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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Purpose/Objective(s): Patients with advanced, refractory Head and Neck Squamous Cell Carcinomas (HNSCC) are often considered for immunotherapy with checkpoint inhibitors subject to PD-L1 expression. We hypothesized that such HNSCC would have unexplored vulnerabilities that could be identified using integrative molecular and cellular investigations (Encyclopedic Tumor Analysis, ETA) and targeted using conventional agents in a label- and organ-agnostic manner. We present findings from the HNSCC sub-cohort of the pan-cancer RESILIENT trial where patients with advanced refractory disease were treated with ETA guided treatments regimens.
Materials/Methods: Freshly biopsied tumor tissue was obtained from all patients. As part of ETA, Tumor Molecular Profiling (TMP) identified druggable gene alterations and dysregulated metabolic pathways. Immunohistochemistry (IHC) identified hormone receptors (HR) that could be targeted with endocrine agents. Chemoresistance and response (CRR) profiling of viable tumor derived cells (TDCs) identified functional vulnerabilities of the tumor against a panel of systemic anticancer agents. Integration of MP, IHC and CRR datasets (i.e., ETA) generated patient-specific, label- and organ-agnostic drug priority lists with projected efficacy and safety. Patients who received such ETA-guided treatments were evaluated radiologically to determine treatment response as well as Objective Response Rate (ORR), Disease Control Rate (DCR) and Progression Free Survival (PFS).
Results: ETA-guided regimens were administered to 30 patients with HNSCC who were evaluable for response *per protocol*. PR was observed in 14 patients (ORR = 46.7%) and 29 patients continued to exhibit PR or SD at study termination (DCR = 96.7%). Median PFS was 147 days at study completion with several patients continuing to remain progression free. Median PFS rate at 90 days was 100%. There were no significant or grade IV therapy related adverse events (AEs) or treatment related mortalities. Most patients reported stable to improved Quality of Life (QoL) in terms of disease-related symptoms and functional status.
Conclusion: ETA-guided treatments outperformed available alternatives such as checkpoint inhibitors in this heavily pretreated HNSCC cancer population by offering meaningful survival benefit.
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Prognostic Significance of Cell Differentiation and Immune Pathway Mutations in Recurrent Laryngeal Squamous Cell Carcinoma

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Purpose/Objective(s): Organ preservation protocols are commonly used as first line therapy for advanced laryngeal squamous cell carcinoma (SCC). Disease free survival after radiation or chemoradiation ranges from 30-60% and recurrent tumors often display an aggressive phenotype resulting in poor patient outcomes. The aim of this study is to identify genetic alterations associated with overall and disease specific survival in patients with recurrent laryngeal SCC undergoing salvage laryngectomy.
Materials/Methods: Sixty-two tumors from patients treated at a single NCI designated cancer center were obtained and sequenced using a targeted panel of 250 genes which were identified as being mutated at >1% frequency in the original head and neck squamous cell carcinoma TCGA project. Alterations were grouped based on the pathways defined in Go-lists curated by MSigDB. Disease specific and overall survival were stratified by mutation status for each pathway and outcomes were compared using log rank analysis and multivariate cox regression.
Results: Patients with alterations in the *Cell Differentiation/Epigenetic* and *Oxidation* pathways had significantly worse five-year disease specific survival compared to patients without alterations in these pathways (47.5%, 95% CI 25.2 − 66.9, vs. 82.3%, 95% CI 61.3 - 92.6, p = 0.007 and 32.3%, 95% CI 4.78 − 64.1, vs. 74.9%, 95% CI 59.8 − 85.6, p = 0.023). Conversely, alterations in the *HN-Immunity* pathway were associated with improved five-year disease specific survival (100% vs. 60.0%, 95% CI 42.2 - 73.8, p = 0.019) and overall survival (80.0%, 95% CI 40.8 - 94.6, vs. 38.2%, 95% CI 22.7 − 53.6, p = 0.048). On multivariate cox regression analysis, the *Cell Differentiation/Epigenetic* pathway remained an independent predictor of disease specific survival (HR 4.38, 95% CI 1.04 − 18.4, p = 0.044). The *HN-Immunity* pathway remained significantly associated with improved overall survival (HR 0.269, 95% CI 0.079 − 0.915, p = 0.035) while the *Oncogenic Kinases* pathway was significantly associated with worse overall survival (HR 3.46, 95% CI 1.26 − 9.45, p = 0.016).
Conclusion: Patients with alterations in the *Cell Differentiation/Epigenetic* pathway had significantly worse disease specific survival and patients with alterations in the *HN-Immunity* pathway had significantly improved overall survival in multivariate analysis. Identification of these prognostic genetic biomarkers may serve to help both identify patients at risk for poor outcomes and identify targetable pathways to improve survival.
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Sentinel Node Status to Guide Adjuvant Radiation Therapy in Patients with Merkel Cell Carcinoma

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Purpose/Objective(s): Wide local excision (WLE) with sentinel lymph node biopsy (SLNB) followed by adjuvant radiation therapy (aRT) to the primary tumor site is the preferred initial management approach for Merkel cell carcinoma (MCC). However, management of the draining lymph node basin is less clear, with some studies suggesting nodal aRT can be omitted if SLNB is negative, but other studies documenting nodal recurrence rates as high as 33%. Here, we report a 20-year experience treating MCC with aRT adapted to SLNB findings, specifically to evaluate whether nodal aRT can be safely omitted in SLNB-negative MCC.
Materials/Methods: We retrospectively identified patients who underwent WLE and SLNB for MCC from 1996-2015. SLNB-positive patients underwent completion lymphadenectomy. aRT to the primary tumor site was routinely recommended. The draining lymph node basin was included in the aRT target volume for SLNB-positive patients but omitted if SLNB was negative. Endpoints included overall survival (OS), disease-specific survival (DSS), locoregional recurrence-free survival (LRRFS), and distant recurrence-free survival (DRFS). Survival rates were estimated using the Kaplan-Meier methodology. Differences were assessed using the log-rank test.
Results: 55 patients underwent WLE and SLNB, including 41 (75%) who had a negative SLNB and 14 (25%) who had a positive SLNB. 33 (80%) SLNB-negative patients underwent aRT to the primary site only and 8 (20%) were observed. 11 (79%) SLNB-positive patients underwent aRT to the primary site plus nodal basin and 3 (21%) were observed. Median follow-up was 43.1 months (range: 5-182). 4-year DSS was 100% for SLNB-negative patients irradiated to the primary site only and 75% for