A Strategy to Assess Contributions of Individual Agents in Combination Immunotherapy Trials.

Traci L. Hilton[3] Christopher Paustian[1,3], Yoshinobu Koguchi[1], Adi Mehta [4], Fridtjof Lund-Johansen[4], Brian Boulmay[2], Rui Li[1], Kyle I. Happel[2], Tarsem Moudgil[1], Sachin Puri [1], Christopher Dubay[1], Brenda K. Fisher[1], Eileen T. Mederos[2], William Redmond[1], Carlo B. Bifulco[1], Augusto C. Ochoa[2], Hong-Ming Hu[1,3], Bernard A. Fox[1,3], Walter J. Urba[1] and Rachel E. Sanborn[1]

[1]Earle A. Chiles Research Institute, Robert W. Franz Cancer Center, Providence Portland Medical Center, Portland, OR, USA; [2]Stanley S. Scott Cancer Center, School of Medicine, LSUHSC, New Orleans, LA; [3] UbiVac, Portland, OR, USA; [4]Oslo University Hospital Rikshospitalet, Oslo, Norway

Background

A recent FDA workshop (1) discussed how to demonstrate the contribution of each individual agent in combination immunotherapy trials. Here we outline a strategy of a coordinated assessment of the kinetics of B and T cell responses to a complex cancer vaccine as a possible model for such an evaluation. This strategy was applied to assess patients receiving DPV-001 DRibbles®, a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and > 200 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. Preclinical studies documented that addition of anti-OX40 significantly (P<0.05) improves survival and apparent cures in difficult to treat animal models we are planning a clinical trial of DPV-001 and anti-OX40 and will use this strategy to monitor the impact of the combination.

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) and cytokines (microsphere affinity proteomics (MAP), Quanterix), peripheral lymphocytes populations (flow cytometry) and TCR repertoires (Adaptive immunoSEQ). Evaluation of primary endpoint immune response occurred at week 12/13.

Results

13 pts were enrolled (Arm A: 5; B: 4; C: 4). IgG levels to 5000 proteins was assessed at 3-week intervals. For some antigens, IgG Ab responses peaked and then returned to baseline with new Ab responses developing or being augmented at each time point. In others Ab responses were maintained at multiple time points. Ab responses were detected against proteins whose genes were commonly upregulated in NSCLC, in some cases this upregulation was associated with significantly reduced survival (TCGA). Evaluation of CD4 and CD8 T cell clones by TCRSeq identified significantly (p=0.002) increased clonal expansion compared to normal controls (n=3). Similar to Ab responses, T cell clonal expansion exhibited expansion followed by apparent contraction.

Conclusions

Vaccination with DPV-001 increased antibody responses to antigens commonly over-expressed in NSCLC and associated with reduced survival, suggesting immunization is targeting relevant antigens. Monitoring the development of responses over therapy provides insights into possible Combination I-O strategies as well as a means to monitor impact of such trials.

Clinical Trial Identifier

NCT01909752

Reference

(1) https://www.fda.gov/Drugs/NewsEvents/ucm562746.htm

Vaccine Trial for Adjuvant Treatment of Definitively Treated NSCLC

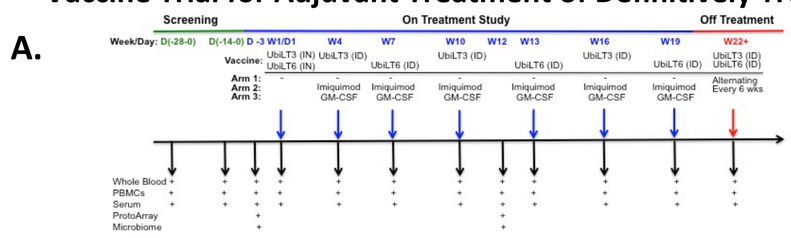


Figure 1.

A. Schematic of DPV-001 NSCLC clinical trial design.

B. Left panel: Antibody response to vaccination with a model antigen. **Center panel:** Antibody responses seen in patients vaccination with DPV-001 according to clinical trial design. **Right panel:** Kinetics observed for TCR clone expansion after vaccination with DPV-001.

