Characterizing Cancer's Dark Matter, Short-lived Proteins, and Defective Ribosomal Products, Presented by Cancer and Contained in the DPV-001 Cancer Vaccine

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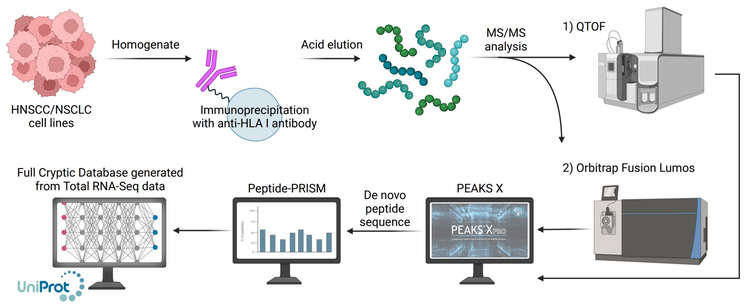
Abstract

Short-lived proteins (SLiPs), defective ribosomal products (DRiPs), and Cancer's dark matter/non-canonical peptides (NCP) are unstable and rapidly degraded, loaded onto MHC and represent a large proportion of the epitopes presented by cancer cells. Recent data suggests that NCP may play a role in molecular control of some malignant processes, further strengthening the rationale for characterizing their presence and developing pharmacologic and immunologic approaches to impact their effect [PMID: 37040070]. Immunologic approaches seem particularly promising as NCP, that are not expressed in the thymus, represent potential shared alternative cancer neoantigens [PMID: 33852826]. Our group developed a vaccine strategy that concentrates SLiPs, DRiPs, and NCP in dendritic cell-targeted microvesicles [PMID: 27190627]. Preclinical combination immunotherapy studies documented efficacy in difficult to treat animal models [PMID: 2787404, PMID: 31747946] and an off-the-shelf human vaccine was developed and has entered clinical trials. The current studies were undertaken to characterize the breadth of peptides presented by head and neck squamous cell cancer (HNSCC) and non-small cell lung cancer (NSCLC), as well as the proteins contained in the DPV-001 vaccine.

Background and Aims

Our group seeks to identify the targets recognized by a therapeutic immune response so that it may facilitate monitoring of patients on immunotherapy studies and aid understanding of the complexity of response to treatment and progression. It may also aid the development of cancer vaccine strategies. Here we present observations combining seromic evaluation of antibody responses in patients receiving a complex vaccine, containing >100 antigens overexpressed by adenocarcinoma and squamous cell cancers, with a characterization of the peptidome presented by an autologous tumor cell line. Our focus on IgG responses to specific antigens is related to evidence that B cell and T cell responses are coordinated, and detection of an antibody response generally identifies a T cell response to an epitope of the same protein. *Kwek S, et al, J Immunol 2012, Tripathi SC, et al, PNAS 2016, Hulett T, et al, J ImmunoTher Cancer 2018*

Methods



Cancer cells were lysed and recovered lysates were treated with detergent in the presence of protease inhibitor. HLA peptides were collected from HLA complexes purified by anti-HLA-I antibody (w6/32). Recovered HLA peptides were analyzed by the Orbitrap mass spectrometer (ORBITRAP FUSION LUMOS with FAIMS-Pro interface (Thermofisher Scientific)). The two components of DPV-001, UbiLT3 and UbiLT6, were assessed via deep proteomic profiling on an Spectra from whole proteome tryptic digests were acquired in data-dependent acquisition (DDA) mode, output by PEAKS Studio, and Peptide-PRISM was used to identify canonical and noncanonical peptides (NCPs).

Phage display immunoprecipitation (PhIP)

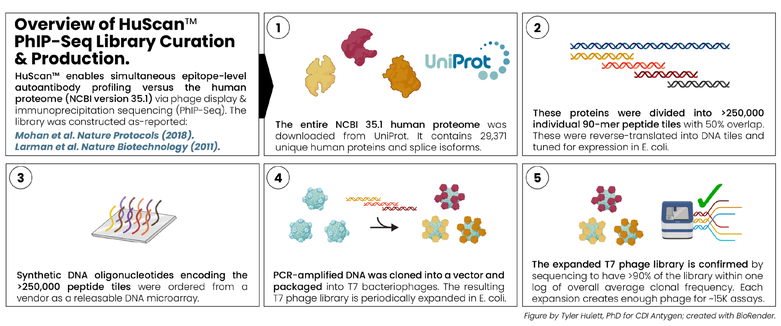
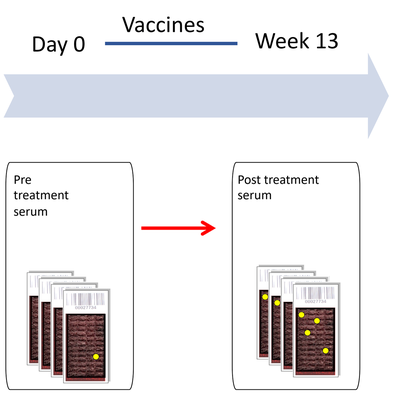


