

### DEVELOPMENT OF AN IMAGE-BASED TUMOR MICROENVIRONMENT ANALYSIS COUPLED WITH PERIPHERAL FLOW CYTOMETRY REVEALS A DISTINCT IMMUNE CELL PHENOTYPE IN RESPONDER PATIENTS IN THE PHASE 1B/2 STUDY ASP-1929–181

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**Background** The identification of robust biomarkers that will allow for the selection of cancer patients who will benefit from a given therapeutic is critically needed for the design of late-stage clinical trials. ASP-1929–181 is an open-label phase 1b/2 clinical trial evaluating the safety, tolerability, and tumor response in patients with squamous cell cancer of the head and neck who receive ASP-1929 photoimmunotherapy in combination with anti-PD-1 therapy. Here we sought to characterize the effect of treatment on patients enrolled in ASP-1929–181 and compare the immune characteristics of responders vs non-responders. ASP-1929 photoimmunotherapy is an investigational treatment that combines cell surface binding of an anti-EGFR antibody conjugated to IRDye<sup>®</sup>700DX with red-light illumination for selective cell killing and has been shown to elicit a measurable anti-tumor immune response in preclinical models.<sup>1, 2</sup>

**Methods** Frozen peripheral blood mononuclear cells (PBMCs) isolated from patient whole blood were stained for flow cytometry to assess fluctuations of adaptive immune cells throughout treatment cycles. Tumor biopsies were evaluated for immune cell density and distribution using a novel multiplex immunofluorescence (mIF) imaging method based on machine-learning and watershed-based segmentation, allowing for an effective identification of the tumor region (figure 1). Using this method, cells within the mIF images were quantified based on population activation state, functionality, and/or proliferation.

**Results** We analyzed PBMCs from 12 patients, taken at up to 7 timepoints and 76 whole-slide images from 22 patients enrolled in ASP-1929–181. At the tumor, we see an increase in CD8+ cell densities (PD-1+, CTLA-4+, CD69+, and Granzyme B+ (GrB)) at C1D9 which is maintained to C2D9 in all patients treated. Importantly, responders show a sustained increase in functional CD8+ populations compared to non-responders at C3D9 ( $p < 0.05$ ). At the periphery, responders have significantly lower levels of circulating CD8+, CD8+GrB+, and CD8+CTLA-4+ T cell frequencies and significantly higher levels of CD8+PD-1+ T cell frequencies at screening and throughout treatment ( $p < 0.001$ ). Further data to be presented.

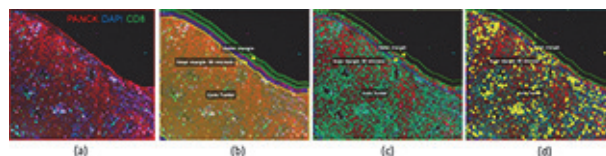
**Conclusions** Together these data reveal a T cell immune response at the tumor across patients and a peripheral T cell phenotype that may predict response for patients treated with ASP-1929 photoimmunotherapy plus anti-PD-1 therapy. Similar to previous work analyzing predictive peripheral biomarkers for immune checkpoint inhibitor therapy (ICI), our results describe a patient population with higher baseline CD8+PD1+ T cells and lower overall CD8+ T cells at screening may respond better to ICI combined with photoimmunotherapy.<sup>3</sup>

**Trial Registration** <https://classic.clinicaltrials.gov/ct2/show/NCT04305795>

### REFERENCES

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**Ethics Approval** This study obtained ethics approval by Integreview IRB for protocol ASP-1929–181. Patients gave informed consent before taking part.



**Abstract 83 Figure 1** Multiplexing immunofluorescence (mIF) imaging method: (A) Acquisition of mIF panel containing 9 markers – PANCK, DAPI, CD8, CD56, CD69, CTLA4, GranzymeB, Ki67 and PD1; (B) Extraction of tumor microenvironment compartments using PANCK channel; (C) Detection of cells in the compartments using DAPI channel; (D) Classification of cells into single-positive and dual-positive phenotypes (such as CD8+PD1+) based on machine learning models and correlation of cell counts with response outcomes.

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