37491232; MDM2 - 31440117; PN01 - 36625087

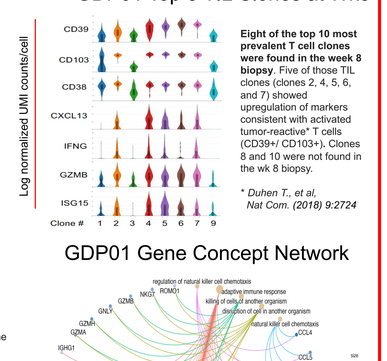
GDP01 T Cell Receptor Analysis



The top 50 TCR clones from blood and biopsy. GLIPH2 clusters TCRs predicted to bind the same MHC-restricted peptide antigen as parent clones. Bubble size correlates with parent clone size. If the number of GLIPH2 clones equals the parent clone numbers (100%) the color is true green. Upper right rectangle: clones with at least 4-fold increase post-vaccine (Y-axis) and a 2-fold increase in biopsy (X-axis).

GLIPH2 - Huang H., Wang C., et al. Nat Biotech 2020 Apr 27

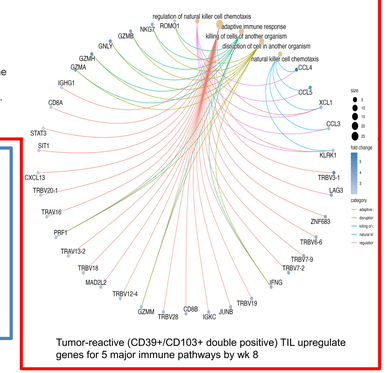
GDP01 Top 8 TIL Clones at Wk8



Eight of the top 10 most prevalent T cell clones were found in the week 8 biopsy. Five of those TIL clones (clones 2, 4, 5, 6, and 7) showed upregulation of markers consistent with activated tumor-reactive* T cells (CD39+/ CD103+). Clones 8 and 10 were not found in the wk 8 biopsy.

** Duhen T., et al, Nat Com. (2018) 9:2724*

GDP01 Gene Concept Network



Tumor-reactive (CD39+/CD103+ double positive) TIL upregulate genes for 5 major immune pathways by wk 8

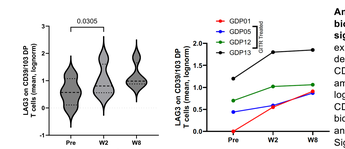
TIL – 4 Pts

Increased Density of TIL Expressing IFNG and/or GZMB, TIM3 and LAG-3 at Wk8

Tumor biopsies were sampled pre-treatment, at week 2 and week 8 (n=4 pts/12 biopsies). A fraction of each biopsy was analyzed by mIF for CD3+ cells/mm². 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).**

Proportion Adjusted Density (PAD) = CD3⁺ cells/mm² x %CD3 expressing scRNAseq

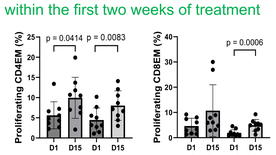
Tumor-Reactive TIL Increase LAG-3 Expression by Wk2



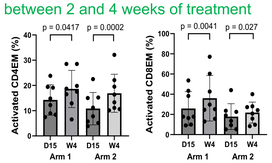
Among CD39/103 DP T cells in the biopsies, LAG3 intensity increases significantly by W2. LAG3 gene expression of tumor biopsies was determined by scRNAseq. CD39 and CD103 double positive cells were selected among the TCR+ T cells (n=4 pts). LAG3 lognorm gene expression for all CD39 and CD103 double positive 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.

Peripheral Blood – 4 Pts

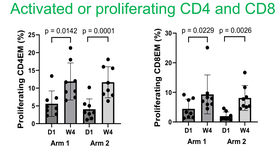
CD4 T effector memory cells are proliferating within the first two weeks of treatment

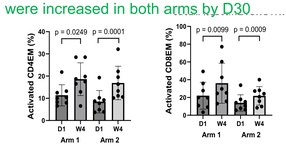


T effector memory cells are activated between 2 and 4 weeks of treatment



Activated or proliferating CD4 and CD8 EM were increased in both arms by D30





PBMC were evaluated by flow cytometry. CD4EM cells were gated as CD45+, CD3+, TCRαβ+, TCRγδ-, CD4+, CD8-, CCR7-, CD45RA-. CD8EM cells were gated as CD45+, CD3+, TCRαβ+, TCRγδ-, CD4-, CD8+, CCR7-, CD45RA-. Activated cells (right) were defined as CD38 and HLA-DR double positive and proliferating cells (left) were defined as KI67+. Both are expressed as a percentage of the entire CD4 or CD8 EM populations. Mean, SD and paired-two-tailed students t-test results shown.

Preliminary Results

CLINICAL:

- 18 pts received backbone therapy of DPV-001 + sequenced PD-1; of these, 9 pts also received GITR starting D1 (Arm 2). Combining both arms, the response rate (RR) for PD-1 naïve pts was 55% (5/9), and 33% (3/9) for PD-1 experienced pts. Two CR's have been observed in Arm 2.

IMMUNOLOGICAL MONITORING:

- **PBMC:** DPV-001 induced increased Ki67+ CD4 EM (p<0.042) by D15, prior to first dose of PD-1. Addition of GITR at D1 further increased Ki67+ CD4 (p=0.008) & CD8 EM cells (P=0.0006) by D15. Activated or proliferating CD4 and CD8 EM were significantly increased in both arms by D30 (p<0.025). Across above specified analyses, pts receiving GITR had the greatest increase.
- **Tumor:** Evaluable pts had significantly (all p<0.032) increased numbers of TIL expressing IFN-γ and/or GZMB, LAG-3, and TIM3 in on-treatment biopsies.
- **Sera:** PhIP-seq against >40,000 human proteins and isoforms identified the development of antibodies against a number of antigens whose genes are associated with poor outcomes in patients with cancer and were associated with onset of tumor regression in GDP01. Consistent with DPV-001's proposed MOA, these data suggest that treatment drove a broad immune response against a large number of relevant cancer antigens, potentially limiting opportunities for cancer to downregulate a single antigen and escape.
- **TCR clones:** Characterization of TIL T cell clones identified expansion or migration of T cells into the tumor by Wk8 in GDP01. Gene Concept Network analysis of the tumor-reactive TIL clones identified upregulation of genes associated with adaptive immunity, cell killing, cell disruption, NK cell chemotaxis, and regulation of NK cell chemotaxis.

Preliminary Conclusions

CLINICAL: We demonstrate the utility of deep immunologic characterization of Dribbles vaccine with delayed PD-1 blockade. Results are shown for the first patient and analysis is ongoing. Immune related toxicity has been manageable and attests to bioactivity. Clinical response rates point to enhanced activity of Dribbles vaccine +/- GITR agonist, over PD-1 blockade alone in this setting.

TUMOR: Increased expression of LAG-3 by week 2 biopsy, and continuing at week 8 biopsy, on CD39⁺/CD103⁺ tumor-reactive TIL, points to additional therapeutic combination.

FUTURE PLANS: Ongoing characterization of coordinated B and T cell response to antigens contained in the Dribbles vaccine, including the non-canonical "Dark Matter" cancer antigens, with functional validation. Development of combinatorial LAG-3 blockade.