Figure by Tyler Hulett, PhD for CDI Antygen; created with BioRender.

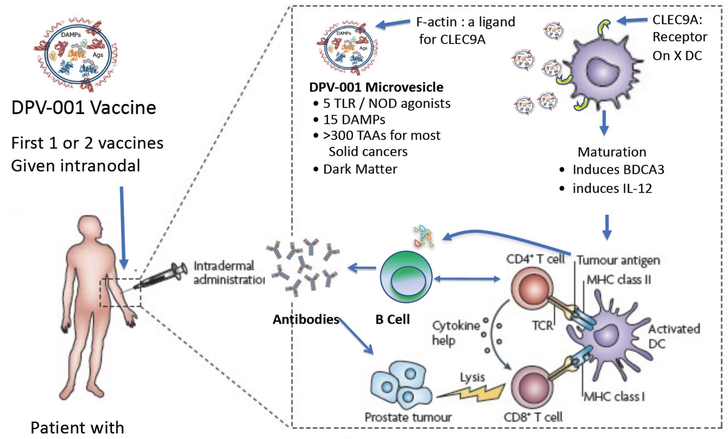
Protein Arrays



Sera were analyzed by protein arrays (Invitrogen) or by phage display immunoprecipitation according to manufacturers recommendations. HuScan™ Covers the NCBI 35.1 human proteome with 29,371 unique human proteins and splice isoforms. Phages express 90mer peptides with 50% overlap. PhIP was performed by CDI Labs (note excellent assistance from Tyler Hulett, PhD and Gabriel Roman, PhD). References: (PMID: 30190553; PMID: 21602805)

Clinical Trial

Randomized Phase II Trial of Cyclophosphamide with Allogeneic NSCLC Dribble Vaccine alone (DPV-001) or with Granulocyte-Macrophage Colony-Stimulating Factor or Imiquimod for Adjuvant Treatment of Treated Stage IIIA or B NSCLC. Trial Registration: NCT01909752



Modified from
Charles G. Drake. Nat Rev Immunol. 2010 Aug;10(8):580-93.
Christopher C Goodnow et al, Nature Immunology. Aug ; 10(11): 681-688

Peptide-PRISM Characterization of DPV-001 Vaccine

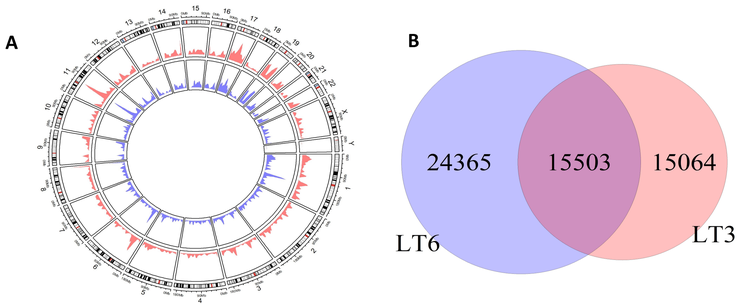
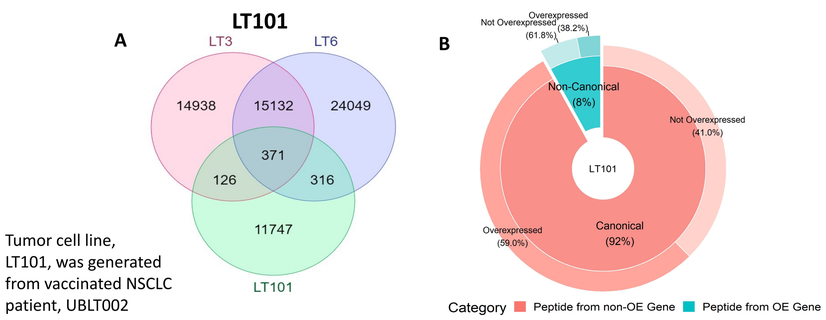


Figure 1. A. Genomic location of all significant peptides (Q <= 0.05), identified by Peptide-PRISM, for LT3 (Salmon) and LT6 (Violet). B. Total number of unique and shared peptides for LT3 and LT6 identified by Peptide-PRISM.

