lungs, the tumor microbiome has been shown to be less diverse than normal tissue, but the effect of microbial composition alterations in airways prior to diagnosis of lung cancer is unknown. We sought to characterize the microbiome in airways of patients found to have lung cancer on follow-up. Following consent, bronchial brushes were obtained from 48 patients at a high risk of lung cancer. With a mean follow-up of 9.4±1.2 years, 5/48 were diagnosed with lung cancer, and 3/48 were diagnosed with lung cancer at bronchoscopy. 16S sequencing was performed on bronchial epithelial taken from the airways of each patient, and the QiIME2 platform was used to classify the bacterial populations. The bacterial taxonomy, alpha, and beta diversity measures were compared according to cancer status, and bacterial metagenome functionality was assessed using PICRUSt2. We found that patients with lung cancer and those who would develop it had lower airway bacterial diversity. Further, individuals who developed lung cancer over time displayed significantly different airway microbiome profiles from those who did not, but similar profiles to those who already had cancer (p<0.0001), with global taxonomic shifts observable at the phylum level. Using gene content inference, we observed that the lung-resident bacterial communities of patients with prevalent and incident cancers had significantly different metabolic profiles when compared to patients with no cancer. In particular, we observed an enrichment in the metabolites associated with cancer pathway (Wnt and Notch) activation (p-adjusted<0.0001), implicating a role of lung-resident bacterial communities in cancer initiation. Validation in an independent cohort consisting of 55 incident cancer, 18 prevalent cancer, and 263 noncancer subjects is ongoing. Here, we profile the microbial community resident to the lung epithelium, and detect changes in this community years prior to the clinical detection of lung cancer. This work lays a foundation for further prospective studies leveraging microbiome profiles to further our understanding of the role of the lung microbiome in the pathogenesis of lung cancer.

A05
ART1, a Mono-ADP-Ribosyltransferase, Regulates Tumor-Infiltrating CD8+ T Cells and Is Highly Expressed in EGFR Mutated Lung Cancers

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Introduction: ADP-ribosyltransferase 1 (ART1), a GPI-linked cell surface protein, is broadly expressed at the protein level in human tumors and has been linked to tumor progression in colon cancer and gliomas. ART1 may regulate the immune microenvironment through mono-ADP-ribosylation of the P2X7 receptor on CD8+ T cells, leading to T-cell apoptosis through NAD-induced cell death. P2X7R expression is prominent on tissue resident memory (Trm) CD8+ T cells, which have increasingly been recognized for their critical role in immune response. We evaluated the role of ART1 in an immune-competent murine model and sought to determine the expression of ART1 in human tumors, particularly EGFR mutated tumors, which are known to be poorly responsive to immunotherapy. Methods: Initial experiments were performed using the KP1 lung cancer cell line derived from lung tumors of KRASG12D/P53/- mice. KP1-shART1 cells with doxycycline (Dox)-inducible knockdown of ART1 were generated by lentiviral constructs and used in flank and tail vein tumor models to determine ART1 effects on tumor growth. A lung adenocarcinoma tissue microarray (TMA) was then stained and scored for ART1 expression in order to determine ART1 expression in human tumors. We also evaluated ART1 expression in patient samples by whole-tumor RNAseq and IHC from a prospective clinical trial of neoadjuvant durvalumab +/- RT, significantly decreased tumor burden in flank and tail vein tumor models. Populations of tumor-infiltrating CD8+ T cells and of P2X7R+/CD8+ T cells were higher with ART1 knockdown, suggesting that ART1 expression on tumor cells may regulate tumor-infiltrating T cells. In human lung cell lines, the EGFR+ cell line H1650 expressed significantly more cell surface ART1 than A549 cells or BEAS cells (2.7- and 6.9-fold, respectively). In the TMA, among 463 stage I patients, 257 patient tumors (55.5%) strongly expressed ART1. Among patients with EGFR mutated tumors (n=79), 69.6% strongly expressed ART1 compared to 52.9% of KRAS+ tumors (n=119, p=0.03). In NCT02904954, among patients with whole-tumor RNAseq performed from preoperative biopsy (n=21), relative ART1 expression was 3-fold higher in EGFR+ patients (n=6). Median post-treatment H-scores for ART1 staining in resected tumors were also higher in EGFR+ tumors (120 vs. 77.5, p<0.0065). No patients with EGFR+ tumors (n=8) had a major pathologic response to neoadjuvant durvalumab +/- RT, compared to a 38% combined arm MPR rate in EGFR+ tumors (n=34). P2RX7 was strongly expressed in post-treatment tumors by whole-tumor RNAseq and trended higher in responders. Conclusions: ART1 expression on lung cancer cells modulates tumor-infiltrating CD8 T cells. Knockdown of ART1 abrogates tumor growth, suggesting that ART1 may be a potential novel immune checkpoint and a therapeutic target. ART1 is particularly overexpressed in EGFR mutated lung cancers and may provide one mechanism to help explain their poor response to immunotherapy.

A06
Tri-complex Inhibitors of the Oncogenic, GTP-Bound Form of KRASG12C Overcome RTK-Mediated Escape Mechanisms and Drive Tumor Regressions in Preclinical Models of NSCLC


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RAS proteins are small GTPases that drive cell proliferation and survival when bound to GTP. Mutant RAS proteins are found in approximately one-third of NSCLC, and exist predominantly in the GTP-bound state, leading to aberrant downstream signaling via interaction with effectors such as RAF. Recently, multiple potent, covalent inhibitors of the oncogenic mutant KRASG12C have entered development and are driving high lung cancer response rates in early clinical trials. These inhibitors target the inactive, GDP-bound form of KRASG12C, KRASG12C(ON), and thus rely on the residual intrinsic hydrolysis of GTP to cycle KRASG12C proteins through the GDP-bound state. This mechanism is vulnerable to adaptive responses in cancer cells that increase upstream signaling to further elevate the relative abundance of the active, GTP-bound state, KRASG12C(ON). An inhibitor that directly targets KRASG12C(ON) would overcome this limitation. We have developed tri-complex inhibitors of KRASG12C(ON) that promote a ternary complex between KRASG12C and the immunophilin cyclophilin A (CypA). KRASG12C(ON) inhibitors attenuate both RAS-MAPK signaling and cell viability in cancer cell lines bearing KRASG12C mutations. In vivo administration of a KRASG12C(ON) inhibitor drives dose-dependent tumor regressions in the NCI-H358 KRASG12C NSCLC xenograft mouse model and is well tolerated. Consistent with targeting the KRAS(ON) and inhibitory activity in vitro is unaffected by RTK activation, whereas the activity of first-generation KRASG12C(OFF) inhibitors is significantly attenuated. In addition, proliferation of NCI-H358 cells in vitro is suppressed for a significantly longer duration with KRASG12C(ON) inhibitor treatment compared to KRASG12C(OFF) inhibitors. The ability to target the GTP-bound form of mutant KRAS permits a broad array of combination opportunities. Combination of