patients (43%). Preliminary clinical antitumor activity was also seen in one additional patient with NSCLC harboring the oncogenic KRASG12C mutation and a presumed hyperactivating SHP2 mutation (SHP2V428M). Plasma exposures of RMC-4630 increased proportional to dose, and at all dose levels were within the range that was projected to have antitumor activity from preclinical studies. Sequential analysis of pERK in peripheral blood cells and paired tumor biopsies showed evidence of RAS signaling pathway inhibition. The safety and tolerability profile of RMC-4630 appear to be consistent with RAS pathway inhibition. RMC-4630 showed reasonable tolerability and preliminary signs of clinical activity in patients with NSCLC harboring KRAS mutations. RMC-4630 continues to be tested as a single agent in patients with tumors harboring RAS signaling pathway mutations. This study is also open to patients with KRASG12C NSCLC who are progressing on KRASG12C(OFF) inhibitors. A study in combination with the MEK inhibitor cobimetinib (Cotellic) is also under way. RMC-4630, and other chemically related SHP2 inhibitors, have demonstrated combinatorial benefit with mutant-selective inhibitors of KRASG12C(OFF), such as AMG 510, in preclinical models. A clinical trial evaluating the combination of RMC-4630 and AMG 510, as well as additional combination studies, are planned.

A13
A Functional Genomics Approach Highlights New Therapeutic Opportunities for KRAS-Mutated Non-Small Cell Lung Cancer

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Despite the introduction of innovative therapeutics, the prognosis of non-small cell lung cancer (NSCLC) remains poor, with an overall survival at five years of only 16%. In recent years, a great effort has been conferred to target oncogenes on which cancer cells rely for survival and proliferation. However, the success of this strategy is often limited by development of drug resistance and by difficult-to-target oncogenes. KRAS-driven lung adenocarcinoma is particularly hard to target, still representing an unmet clinical need and an open challenge. In this context, based on the notion that tumors rely for their survival also on genes that are not classical oncogenes, an innovative strategy is to move the focus from oncogenes to "non-oncogene addiction." Because of their aberrant biology, cancer cells are more sensitive than normal cells to inhibition of those nononcogenic pathways. In this work, we aimed to identify nononcogene dependencies that can be exploited to develop novel therapeutic strategies for KRAS-mutated NSCLC. To this end, we used a CRISPR/Cas9 genome-scale knockout approach in KRAS-mutated NSCLC cells. After normalization with CERES algorithm, 705 genes were identified as nononcogene addictions. Next, we compared our results with data available through the Cancer Dependency Map Portal (DepMap), which collects dependency data of 73 lung cancer cell lines. From this analysis, we obtained two outputs: a list of common dependencies in lung cancer cell lines and a list of KRAS-mutated NSCLC-specific vulnerabilities. Reactome enrichment analysis on these genes identified pathways related to mRNA metabolism as key dependencies. We showed that a subset of these genes is overexpressed in tumor samples and associated with worse prognosis in adenocarcinoma patients. These candidates represent excellent therapeutic targets. Starting from our lists of essential genes, we also identified already available chemical compounds that inhibit the activity of those genes. Some of the drugs are already approved or currently in clinical trials for NSCLC, supporting the validity of our analysis. Intriguingly, we also identified druggable genes whose role in lung carcinogenesis is controversial or has been poorly investigated.

These drug-target interactions may be used to repurpose already available drugs for NSCLC treatment. Through a functional genomics strategy, we highlighted novel KRAS-mutated NSCLC vulnerabilities that can be used both for drug repurposing and for developing new therapeutics.

