IC50), but high dose EN460 didn’t sensitize cells to CDDP. In summary, high ERO1L expression was associated with poor prognosis of non-small cell lung cancer and resistance to CDDP therapy, which may be ameliorated with addition of EN460. These data suggest that ERO1L may promote lung cancer cell survival and resistance to chemotherapy by reduction of ER stress, and that ERO1L inhibitor may sensitize CDDP-resistant cancer cells to CDDP.

Intratumoral CCL21 and checkpoint blockade cooperatively inhibit NSCLC tumor growth in vivo to a greater extent than either monotherapy alone

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Recent studies reveal responses in approximately 20% of non-small cell lung cancer (NSCLC) patients treated with inhibitors of the PD-1/PD-L1 checkpoint. This includes robust and durable responses in previously treated patients with progressive locally advanced or metastatic NSCLC. However, a large percentage of patients do not respond to checkpoint inhibitors delivered as single agents. Studies demonstrate that tumor-infiltrating CD8+ T cells are requisite for antitumor responses to antibody-mediated therapies that block PD-1 or PD-L1. One potential approach to extend the effectiveness of checkpoint inhibitors to additional NSCLC patients is to enhance T cell responses by in situ vaccination that takes advantage of the full repertoire of available tumor antigens. In preclinical and clinical trials, we discovered that CCL21 has antitumor properties and CCL21-DC has the capacity to induce both local T cell recruitment and systemic immune responses. We hypothesized that in situ vaccination with CCL21-DC could serve as a tool to restore T cell infiltration, tumor antigen presentation, and T cell responsiveness, thereby sensitizing non-responsive NSCLC tumors to checkpoint blockade. To test this hypothesis, we first evaluated CCL21-DC as a monotherapy in the well-characterized syngeneic KRASG12D murine model of lung cancer. We observed decreased tumor growth, increased tumor-infiltrating lymphocyte (TIL) cytolytic activity against the autologous tumor, and increased IFNγ/TNFα in the tumor, as well as systemically in the spleen. Using the same LKR13 murine model, we observed that anti-PD-1 monotherapy also inhibited tumor growth, increased TIL cytolytic activity against the autologous tumor, and increased IFNγ/TNFα in the tumor, as well as systemically in the spleen. To determine if TIL activity from the CCL21-DC treatment group could be enhanced by PD-1 blockade, we performed an in vitro cytolytic assay. TIL from the CCL21-DC group had significantly greater cytolytic activity against the autologous tumor in the presence of PD-1 antibody relative to control antibody. We next evaluated intratumoral CCL21 and intraperitoneal anti-PD-1 administered in combination to LKR13 tumor-bearing mice. Both monotherapies reduced final tumor volume approximately three-fold, and the combination proved more efficacious than either agent alone. We obtained similar results utilizing the syngeneic 3LL murine lung cancer model. Both monotherapies significantly reduced tumor growth, such that the final 3LL tumor volume at the time of necropsy was approximately half that of the control group, and the combination of checkpoint blockade and CCL21 augmented the antitumor activity nearly two-fold more. Collectively, these data support the hypothesis that intratumoral administration of CCL21-DC and checkpoint blockade therapy cooperatively inhibit NSCLC tumor growth to a greater extent than either monotherapy alone. We anticipate that the capacity of in situ vaccination to drive both DC and T cell effector infiltration of NSCLC tumors will play an important role in increasing the number of patients responding to immunotherapy in the future.

Diagnostic and predictive quantitative-imaging features in lung cancer screening

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Background: Although the National Lung Screening Trial (NLST) found a 20% reduction for lung cancer mortality among participants screened with low-dose computed tomography (LDCT) compared to standard chest radiography, there are many limitations of LDCT screening. First, LDCT screening identifies large numbers of indeterminate pulmonary nodules (IPNs) of which only a fraction develop into cancer. At present non-invasive approaches do not exist to determine whether these IPNs are cancerous or benign. Next, if a nodule is detected, clinical guidelines provide for the evaluation and follow-up of nodules, but do not provide clinical decision tools to predict risk and probability of cancer development. Cumulatively, these limitations of LDCT screening critically require development of non-invasive, accurate quantitative imaging-based classifier models that i) can be reduce false positives by differentiating...
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 were 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 (concavity, 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.

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**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 adeno-CRE 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 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.