Preliminary Analysis of Shared Peptides of DPV-001 and LT101 Cell Line



Tumor cell line, LT101, was generated from vaccinated NSCLC patient, UBLT002

Figure 2. A. Venn-diagram depicting the shared peptides between the DPV-001 vaccine components (LT3 and LT6), and the LT101 cell line. B. The inner pie chart represents peptides from unique proteins originating from either the coding sequence (Canonical) or from elsewhere (Non-Canonical). The outer ring represents whether that protein was overexpressed based on sourced TCGA data.

Development of IgG Responses to Characterize Immunogenicity and Identify Potential Antigens Recognized by Tumor-Reactive T cells.

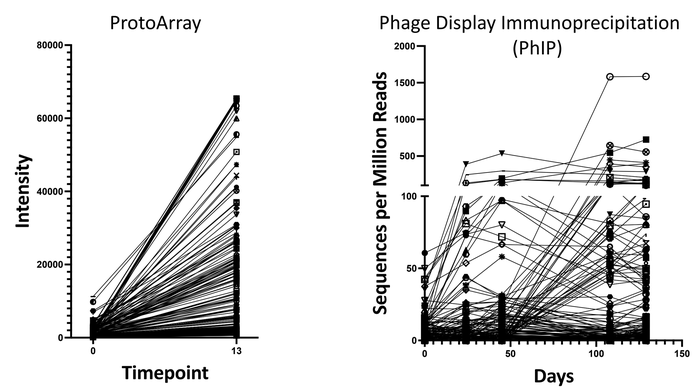


Figure 3: **A.** Left – 4 fold increase in serum antibodies recognizing full-length protein at WK 13 measured by ProtoArray. Right – 4 fold increase in serum antibodies recognizing peptides between WK4 and WK19 measured by Phage Display Immunoprecipitation and next generation sequencing. Responses meeting a minimum detection threshold of 50 sequences per million reads.

Preliminary Evaluation of Antibody Responses of Patient UBLT002 Using ProtoArray and PhIP Methodologies

Table 1. Vaccinated patient makes antibody responses to a large number proteins with a peptide detectable on the surface of their autologous cancer cell line

Assay	Target	Timepoints	# of Responses > 4x (Unique Proteins)	Responses with peptide on patient cell line
PhIP	Linear Peptides	6	136 (121)	58
Protoarray	Whole Protein	2	107 (97)	28

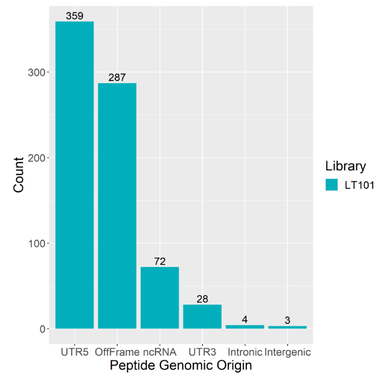
Table 1. Summary of HLA presented peptides for tumor cell line LT101 derived from patient tumor, corresponding to responses detected by Protoarray and PhIP. Some of the differences between the number of antibody responses detected by ProtoArray and PhIP can be explained by the technologies coverage of the human proteome. PhIP has more than twice the coverage of the proteome, Additionally, more timepoints were sampled with PhIP. There are also substantial differences in the identity of proteins identified by ProtoArray and PhIP methods, some of which may be related to the linear versus intact target.