A14
Circulating Ensembles of Tumor-Associated Cells Are Ubiquitous in Lung Cancers

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Detection of lung cancers is based on histopathologic analysis of tumor tissue obtained by invasive biopsies following findings on low-dose computed tomography (LDCT) or other symptomatic presentation in suspected cases. There is presently no noninvasive nonradiologic blood-based test with high specificity and sensitivity for detection of lung cancers. Considering that unprovoked thromboembolism is a significant risk in multiple cancers, we hypothesized that tumor-derived circulating emboli in peripheral blood could comprise cancer cells and would serve as a reliable biomarker for detection of lung cancers. These circulating ensembles of tumor-associated cells (C-ETACs) are defined as clusters of 3 or more cells of tumorigenic origin (EpCAM+, CK+, and CD45-). We obtained 15ml of blood from 11,063 individuals, including 438 cases of non-small cell lung cancer (NSCLC) as well as from 10,625 asymptomatic individuals with age-related elevated risk, prior to LDCT scan. PBMC were isolated by centrifugation. C-ETACs were enriched using an epigenetically activated medium that eliminates normal cells (nontumorigenic origin) and confers survival privilege on apoptosis-resistant cells of tumorigenic origin (C-TACs, circulating tumor-associated cells) and their clusters (C-ETACs). Surviving C-ETACs were confirmed by immunostaining (EpCAM, pan-CK, CD45, TTF-1, Napsin-A). C-ETACs were detected in 374 (85.4%) of 438 lung cancers irrespective of extent (stage/metastatic status) of disease and prior treatments. Among the 587 asymptomatic individuals with suspicious findings on LDCT (Lung RADS category ≥ 2), C-ETACs were detected in 21 individuals (3.6%). Among the 10,038 asymptomatic individuals with no suspicious findings on LDCT (Lung RADS = 1), C-ETACs were detected in 371 individuals (3.7%). C-ETACs were ubiquitous in NSCLC regardless of extent and treatment status, and pose significant latent risk of metastasis/recurrence. Simultaneously, the relative undetectability of C-ETACs in asymptomatic cohort indicates causative connection of C-ETACs with lung malignancies. C-ETACs are suitable for screening suspected populations for lung cancers.

A15
Cancer-Associated Mesenchymal Cells Influence Lung Cancer Metastatic Phenotypes in Vitro and in Vivo

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Lung cancer is the leading cause of cancer deaths worldwide among both men and women. The vast majority of all cancer deaths are caused by metastatic dissemination of the disease. The extracellular environment surrounding and within a tumor, the tumor microenvironment, comprises a variety of components and multiple cell types. The interactions between different cell types and their associated extracellular matrices (ECM) are thought to play a role in cancer progression and metastasis, as well as therapeutic responses.

These drug-target interactions may be used to repurpose already available drugs for NSCLC treatment. Through a functional genomics strategy, we highlighted novel KRAS-mutated NSCLC vulnerabilities that can be used both for drug repurposing and for developing new therapeutics.
to cytotoxic and immunotherapies. The precise molecular details for how interactions between individual components of the tumor microenvironment impact cancer progression and metastasis are not well understood. Elucidating complex interactions within the tumor microenvironment is essential for identifying novel therapeutic targets but has proved challenging because isolating and studying cancer-associated stromal cells from primary lung tumors has remained difficult. To begin to fill this knowledge gap, we developed a rapid, reliable, and reproducible culture system that allows for the isolation and expansion of large quantities of primary cancer-associated mesenchymal (CaM) cells. Briefly, by combining fibroblast-derived extracellular matrices with hypoxic culture conditions, we developed a microenvironmental mimetic cell culture system. Primary lung cancer biopsies are dispersed and put into this system, and the resulting cells that expand are CaM cells with stem-like properties. We show that CaM cells and their deposited extracellular matrices alter signaling pathways and migration of lung cancer cells in vitro, and when co-injected with lung cancer cells, CaM cells induce metastasis in vivo. Thus, the overall hypothesis of this work is that CaM cells drive lung cancer metastasis by reprogramming the extracellular milieu and inducing metastatic signaling within the cancer cells. We are now working to determine the specific mechanisms by which primary CaM cells influence metastatic phenotypes of cancer cells. Further, these experiments will lead to novel therapeutic targets by identifying interactions and signaling events that are only initiated by CaM cells and their deposited ECM within the tumor microenvironment.

