Evaluating Immune Responses of Patients Receiving the DPV-001 Cancer Vaccine

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Background DPV-001 DRibble® is a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and >130 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. We hypothesize that the efficacy of DRibbles' vaccination can be attributed to tumor-derived short-lived proteins (SLiPs) and defective ribosomal products (DRiPs). SLiPs and DRiPs are typically not processed and presented by professional antigen presenting cells therefor the host may be less tolerant. The large number of potential antigens in the vaccine necessitate new techniques to monitor responses.

Materials and Methods Patients received induction cyclophosphamide, then 7 vaccines at 3-week intervals. First vaccine was given intranodally; subsequent vaccines intradermally. Patients were randomized to receive DRibble alone (A), or with imiquimod (B) or GM-CSF (C). PBMCs and serum were collected at baseline and at each vaccination to assess changes in antibodies (Protoarray, microsphere affinity proteomics (MAP)) and cytokines (Quanterix), peripheral lymphocytes populations (flow cytometry) and TCR repertoires (Adaptive immunoSEQ).

Results 13 pts were enrolled (Arm A: 5; B: 4; C: 4). Serum cytokines (IL1β, IL8, IFNα, IFNγ, IL6, IL17 and TNFα) were measured and normalized and the sum plotted against time. The slope of the resultant trend line was used as an indicator of either increased (positive slope) or decreased (negative slope) systemic inflammation. DPV-001 alone did not change net cytokine load while the addition of the adjuvant immiquimod increased, and the addition of GM-CSF significantly lessened the slope. Vaccination induced or increased IgG Ab responses against targets over-expressed by NSCLC, correlating with activated Th1 cells in whole blood samples. New or augmented Ab responses were observed with continued vaccination. Pts receiving DPV-001 had a significant ($p<0.04$) increase in total (CD4 + CD8) TCRs that increased 10 fold over baseline compared to normal controls (independent from trial, n=3) and the increase in CD4 clones was similar to that seen following Ipilimumab (melanoma pts, independent from trial, n=15). Patients receiving DPV-001 alone had the largest increase in CD8 T cell clones.

Conclusions Vaccination with DPV-001 increased the number of strong antibody responses to antigens commonly over-expressed in NSCLC and expanded populations of T cells. DPV-001 alone provided the greatest increase in CD8 TCRs. Interval monitoring of PBMCs/serum identified the complexity of the immune response to this vaccine and suggests possibilities to boost or sustain immunity.

Trial Registration NCT01909752

Figure 3. DPV-001-vaccinated NSCLC patient T cell repertoire changes exceed that observed for normal donors

A.

	UBLT001	UBLT002	UBLT003	UBLT004	UBLT005	UBLT006	UBLT007	UBLT008	UBLT009	UBLT011	UBLT012	UBLT013	Mean	Median
First post timepoint	13	13	9	7	10	13	10	4	7	7	10	10	9.42	10
Second post timepoint	19	16	16	12	19	43	19	13	16	10	19	16	18.17	16

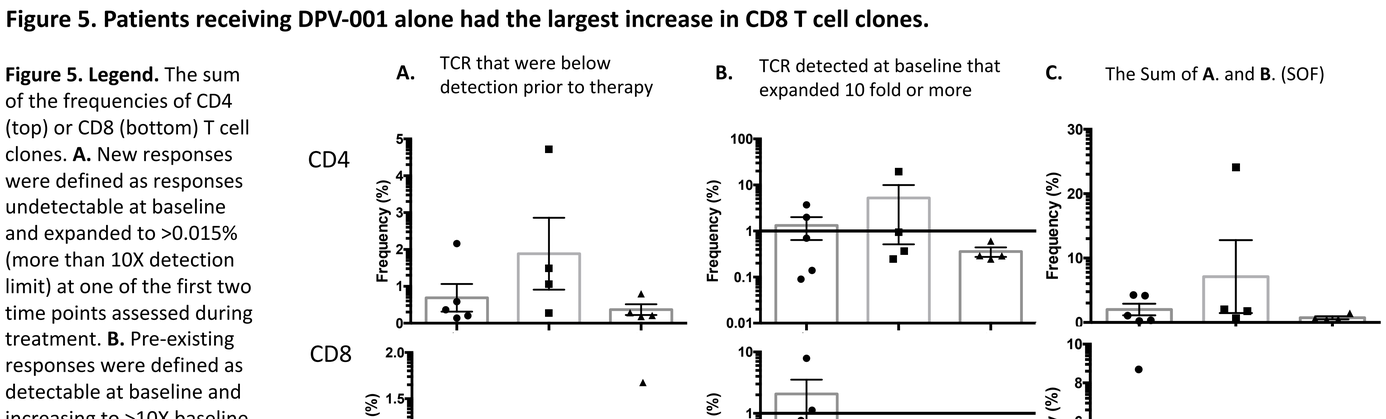
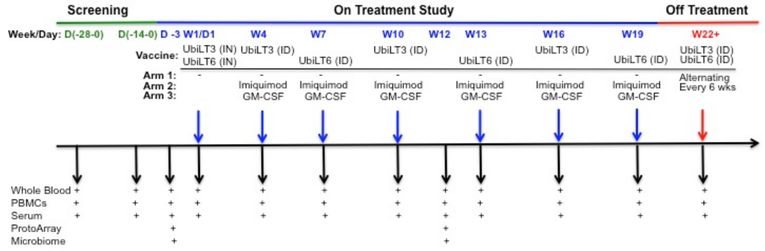