Ongoing Monitoring of Humoral Responses with MAP Provides Kinetic Data and Reveals Responses Missed at Single Time Point Evaluation

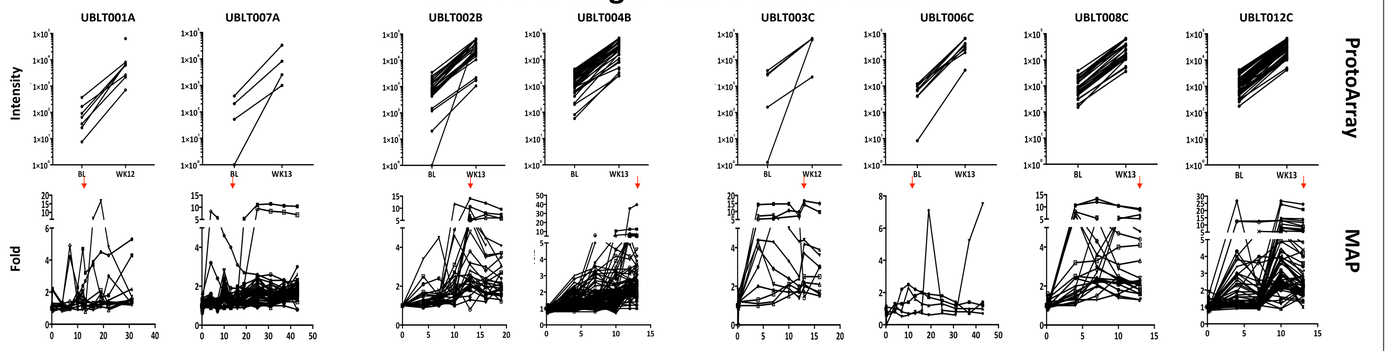
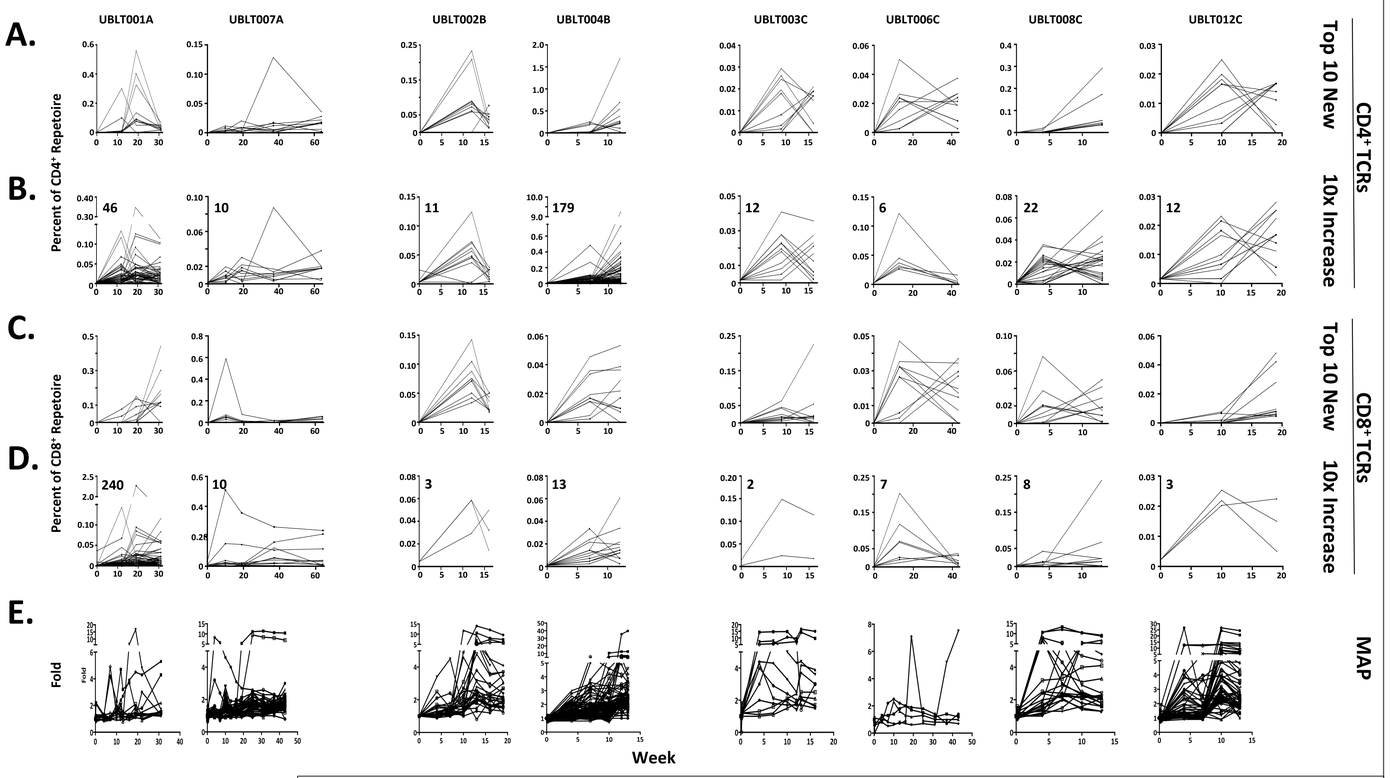


