

MULTI-PARAMETRIC ASSESSMENT OF THE IMMUNE RESPONSE TO A TRIO IMMUNOTHERAPY IN PATIENTS WITH RECURRENT OR METASTATIC HEAD AND NECK SQUAMOUS CELL CARCINOMA

¹Christopher Paustian*, ²Tarsem Moudgil, ³Venkatesh Rajamanickam, ⁴Noah Simons, ⁵Ryan Meng, ⁶Marcus Couey, ⁴Matthew H Taylor, ⁴Yoshinobu Koguchi, ⁴Annie Stadum, ⁴Tanisha Christie, ⁷Tyler Hulett, ⁸Noriko Iwamoto, ⁸Takashi Shimada, ⁹Yuriko Minegishi, ¹⁰Quyen Vu, ⁴William L Redmond, ⁴Lessli Rushforth, ⁴Abigail Peterson, ⁴Brady Bernard, ⁴R Bryan Bell, ⁴Carlo Bifulco, ¹¹Koji Ueda, ¹²Traci Hilton, ²Shawn M Jensen, ¹³Hong-Ming Hu, ⁴Brian Piening, ⁴Walter J Urba, ⁴Bernard A Fox, ⁴Rom S Leidner. ¹EACRI PPMC, Portland, OR, USA; ²Earle A. Chiles Research Institute, Portland, OR, USA; ³Providence Health, Portland, OR, USA; ⁴Earle A. Chiles Research Institute, Providence Cancer Institute, Portland, OR, USA; ⁵Providence Cancer Institute, Portland, OR, USA; ⁶Boston University, Boston, MA, USA; ⁷CDI Laboratories, Mayaguez, PR, USA; ⁸Shimadzu Corporation, Kyoto, Japan; ⁹Japanese Foundation for Cancer Research, Ariake, Japan; ¹⁰Providence Cancer Center, ¹¹Japanese Foundation for Cancer Research, Ariake, Tokyo, Japan; ¹²UbiVac, Portland, OR, USA; ¹³Earle A. Chiles Research Institute, Providence Cancer Center, Portland, OR, USA

Background Our group developed a strategy for generating an ‘off-the-shelf’ multivalent proteasome-blocked autophagosome vaccine that contains proteins for many genes commonly over-expressed 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, DPV-001, 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 pre-treatment 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 IFN- γ 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 $\alpha\beta$ 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.

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