(ORR) (43% *vs* 11%, OR=6.22 [CI=1.31-29.44], p=0.021) and progression-free survival (HR 0.36 [CI= 0.14-0.90], p=0.028) but not with overall survival (p=0.117). PD-L1 IHC expression was available in 35 cases, of which 46% had high (≥50%) expression levels. A moderate concordance (0.49) was observed between PD-L1 IHC and *PD1*-mRNA. In this subset analysis, high PD-L1 IHC was significantly associated with response (50% *vs* 11%, OR=8.50 [CI= 1.45-49.53], p=0.043). Importantly, when combining predefined high/low-sets for both biomarkers (PD-L1 IHC/*PD1*-mRNA), response was significantly increased in PD-L1-high/*PD1*-high compared to PD-L1-low/*PD1*-low (ORR 58% *vs* 0%, p=0.019). **Conclusion:** *PD1*-mRNA expression is associated with response to anti-PD1 monotherapy and can increase the predictive ability of PD-L1 IHC. Further validation of these findings in pivotal clinical trials evaluating IO in advanced NSCLC pts seems warranted. **Keywords:** nCounter, PD1, Immunotherapy

P2.04-23
Tumor-Stroma Interactions Promote cGAS-STING Driven Inflammation in Lung Tumor Microenvironment

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Background: The tumor stroma is an essential component of the tumor microenvironment (TME) and has critical roles in promoting resistance to immunotherapies. Most anticancer therapies target cancer cells specifically, however, it is important to also study signaling contributions from the TME. The recruitment of immune cells following intratumoral administration of Stimulation of Interferon Genes (STING) agonists in the TME is a critical event in the cGAS-STING-driven antitumor immune response, a pathway with great relevance in the context of cancer immunotherapy. Towards this, the infiltration of immune cells rely on functional vasculature to infiltrate into the tumor tissue. We have previously demonstrated that LKB1 mutation is associated with suppression of tumor cell STING levels due to mitochondrial dysfunction and reduced production of T-cell chemoattractants such as CXCL10 in KRAS-driven non-small cell lung cancer (NSCLC). Consistently, immunohistochemical staining of patient samples showed poor infiltration of CD3, CD4, and CD8 T cells into LKB1 negative versus LKB1 intact cancer epithelium, and instead, retention of T-cells in stroma. **Method:** 3-D microfluidic device was fabricated using cyclic olefin polymer (COP) at AIM BIOTECH. NCI-H1355 cells were cultured for 24h in ultra low-attachment culture plates for spheroid formation. To form the 3D tumor microvascular model, cancer spheroids, human lung fibroblasts (hLFBs) and human umbilical vein endothelial cells (HUVECs) were resuspended in an extracellular matrix-like fibrin/collagen gel and loaded into the central channel and cultured for 7 days and hydrated with culture medium (Vasculife, lifeline). Cytokine profiling (Human Cytokine 40-plex panel) was performed with media collected from 3D culture. **Result:** To examine how LKB1 alters immune cell recruitment, we used a 3-D microfluidic co-culture system to study interactions between vasculature and tumor spheroids derived from a KRAS/LKB1 mutated (KL) cell line with LKB1 reconstitution +/- STING deletion. Co-culturing tumor spheroids and vasculature, we identified changes in morphology, cytokine production, and gene expression that occur during the co-culture. We found that co-culture induced cooperative production of multiple immune cell chemo-attractants such as CXCL10, CCL2, CCL5, and G-CSF (Fig.1b,c). Interestingly, this more physiologic ex vivo tumor model of LKB1 reconstitution revealed particularly strong cooperative production of STING-dependent cytokines such as CXCL10 in the vasculature. Moreover, knocking down STING in the LKB1-reconstituted cancer cells did not significantly attenuate production of CXCL10 and other cytokines in co-culture, suggesting that tumor/vasculature interaction may promote STING activation in the vasculature regardless of cancer cell-intrinsic STING function. Furthermore, although there was no appreciable response after treatment of KL cancer cells with cGAMP based STING agonists, treatment of isolated 3-D vascular networks with cGAMP enhanced vascular permeability and increased production of CXCL10 and CCL5, possibly contributing to defective chemokine gradients that retain T cells near the vasculature. **Conclusion:** Developing these more complex models that incorporate 3-dimensional tumor and self-assembled microvasculature may elucidate important aspects of cGAS-STING biology in KL lung cancer microenvironment, and may ultimately aid further development of effective immunotherapies targeting this signaling pathway. **Keywords:** cGAS-STING, KRAS/LKB1 lung cancer, tumor-vascular interactions

P2.04-24
Transcriptional Profiling of Neoantigen Specific T Cells in Resectable NSCLC Treated with Neoadjuvant Anti-PD-1

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Background: Neoadjuvant nivolumab has a manageable safety profile and can be effective in patients with resectable non-small cell lung cancer (NSCLC). To characterize the immune response in these patients, we sought to evaluate the existence and dynamics of neoantigen specific tumor-infiltrating T cells and identify their molecular phenotype including co-inhibitory checkpoint expression. **Method:** We evaluated peripheral blood and tumor infiltrating lymphocytes from seven patients treated with nivolumab. To identify neoantigen-specific T cell responses, we used MANAFEST (Mutation Associated Neoantigen Functional Expansion of Specific T cells), an assay we developed that links antigen specificity with unique CD8+ TCR Vβ CDR3 identities. We then carried out single cell TCRseq/RNAseq of tumor infiltrating T lymphocytes (TIL) to enumerate the genome wide digital gene expression and T cell clonotypic identity of each single cell (VDJ+DGE analysis), and particularly those with Vβ CDR3 regions identical to those identified as neoantigen-specific by MANAFEST. **Result:** Neoantigen-specific TCRs were detected in peripheral blood in all 3 patients with major pathologic response (MPR) and in 3 of 4 patients without MPR. Several of these clonotypes were found in the resected tumor and underwent peripheral expansions upon PD-1 blockade. In one notable patient, MD043-011, MANAFEST detected a T cell clonotype specific for a *CARM1* R208W mutation, despite this patient having no evidence of pathologic response. This neoantigen-specific clonotype represented 3.4% of TIL. Two years later, this patient recurred with a solitary brain metastasis. Single cell analyses of TIL in the primary lung lesion and brain metastasis revealed the same neoantigen-specific T cell clonotype was detected in the metastatic lesion. Strikingly, this clonotype exhibited a differential expression profile in the primary and recurrent lesion, with the clonotype in the primary tumor having an enrichment and upregulation of heat shock proteins indicating molecular stress and the clone in the metastatic lesion having an upregulation of checkpoint molecules, including CTLA4, TIM3, and LAG3. T cell cloning and validation experiments, as well as identification of transcriptional programs associated with MPR, are ongoing. **Conclusion:** The coupling of MANAFEST with single cell VDJ+ DGE analysis enabled us to characterize antigen specific clonotypes after differential expansion using the TCR as a molecular barcode. The presence of alternate co-inhibitory immune checkpoints on neoantigen-specific TIL from non-responding tumors suggests a potential driver of resistance to anti-PD-1 in early stage NSCLC. Ultimately, this integrative approach may provide key insights in predicting and understanding clinical response to neoadjuvant PD-1 blockade in NSCLC. **Keywords:** NSCLC, neoantigens, tcells