tumor response. In addition, a machine-learning derived multidimensional biomarker showed high predictive performance (83%), positive predictive value (100%), and negative predictive value (80%). The multidimensional marker had superior ability to predict tumor response, with 15 of 18 patients characterized correctly. The predictive performance of this approach was compared to the tumor proportion score (TPS) with the on-label PD-L1 IHC assay in 15 of the 18 patients, which showed only 33% success in predicting tumor response.

**Conclusion:** This retrospective study, using a well-defined patient cohort, demonstrates that new methods employing RNA expression and immune health expression models generated a comprehensive multidimensional biomarker model resulting in significant improvements in predicting tumor response, compared to PD-L1. Additional patients will be analyzed to increase the cohort to at least 100 patients, and this data will be presented alongside the preliminary data described above.

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### 122 Profiling the Spatial Composition of the Hypoxic Tumor-Micro Environment through Multiplex Immunohistochemistry in a Prospective cohort of HPV Associated Oropharynx Cancer

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**Purpose/Objective(s):** Hypoxia is associated with radio-resistance and an immunosuppressive tumor microenvironment (TME). In a prospective trial using hypoxia as biomarker for radiation dose de-escalation to 30 Gy, we aimed to interrogate the spatial relationships between tumor and immune cells in the microenvironment of human papilloma virus (HPV) associated oropharyngeal carcinoma. We hypothesized that the presence of hypoxia impacts the composition of immune infiltrates as well as the spatial relationships of tumor and immune cells.

**Materials/Methods:** Blood samples were obtained from 15 ml blood from 762 known and previously treated HNSCC, which included 635 (83.3%) males and 127 (16.7%) female patients just prior to a PET-CT scan. Peripheral blood mononuclear cells (PBMCs) were harvested by centrifugation. Circulating Ensembles of Tumor Associated Cells (C-ETACs) which are clusters of heterotypic apoptosis resistant cells of tumorigenic origin were enriched by a novel process using combination of commercially available stabilizing agents. C-ETACs were characterized by immunostaining for EpCAM, panCK and CD45.

**Results:** Out of 762 patients who underwent PET-CT scan 142 patients (18.6%) had no detectable disease. Astonishingly, in this cohort of 142 patients C-ETACs were detected in 133 (93.7%). There appeared to be no association between metastatic status and presence of C-ETACs.

**Conclusion:** The presence of CMD in the form of viable tumor cells or clusters might be a feature of persisting HNSCC. Effective systemic therapy, we hypothesized that Circulating Metastatic Disease (CMD) in the form of viable tumor cells or clusters might be a feature of persisting HNSCC.