Heat Map of Top 49 PhIP Responses

Protein	BL	WK4	WK7	WK16	WK19	2YR
A	6	9	24	1581	1585	87
B	24	90	196	545	724	145
C	0	5	10	643	556	30
D	3	10	0	448	410	10
E	17	63	133	353	398	102
F	0	7	3	393	352	16
G	35	387	535	295	286	74
H	12	246	297	248	207	43
I	0	20	26	132	189	31
J	10	20	22	206	167	40
K	37	119	180	161	166	20
L	17	14	16	113	138	6
M	3	0	0	147	128	4
N	9	23	32	141	125	6
O	0	0	1	120	119	0
P	5	134	183	135	117	16
Q	1	0	8	79	111	2
R	1	0	0	83	101	26
S	8	73	98	81	98	27
T	1	2	9	72	95	26
U	4	0	4	117	86	1
V	0	0	0	87	85	3
W	0	3	7	51	81	147
X	5	1	0	80	80	6
Y	19	93	142	71	80	8
Z	0	1	2	50	74	6
AA	0	0	0	82	69	0
AB	9	38	30	59	67	17
AC	0	1	0	76	63	1
AD	0	0	0	45	63	0
AE	7	60	97	55	63	9
AF	0	2	4	47	59	21
AG	61	75	130	60	59	1034
AH	0	3	0	74	57	0
AI	0	4	0	47	53	0
AJ	3	13	16	59	51	11
AK	8	7	21	52	50	15
AL	0	1	5	53	48	4
AM	43	83	97	46	48	1021
AN	2	3	1	61	44	0
AO	1	2	0	57	43	0
AP	42	81	72	45	42	809
AQ	50	74	80	39	42	203
AR	6	73	67	65	40	3
AS	0	0	0	42	40	68
AT	0	0	2	52	37	0
AU	37	54	67	26	35	398
AV	14	38	58	23	34	687
AW	0	0	1	51	32	2

Figure 4. Heat map of PhIP responses of patient UBLT002

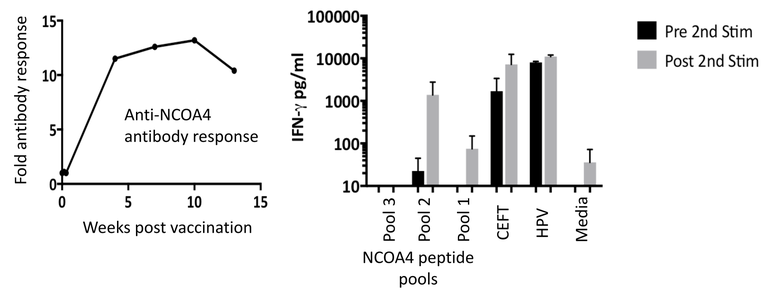
Figure 5. Peptides derived from 5'UTR represented the greatest number of non-canonical peptides



- **PhIP detected Ab responses to 60 canonical sequences where a 5'UTR epitope for that protein was identified as being presented by HLA of LT101.**
- **For 29 of those responses the canonical protein was identified in DPV-001.**

Figure 6. Antibody Identifies Protein Targeted by T cell Response

Coordinated IgG and T cell response to NCOA4 in patient UbiLT012



Preliminary Conclusions and Future Studies

- Peptide-PRISM analysis of MS/MS PEAKS X spectra obtained on the UbiLT3 (LT3) and UbiLT6 (LT6) cell lines identified a large number of proteins that are contained within the proteasome-blocked autophagic microvesicles that constitute the DPV-001 vaccine.
- Immunopeptidome analysis of the LT101 cell line reveals approximately 800 peptides presented by the LT101 cell line that are detected in the DPV-001 vaccine (LT3 and LT6 are components of the DPV-001 vaccine.
- Immunopeptidome analysis by Peptide-PRISM identifies 8% of the total peptidome for the LT101 NSCLC cell line is made up of non-canonical peptides.
- Analysis of the antibody response in a patient receiving the DPV-001 vaccine detected responses to a number of proteins for which a peptide of that protein was presented by HLA of the tumor cell line. This suggests that there is a T cell response to the presented peptide (*Kwek S, et al, J Immunol 2012, Tripathi SC, et al, PNAS 2016, Hulett T, et al, J ImmunoTher Cancer 2018*).
- Antibody responses were also detected to 60 canonical sequences where a 5'UTR epitope (NCP) for that protein was identified as being presented by HLA of LT101 (Figure 5). The DPV-001 vaccine contained 29 of the 60 canonical proteins.
- Future studies will evaluate whether T cell responses to these 5'UTR epitopes exist in patients prior to or following vaccination.
- Future studies will utilize the Orbitrap Fusion Lumos platform to analyze HNSCC cell lines for NCPs, in addition to further increasing the sensitivity for peptide identification for both the vaccine components and other lung cancer cell lines.

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