Figure 3. Top row: 15 fold increase in serum antibodies at WK 12/13 measured by ProtoArray. Primary endpoint for the clinical trail was the number of 15 fold increases in antibodies at WK12/13. Patients UBLT009 and UBLT011 did not have WK 12/13 samples. Bottom row: Patient sera from all time points were assessed for autoreactive antibodies by Microsphere Affinity Proteomics (MAP) to >5000 proteins by flow cytometry. MAP data is not yet available for patients UBLT005 and UBLT013.

Utilizing MAP to Choose Time Points for TCR Sequencing



Post-vaccination detection of previously undetected TCRs and TCRs with >10 fold increase in frequency correlated to the kinetics of humoral responses identified by MAP.

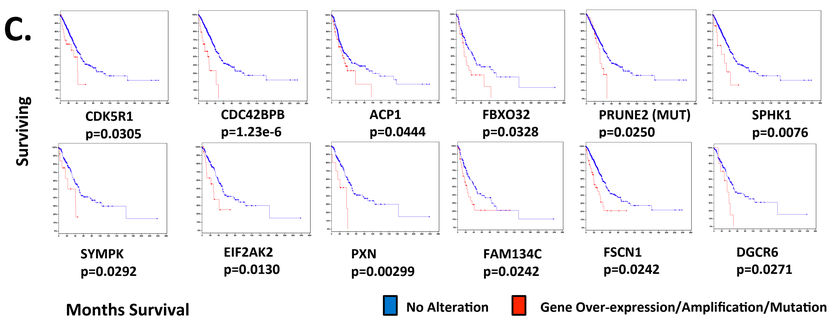
Figure 4. TCRSeq (Adaptive) analysis of CD4+ and CD8+ peripheral T cells sorted by flow cytometry **(A-D).** The timing of humoral responses by MAP **(E)** and number of available PBMCs informed the time points selected for analysis. TCR that were below detection prior to therapy were sorted for maximal frequency at any point during treatment. The top 10 undetected TCRs at baseline are presented for CD4+ (**A.**) and CD8+ **(C.)** T cells. TCR detected at baseline were sorted for maximal frequency at any on-treatment time point evaluated and divided by their baseline frequency. CD4+ (**B.**) and CD8+ (**D.**) that increased at least 10 fold were measured. **E.** Patient sera from all time points were assessed for autoreactive antibodies by Microsphere Affinity Proteomics (MAP) to >5000 proteins by flow cytometry. MAP data is not yet available for patients UBLT005 and UBLT013. patients UBLT009 and UBLT011 did not have WK 12/13 samples.

**See poster P511 for additional analyses of the immune response in patients receiving DPV-001.*

Shared Humoral Responses/Responses to Relevant NSCLC Antigens After DPV-001 Vaccine

A.

	UBLT001	UBLT005	UBLT007*	UBLT010	UBLT013	UBLT002	UBLT004	UBLT009**	UBLT011***	UBLT003	UBLT006	UBLT008	UBLT012
UBLT001		6	3	1	0	6	5	8	4	6	6	0	0
UBLT005	6		4	0	0	4	5	6	4	6	6	0	0
UBLT007*	3	4		0	3	8	11	23	6	4	3	12	9
UBLT010	1	0	0		0	1	0	1	0	0	0	1	0
UBLT013	0	0	3	0		4	5	5	4	1	0	7	4
UBLT002	6	4	8	1	4		6	10	5	5	5	2	3
UBLT004	5	5	11	0	5	6		16	6	6	5	6	11
UBLT009**	8	6	23	1	5	10	16		9	9	8	6	9
UBLT011***	4	4	6	0	4	5	6	9		4	4	2	3
UBLT003	6	6	4	0	1	5	6	9	4		6	0	0
UBLT006	6	6	3	0	0	5	5	8	4	6		1	1
UBLT008	0	0	12	1	7	2	6	6	2	0	1		4
UBLT012	0	0	9	0	4	3	11	9	3	0	1	4	