Figure 3. Legend. Pooled frequencies of CD4 + CD8 T cells from Normal Donors (ND, n=3) and DPV-001-treated patients (n=12) were assessed. **A.** and **B.** To create a fair comparison between our patient samples and ND we used the first two timepoints assessed in our patient samples and chose ND timepoints (weeks 8 and 20) based on these values. **C.** CD4 and CD8 TCR frequencies were multiplied by the percent of CD4 or CD8 subset among total CD4 + CD8. Pre existing responses were defined as detectable at baseline increasing to >10X baseline at one of the first two time points assessed during treatment. New responses were defined as undetectable at baseline and expanded to >0.015% (more than 10X detection limit) at one of the first two time points assessed during treatment. Sum of frequencies (SOF) were defined as the sum of increases in pre-existing and new responses. **D.** Same as C but comparing the number of TCR that increased 10 fold by these methods. ND data provided by Adaptive Biotechnologies' immuneACCESS tool. Significance assessed with the Mann-Whitney test.

A. DPV-001 NSCLC Clinical Trial Design



B. Proteasome Blockade (Velcade) Concentrates DRiPs/SLiPs In Autophagosomes which are used as a vaccine

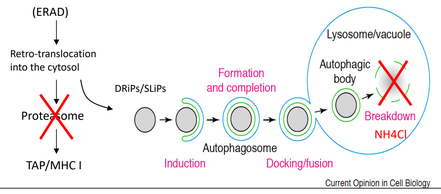


Figure 1. DPV-001 Vaccine. A. Schematic of DPV-001 NSCLC clinical trial design. **B.** By blocking the proteasome degradation pathway DRiPs/SLiPs are shuttled into the autophagy pathway. NH_4Cl impairs fusion of autophagosomes to lysosomes and protein degradation by the lysosome, leading to an accumulation of autophagosomes with DRiPs/SLiPs in the cell. DPV-001 is an off-the-shelf vaccine containing more than 100 antigens common in most cancers.

Figure 4. DPV-001-vaccinated NSCLC patient T cell repertoire changes resemble metastatic melanoma patients receiving Yervoy

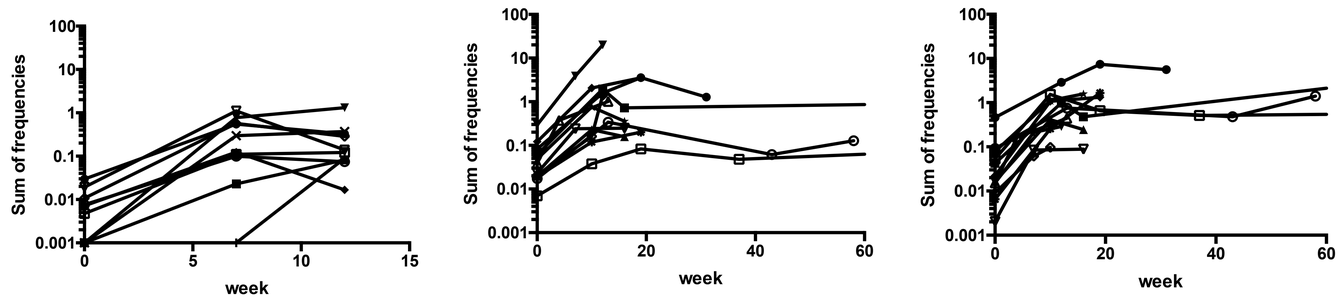


Figure 4. Legend. The sum of the frequencies of all T cell clones that expanded 10 fold or greater for the treatments specified. Each line summarizes the increases in T cell clones for an individual patient over time. **A.** Expansion of CD4 T cells in 15 metastatic melanoma patients during Yervoy treatment, **B.** Expansion of CD4 T cells in 12 NSCLC patients during DPV-001 treatment, **C.** Expansion of CD8 T cells in 12 NSCLC patients during DPV-001 treatment.

Figure 2. Changes in serum cytokines during DPV-001 treatment correlate with adjuvant choice and CD4 vs CD8 expansion.

