immunotherapy in this preclinical GEMM model. We ultimately plan to test direct MYXV intralesional injection by navigational bronchoscopy combined with immunotherapy to enhance immune mediated targeting of SCLC.

**MET:GRB2 complexes define a subset of lung cancer with potential vulnerability to MET inhibition**

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We previously demonstrated that proximity ligation assays (PLA) can be utilized to detect EGFR in complex with its major signaling adaptor GRB2, which couples the receptor to the MAP kinase pathway to drive oncogenic proliferation. These "signaling-associated complexes" correlate with EGFR activity, reveal erlotinib pharmacodynamics and are predictive of improved outcomes to EGFR-directed therapies (Smith et al Science Signaling 2015). Here, we use PLA to assess cMET signaling, which is being actively investigated in late stage clinical trials in lung cancer and other solid tumors. We found that the presence of cMET:GRB2 complexes correlates with sensitivity to cMET tyrosine kinase inhibitors (TKI) and the interaction is specifically abrogated by MET TKI as measured by biochemical approaches, cellular viability and PLA. In MET-amplified patient-derived xenograft models of lung cancer (N=6) we observe MET:GRB2 complexes in regions that also stain strongly using pMET(Y1234/5) immunohistochemistry (IHC). Treatment of these models with single agent crizotinib led to variable responses as measured by RECIST criteria. Ongoing experiments are correlating patterns of PLA positivity with magnitude of response to MET inhibition. In clinical cohorts of unselected non-small cell lung cancer patients (N=409), MET:GRB2 signaling complexes are rare (observed in <1%), even among patients whose tumors are highly positive for cMET protein expression as detected by IHC. In patients with MET gene amplification verified by FISH, presence of MET:GRB2 complexes were observed in 6 of 8 patients with variable intensity and significant spatial heterogeneity. The low rates of MET:GRB2 signaling complexes observed in patient tissues may potentially explain the poor response rates observed in clinical trials targeting cMET in lung cancer. Assays that can detect therapeutically-relevant protein complexes have the potential to improve patient stratification strategies and enable precision medicine in oncology.

**Natural antisense transcript deregulation in non-small cell lung cancer**

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**Background:** Lung cancer represents an enormous health burden, representing the most common cause of cancer death worldwide. The poor therapeutic outcome is largely due to a complex molecular background as well as late stage diagnosis, with most patients presenting unresectable local tumors, or metastatic disease. While mutations of driver genes is a well-known mechanism, approximately half of all non-small cell lung cancer (NSCLC) tumors harbor no known clinically relevant oncogenic drivers, emphasizing the need to explore alternative mechanisms such as non-coding RNAs (ncRNAs).

ncRNAs are RNA molecules that do not encode for protein, but have the ability to regulate DNA, proteins, as well as other RNA species. These genes exhibit tissue specific regulation and have emerged as important players in several tumor types including lung cancer. Natural antisense transcripts (NATS) are ncRNAs that are transcribed from the opposite strand of protein coding genes. These NATs overlap with, and are often involved in the regulation of, their sense counterparts. NATs can recruit regulatory complexes to their transcriptional locus, leading to silencing of overlapping sense partner gene transcription, and have recently been described in cancer to silence tumor suppressor genes such as CDKN2A/B. NATs are quite prevalent as it is estimated that 25-40% of genes display overlapping transcriptional partners, emphasizing their potential in gene regulation. However, only a few NATs have been characterized in cancer. Here we take an unbiased approach to study NAT deregulation as a mechanism for altered sense partner expression in NSCLC.

**Methods:** We performed RNA-sequencing on a set of 65 NSCLC tumors including 36 adenocarcinomas and 29 squamous cell carcinomas as well as matched nonmalignant lung tissues. A sign-rank test was used to identify NATs and partner genes with significantly altered expression between tumor and matched normal tissues. These findings were validated in an external dataset of lung tumors from TCGA. Survival analysis was performed using a Cox Proportional hazard model, as well as the log-rank method.