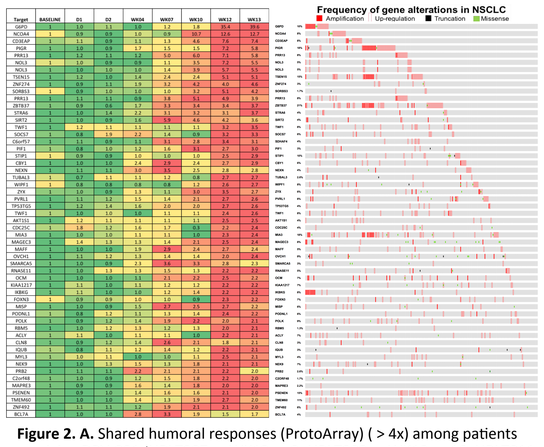
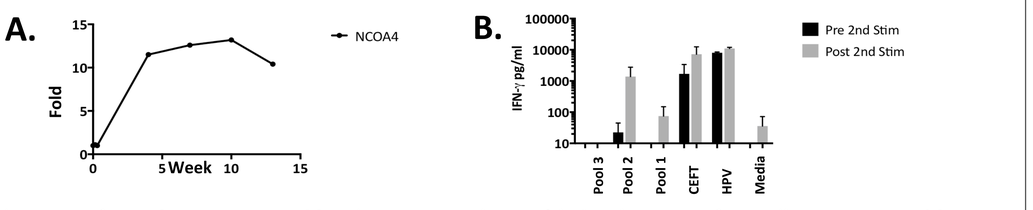


Figure 2. A. Shared humoral responses (ProtoArray) (> 4x) among patients receiving DPV-001 (UBLT007 at WK13 or 3; UBLT009 at WK10 or 16; UBLT011 at WK10). **B.** UBLT004 humoral responses (MAP) over time with TCGA LUAD data on patients over-expressing (z-score > 2) gene or with a gene amplification. **C.** Humoral responses identified in more than one patient associated with poor outcomes in TCGA LUAD patients.

IgG Ab Response Identifies T Cell Response to Same Antigen – NCOA4



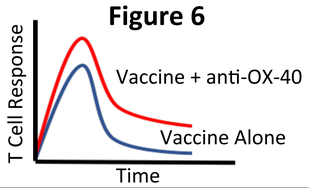
Development of an IgG response to NCOA4 by MAP correlates with development of a T cell response to NCOA4.

Figure 5. A. UBLT012 sera from all time points were assessed for antibody responses to >5000 proteins by Microsphere Affinity Proteomics (MAP). NCOA4 antibody binding is shown. **B.** IFN-γ ELISA after secondary peptide stimulation. Patient PBMCs from Baseline and WK7 in X-VIVO 15 with 1.25% human AB serum 6.25ng/mL IL-15 with were stimulated with peptides for 24hrs. Cells were expanded in IL-2 (10CU) for 9 days. Secondary stimulation was for 24hrs with peptides and anti-CD28/CD49d BD FASTIMMUNE.

Conclusions

- Vaccination with DPV-001 induces the development or augmentation of IgG response to many antigens contained in DPV-001 and known to be over-expressed by NSCLC. The response to these antigens can be variable, with some undergoing peaks and then reduction in titer, while others remain at a high titer or show a gradual reduction in titer over time.
- A similar trend is noted for T cell responses, with contraction of many TCR clones in spite of continued vaccination.
- Repeated vaccination drives development of new IgG responses against a spectrum of relevant NSCLC antigens.
- MAP identified Ab response to NCOA4 allowed identification of a T cell response to the same antigen.
- Identification of these patterns provide insights into monitoring combination immunotherapy trials that include DPV-001 and T cell agonists, that can increase B and T cell responses and sustain T cell clones (Maxwell JR 2000; Curti BD 2013; Redmond W 2009; and Figure 6). This also suggests mechanism to explain the increased therapeutic effect when this vaccine strategy is paired with the T cell agonist, anti-OX40 (Yu G et al Sci Rep 2016).

Figure 6



Future Directions

- Initiate a clinical trial combining DPV-001 and anti-OX40 and use MAP and TCR analyses to assess ability of combination therapy to sustain B and T cell responses
- Evaluate Ab responses directed against membrane proteins and investigate their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) and provide tumor destruction in face of antigen presentation defects and HLA loss.

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