A16
Autoantibody-Antigen Complexes Can Detect Limited-Stage Small-Cell Lung Cancer

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Small-cell lung cancer (SCLC) claims 30,000 American lives each year with five-year survival rates of just ~7%. Somewhat lost in these dismal statistics is the fact that patients diagnosed early (limited stage) display vastly superior survival metrics when compared to those diagnosed late (extensive stage). Since nearly 20% of limited-stage SCLC can be cured with conventional cytotoxic chemotherapy and/or surgery, earlier diagnosis could have a clinical impact. Unfortunately, the computed tomography screening approaches capable of early detection for non-small cell lung cancer (NSCLC) have not proven effective for SCLC. We have found that overall levels of plasma autoantibody-antigen complexes are >2x higher in SCLC compared to other cancer types including NSCLC, colon, breast, and pancreas cancer. Thus, we hypothesized that a blood-based autoantibody test could reliably detect SCLC while still at limited stage. Using high-density antibody arrays, we discovered and twice validated 8 IgG and 11 IgM highly specific autoantibody-antigen complexes for SCLC in 3 independent cohorts (1 pre-diagnostic and 2 diagnostic, total N=240). Using optimized logistic regression, we identified 4 autoantibody-antigen complexes that performed well in each study with an AUC of 0.915 (53% sensitivity at 90% specificity) in the prediagnostic set, 1.0 (100% sensitivity at 90% specificity) in the first diagnostic cohort and 0.866 (64% sensitivity at 90% specificity) in the second diagnostic cohort. Panel autoantibodies were similarly effective when the plasma was drawn up to 1 year prior to diagnosis, at limited-stage diagnosis, or at extensive-stage diagnosis. We have evidence that each panel autoantibody is specific for SCLC as none are upregulated in NSCLC (N=45) samples or in other comorbidities examined, including COPD (N=31) and autoimmunity (N=15). Our findings suggest these autoantibodies have the potential to be used at the time of lung cancer screening to identify limited-stage SCLC to increase the survival of this recalcitrant cancer.

A17
Inhibition of RUVBL1/2 ATPase Activity Drives Immune Infiltration and Radiosensitizes Non-Small Cell Lung Cancer

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Purpose of Study: Prior work in non-small cell lung cancer has shown that RUVBL1 and RUVBL2 (herein RUVBL1/2) are overexpressed in patient tumors and high expression predicts poor patient prognosis. Additionally, the inhibition of RUVBL1/2 ATPase activity using small molecules has shown modest activity as a monotherapy in some preclinical models. In this study we evaluated what clinically relevant agents could be combined with RUVBL1/2 inhibitors to improve therapeutic efficacy in NSCLC. Experimental Procedures: Patient-derived NSCLC lines were treated with radiation, chemotherapy, and targeted inhibitors in combination with a RUVBL1/2 inhibitor, and viability was measured to determine synergy/potentiation using Combenefit (Bliss and Loewes synergy). Both the kinetics and magnitude of DNA damage after RUVBL1/2 inhibition and radiation was measured using immunofluorescence and Western blot in NSCLC and normal human bronchial epithelial cells. Humanized mice bearing NSCLC xenografts were treated with a RUVBL1/2 inhibitor, and intra-tumoral immune infiltrate was measured using flow cytometry. Activation of the cGAS/STING pathway was monitored after RUVBL1/2 inhibition using small molecule inhibitors in combination with a highly specific RUVBL1/2 inhibitor or its enantiomer control and cell viability was measured. RUVBL1/2 inhibition significantly enhanced the killing of NSCLC following radiation, but not chemotherapy or other targeted agents. This enhancement was specific to NSCLC, not normal bronchial epithelial cells, and occurred by inhibiting the ability of NSCLC to efficiently repair their DNA. To gauge the effect of RUVBL1/2 inhibitors on the immune system, and thus potentially immunotherapy, humanized mice bearing NSCLC xenografts were treated with a RUVBL1/2 inhibitor. Treatment with a RUVBL1/2 inhibitor caused infiltration of T cells, B cells, and dendritic cells, suggesting that RUVBL1/2 inhibition stimulated the immune system. Additionally, treatment of NSCLC lines in vitro with a RUVBL1/2 inhibitor and radiation activates the cGAS/STING pathway, suggesting that RUVBL1/2 inhibitors could be combined with radiation and immunotherapy.