**Results:** We have identified a NAT of OIP5, a lung cancer oncogene required for chromatin segregation, to be significantly underexpressed in NSCLC. In the same tumors we find the overlapping partner gene, OIP5 mRNA,
ART1, an extracellular ADP-ribosyltransferase, is over-expressed in non-small cell lung cancer and facilitates cancer cell survival by immune-mediated mechanisms

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Cell surface mono-ADP ribosyltransferases (ARTs) transfer the ADP-ribose moiety from NAD$^+$ to amino acid residues on target proteins and post-translationally regulate their function. Most mammalian mono-ARTs, including ADP-ribosyltransferase 1 (ART1) reside on the cell surface, however the scope of extracellular mono-ADP-ribosylation is largely unknown. It has been suggested that epithelial cells in the injured or inflamed lung may overexpress ART1 as a mechanism of cell survival to protect against cell clearance by inflammatory cells. We hypothesize that ART1 expression is cytoprotective to lung cancer cells and facilitates metastatic growth.

We found ART1 to be expressed in multiple human NSCLC cell lines of distinct driver mutation status by RT-PCR, western blots, and immunofluorescent staining. Using biobanked human materials, we also found evidence of ART1 expression in human NSCLC tumors by whole tumor PCR, western blots, and immunohistochemistry. Compared to matched adjacent normal lung (n=40), by RT-PCR there is over a 2-fold increase (p=0.01) in median tumor expression of ART1, suggesting a role in tumorigenesis or tumor progression. We subsequently stained a tissue microarray containing 184 cases of predominantly stage I NSCLC to determine the prevalence of NSCLC tumors staining positive for ART1. ART1 staining was moderate or strong in 83% of adenocarcinomas (n=145) and in 45% of squamous cell cancers (n=39, p<0.001).

We next used murine cell lines derived from inducible KRASG12D/+ /p53/- mice and an in vivo model to determine the effect of ART1 expression on metastatic outgrowth. In a tail vein injection model in immunocompetent mice, we noted a highly significant decrease in metastasis in the ART1-knockdown cell line (sh175) compared to the parent KP1 line. To determine whether the immune protective effect mediated by ART1 may be T cell dependent, we implanted flank tumors in immunocompetent (B6) and lymphocyte-depleted nude mice. Despite slightly faster in vitro rates of growth, sh175KP1 cells lacking ART1 expression were largely unable to form tumors when injected into the flanks of immunocompetent B6 mice. Only 1 of 5 immunocompetent mice injected with sh175KP1 cells developed a tumor, compared to 5 of 5 mice injected with KP1 control cells. However, in lymphocyte-depleted nude mice, there was robust flank tumor growth with both cell lines. Because the sh175KP1 cells lacking ART1 only had a disadvantage to growth in mice with lymphocytes, we can infer that ART1 expression may have a strong immunomodulating effect on that cell population. We also evaluated the effect of ART1 expression on neutrophil cytotoxicity. At a neutrophil:tumor cell ratio of 20:1, the knockdown cell line sh175KP1 lacking ART1 expression is more sensitive to neutrophil-induced apoptosis in the co-culture assay (87% vs. 56% Annexin V positive, p=0.05). Importantly, chemical inhibition of mono-ADP-ribosylation in the parent KP1 cell line with two well established inhibitors, novobiocin and meta-iodobenzylguanidine, facilitated neutrophil-induced apoptosis, implying that the enzymatic activity of ART1 is critical to the phenotype.

We provide evidence that ART1 is overexpressed in NSCLC. ADP-ribosylation may serve as a defense against immune-mediated cytotoxicity. Cells without ART1 expression are more sensitive to cytotoxicity induced by immune cells and these cells have a markedly decreased capacity to grow in the lungs in an immunocompetent metastatic model. Because ART1 is an extracellular enzymatic target, it is expected to be highly druggable and susceptible to therapeutic intervention. We envision using targeted inhibition of ADP-ribosylation in ART1-overexpressing tumors to facilitate immune-mediated destruction of established cancers or of micrometastatic disease.

Routine molecular testing of resected early-stage lung adenocarcinoma with targeted next-generation sequencing demonstrates a high rate of actionable mutations


Introduction: Molecular testing is routinely performed in patients with metastatic non-small cell lung cancer. As the