between benign and cancerous nodules, and ii) quantitatively predict risk of lung cancer incidence.

Methods: Using data and images from the NLST, we performed post hoc nested case-control analyses. The first analysis was conducted to identify diagnostic quantitative imaging features that differentiate between malignant tumors and benign nodules. This study included 88 incidence lung cancer cases diagnosed at the first follow-up interval (T1) and 172 “controls” that had a nodule+ scan at T1 that was not lung cancer. The second analysis was conducted to identify predictive quantitative imaging features that are predictive of lung cancer risk. This study utilized baseline scans (T0) from 74 subjects who developed an incidence lung cancer in follow-up intervals and 125 “controls” that had a nodule+ result in follow-up intervals that was not lung cancer. The LDCT scans were subjected to an in-house “Radiomic Pipeline” that converts images to mineable data (>400 quantitative features). Two classes of features were extracted: semantic and agnostic. Semantic features are commonly used in the radiology lexicon to describe regions of interest. Agnostic features are mathematically extracted quantitative descriptors that capture lesion heterogeneity. Separate statistical analyses were performed for the diagnostic and predictive features. Univariable analyses and false discovery rate (FDR) were utilized to identify which were features statistically significant. To generate a parsimonious model, we performed a backward elimination process using a 0.1 threshold for inclusion.

Results: Although nodule size has diagnostic utility, especially among the largest nodules, >80% of cases and controls had nodules <15 cm. For size alone, we found a modest AUC of 0.79 when nodules were <15 cm. We sought to improve the diagnostic capability of size by adding imaging features. Univariable analyses revealed that 17 of the features were significantly different between cases and controls. Backward elimination process revealed a model with 3 imaging features (radius of smallest enclosing ellipse, radius of largest enclosed ellipse, and tumor thickness-pixel) that yielded an AUC of 0.88; and a model with those 3 features, size, and demographics yields an AUC of 0.89. For the risk prediction analysis, univariable analyses revealed that 10 of the features were significantly associated with lung cancer risk which remained significant when included in a single model including demographics/risk factors. Backward elimination process identified a model with six imaging features (concauity, border definition, attachment to vessel, perinodule emphysema, perinodule fibrosis, nodules in both lungs) and demographics yielding an AUC of 0.87 compared to 0.58 for demographics alone.

Conclusions: These results demonstrate that we can improve the diagnostic utility of size alone by including additional imaging features. Moreover, these data provide strong and compelling evidence for the utility of imaging features for risk prediction.

Myxomavirus (MYXV) therapy for small cell lung cancer (SCLC) using patient samples and a genetically engineered SCLC mouse model

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Introduction: Advanced SCLC is an aggressive neuroendocrine subtype of lung cancer that kills approximately 20,000 Americans per year. Despite multiple clinical trials, there have been few improvements in standard treatments for the past 3 decades. Therefore, there is a need for new therapeutic strategies. We have undertaken a multidisciplinary project to test the efficacy of an oncolytic MYXV to preferentially infect, replicate, and kill SCLC cells in human SCLC primary tumors and derived human and mouse SCLC cell lines in vitro and in a SCLC GEMM mouse model in vivo.

Results: We optimized a conditional RB/p130/p53 knock-out mouse SCLC tumor model (GEMM) using limiting dilutions of intra-tracheal Adeno-CRE virus to reduce the number of primary lung SCLC foci in order to simulate human disease and to generate mouse SCLC cell lines from individual tumor clones. We tested MYXV in vitro using 14 different human and mouse SCLC cell lines and observed productive infection and viral replication associated with cytotoxicity in tumors cells that grow both adherently and as non-adherent spheroids. In contrast, we did not detect productive infection nor cytotoxicity in non-cancerous cells. We analyzed groups of mice at 1 month interval after adenovirus delivery and determined the optimal time to start intrapulmonary MYXV delivery when small tumors were present but not yet metastatic. At 3 days post-treatment, we observed that MYXV localized to the lungs when tumors were present, but in control GEMM mice lacking CRE-mediated tumor induction, MYXV was not detected. Necropsy examination and TUNEL staining showed apoptosis and necrosis in murine SCLC tumors cells that grow both adherently and as non-adherent spheroids. At 3 days post-treatment, we observed that MYXV localized to the lungs when tumors were present, but in control GEMM mice lacking CRE-mediated tumor induction, MYXV was not detected. Necropsy examination and TUNEL staining showed apoptosis and necrosis in murine SCLC tumors within 5 days of MYXV treatment, and the effect persisted with tumor necrosis at 30 days post-treatment. We are currently testing the effect of MYXV delivery on overall survival in this murine SCLC model system.

Conclusion: MYXV enhances cell killing of human and murine SCLC cells in vitro and targets SCLC tumors in an immunocompetent GEMM. Our goal is to study the effect of MYXV combined with cisplatin cytotoxic chemotherapy as well as MYXV combined with anti-PD1 and anti-CTLA...
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,