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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Profiling the Spatial Composition of the Hypoxic Tumor-Immune Microenvironment 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: 21 immuno-histochemical markers were used to evaluate the pre-treatment TME in a cohort of n = 10 HPV-associated oropharynx squamous cell carcinoma patients enrolled on a prospective trial (n = 19) of hypoxia-guided radiation dose de-escalation. Hypoxia negative status was determined by the absence of uptake of Flurorine-18 labeled Fluoro-Misonidazole (18F-FMISO) PET/CT imaging. Formalin fixed paraffin embedded resected primary tumor was reviewed in conjunction with a pathologist. Slides were stained using the Vectra Opal Multiplex immunohistochemistry system. Tumor and immune cell populations were phenotyped and quantified using semi-automated cell segmentation with the Halo digital pathology platform. Spatial analysis was conducted by evaluating immune cells within 50 micrometers of tumor cells. Two-sided student's T-test was used for statistical analysis between hypoxic and non-hypoxic primary tumors.

Results: 50% (n = 5) of patients were initially hypoxia negative, 30% (n = 3) converted from hypoxia positive to hypoxia-negative after 10 fractions of radiation, and 20% (n = 2) remained persistently positive. TME of initially hypoxia negative and patients who converted to hypoxia-negative was associated with an increased density of exhausted CD8+/PD1+/EOMES+ T-cells ($p = 0.027$), lower density of CD68+/CD163+ M2-macrophages ($p = 0.032$), and a lower density CD4+/FOXP3+ T-regulatory cells ($p < 0.001$). There were no significant differences in tumor PDL1 expression, as well as density of CD8+, proliferating CD8+/Ki67, or activated CD8+/Ki67+/granzyme B+ T cells.

Conclusion: Absence of hypoxia in the TME of HPV associated oropharynx carcinoma is associated with a decreased density of immunosuppressive T-regulatory cells, M2 macrophages, and an increased density of infiltrated exhausted T-cells. Insight into tumor-immune cell relationships, may increase understanding for treatment resistance in hypoxic TMEs is being investigated in a larger prospective study.

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Viable Circulating Ensembles of Tumor Associated Cells Persist in Patients with No Radiologically Detectable Disease after Treatment in Head and Neck Cancer

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Purpose/Objective(s): Advanced (metastatic) Head and Neck Squamous Cell Carcinomas (HNSCC) have limited systemic treatment options and such patients are often referred for palliative care. Response evaluation in HNSCC is determined by clinical and radiological parameters with FDG PET-CT being the modality of choice. However, recurrence or emergence of new metastases are frequently encountered in cases where radiological scans previously implied complete response to systemic treatments. To explore the mechanistic basis of disease recurrence in spite of apparently 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.

Materials/Methods: We obtained 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, pan-CK 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 a significant proportion of cases with no evidence of metabolically active disease implies that the majority of patients in whom conventional parameters of disease are negative have viable residual systemic disease and are not biologically cured.

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Encyclopedic Tumor Analysis Guided Treatments with Conventional Drugs Outperform Available Alternatives in Refractory Head and Neck Cancers

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