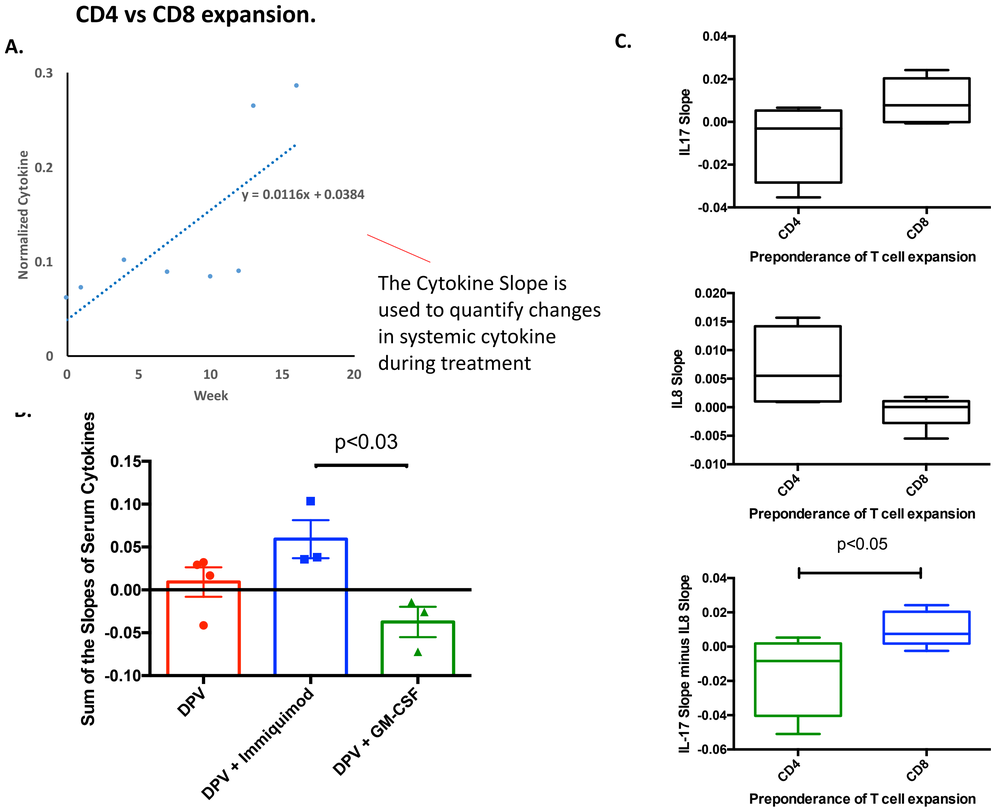


Figure 2. Legend. A. Serum cytokines (IL1β, IL8, IFNα, IFNγ, IL6, IL17 and TNFα) were measured and normalized and the sum plotted against time. The slope of the resultant trend line was used as an indicator of either increased (positive slope) or decreased (negative slope) systemic inflammation. **B.** DPV-001 alone did not change net cytokine load while the addition of the adjuvant imiquimod increased, and the addition of GM-CSF lessened the slope. **C.** Patients were divided into two groups: those expanding more CD4 T cells vs those expanding more CD8 T cells as assessed by TCR SEQ of sorted patient samples. Increases in IL8 and IL17 correlate with expansion of CD4 or CD8 T cells respectively. Significance assessed with the Mann-Whitney test.

Figure 5. Patients receiving DPV-001 alone had the largest increase in CD8 T cell clones.

Figure 5. Legend. The sum of the frequencies of CD4 (top) or CD8 (bottom) T cell clones. **A.** New responses were defined as responses undetectable at baseline and expanded to >0.015% (more than 10X detection limit) at one of the first two time points assessed during treatment. **B.** Pre-existing responses were defined as detectable at baseline and increasing to >10X baseline at one of the first two time points assessed during treatment. **C.** Sum of frequencies (SOF) were defined as the sum of increases in the new and pre-existing responses.

Conclusions and Future Plans

- While time points for assessing serum cytokines were not optimal, changes in serum cytokines during DPV-001 treatment correlate with adjuvant choice and CD4 vs CD8 expansion, and merit further investigation.
- Vaccination with DPV-001 significantly ($p<0.005$) increased the number of T cell clones that expanded ≥10x compared to normal donors studied during a similar span of time.
- T cell expansion was similar between cancer patients receiving Yervoy and DPV-001.
- Addition of adjuvants, imiquimod or GM-CSF, appeared to reduce the ability of DPV-001 vaccine to expand CD8 T cells.
- Other data (Biomarker Poster P38, Hilton et al.) document that vaccination with DPV-001 primes and/or augments T and B cell responses, but many T cell responses contract and IgG titers diminish. Preclinical studies suggest that T cell agonists, like anti-OX40, can sustain these responses. Additionally, when the preclinical version of the DPV-001 vaccine strategy is combined with anti-OX40, a significant increase in therapeutic efficacy is observed (Yu, G. et al., Sci Rep 2016). Based on these data we are preparing to initiate clinical trials combining DPV-001 with T cell agonists or with checkpoint blockade. We plan to employ the evaluations studied here and in poster P38 to assess the impact of these combination immunotherapy strategies.