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

K.J. Lastwika,1 Y. Zhang,1 J.J. Ladd,1 P.P. Massion,2 A.M. Houghton,1 P.D. Lampe1 1Fred Hutchinson Cancer Research Center, Seattle, WA/US, 2Vanderbilt University, Nashville, TN/US

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 recurrent cancer.

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

P. Yeneralli,1,2 A.K. Das,2 S. Wang,1,3 R.K. Kollipara,1 H. Li,2 L. Shan Li,1 P. Villalobos,4 J. Flaming,1 K. Huffman,1 B.C. Timmons,2 C. Gilbreath,3 R. Sonavane,1 J. Rodriguez-Canales,2 C. Moran,1 C. Behrens,5 M. Hirasawa, T. Takata,1 R. Murakami,1 K. Iwana,8 G.V. Raj,2,3,10 A.I. Wistuba,4 J.D. Minna,10,11,12 R. Kittler1,10,11,12 Eugene McDermott Center for Human Growth and Development, UT Southwestern Medical Center, Dallas, TX/US, 2Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX/US, 3Department of Urology, UT Southwestern Medical Center, Dallas, TX/US, 4Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX/US, 5Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, TX/US, 6Department of Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX/US, 7Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi-Sankyo Co., Ltd., Tokyo/JP, 8Oncology Medical Science Department, Medical Affairs, Daiichi-Sankyo Co., Ltd., Tokyo/JP, 9Oncology Research Laboratories II, Daiichi-Sankyo Co., Ltd., Tokyo/JP, 10Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX/US, 11Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX/US, 12Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX/US

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 intratumoral immune infiltrate was measured using flow cytometry. Activation of the cGAS/STING pathway was monitored after RUVBL1/2 inhibitor treatment in NSCLC lines using Western blot. Summary: Patient-derived NSCLC lines were treated with clinically relevant chemotherapies, targeted inhibitors, or radiation 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.
Conclusions: Inhibition of RUVBL1/2 as a monotherapy has modest efficacy due to a narrow therapeutic window. This work demonstrates that RUVBL1/2 inhibitors can enhance the cancer-killing effect of radiation, but not other clinically relevant agents, specifically in tumor cells (i.e., spares normal cells). Additionally, we show that treatment with a RUVBL1/2 inhibitor can cause immune infiltration in NSCLC tumors, and a RUVBL1/2 inhibitor in combination with radiation can activate the cGAS/STING pathway. In totality, our results suggest that further research should be done looking at the efficacy of RUVBL1/2 inhibitors with immune checkpoint inhibitors, both with and without radiation, in NSCLC.

A18
Culture of Immortalized Human Alveolar Epithelial Cells in 2D and 3D to Model Lung Adenocarcinoma Progression in Vitro

Background: Lung cancer is the leading cause of cancer death in the United States. Lung adenocarcinoma (LUAD) is the most common histologic subtype, arising from epithelial cells of terminal respiratory units called alveolus. The overall 5-year survival of lung cancer remains low at 19%. There is an urgent need to understand early events in LUAD development, as well as to develop new 1st-, 2nd-, and 3rd-line targeted therapies. Human organoids are powerful research tools for the molecular and mechanical manipulation of genetically diverse cells without exposing human subjects to treatment. Establishment of normal cell lines from human alveolar epithelial cells (AECs) has remained challenging due to the difficulty of growing primary cells in long-term culture. Human alveolar organoids would provide a powerful tool to 1) study LUAD development, progression, and drug resistance; 2) screen for new therapeutics; and 3) study the effects of environmental exposures on AECs. Goal: Optimize growth and genetic conditions to derive human AEC lines from purified primary cells for the stepwise modeling of LUAD. Approach: We tested different immortalization strategies using primary purified AECs to determine which condition allowed cells to continue proliferating while retaining their epithelial phenotype in two-dimensional (2D) culture and their ability to form spheroids in three-dimensional (3D) culture. Results/Discussion: Using purified primary AECs from three deceased, deidentified human subjects, we found that the initial propagation of AECs in media containing Y-27632 and subsequent transduction with Simian virus 40 Large T antigen allowed cells to divide readily in 2D as a monolayer, while expressing epithelial marker E-cadherin but not mature lung genes. When placed in 3D Matrigel culture with fibroblasts, these cells form multilobulated structures expressing mature AEC markers, reminiscent of the peripheral lung. We are currently optimizing this system to allow stepwise modeling of LUAD.

A19
Epithelial Beta 1 Integrin Regulates Lung Cancer Susceptibility Through NF-kB Signaling
E. Plosa, J. Sucre, P. Gulleman, T. Blackwell Vanderbilt University Medical Center, Nashville, TN/US

Rationale: Cell-extracellular matrix (ECM) interactions are essential for maintenance of alveolar homeostasis in the adult lung. Alveolar epithelial cells (AECs) connect to the ECM through integrins. β1 integrin is the most common lung epithelial integrin subunit, and forms the receptors for collagen, laminin, and fibronectin. We have previously shown that epithelial β1 integrin regulates AEC inflammatory signaling during alveolar homeostasis. However, the role of epithelial β1 integrin during repair after injury remains undefined. We hypothesize that epithelial β1 integrin is required for alveolar repair by regulating AEC proliferation and survival in response to injurious stimuli. Methods: We deleted β1 integrin in type 2 AECs in the adult lung in SP-C rtTA; TetO-Cre; β1f/f mice using doxycycline to induce Cre expression (called β1rtTA mice). Three-month-old β1rtTA and β1f/f mice were challenged with 3 μg/g intratracheal lipopolysaccharide (LPS) or PBS. β1f/f lettermate mice and doxycycline naïve SP-C rtTA; TetO-Cre; β1f/f mice were used as controls and mice were crossed onto the CCR2-/- background. Lung slices obtained from β1rtTA and β1f/f lungs were cultured with the NF-kB inhibitor BAY-11-7082, LPS, or both. Results: We previously reported that β1-deficient type 2 AECs are inflamed in the absence of injury, exhibited increased reactive oxygen species production, increased NF-kB activation, and secreting inflammatory cytokines. Aged 2-year-old β1rtTA mice exhibited chronic progressive macrophage dominant inflammation and developed adenocarcinoma. We next challenged 3-month-old β1rtTA mice with intratracheal LPS. LPS-treated β1rtTA mice had increased mortality at 7 days (43% β1rtTA vs. 91% β1f/f survival, p < .05) and an escalation in the number of recruited inflammatory cells from 24 hours to 7 days post-LPS challenge. In the β1rtTA survivors, histologic examination 21 days after LPS challenge resulted in emphysema in β1rtTA lungs, fibrotic regions identified by trichrome staining, and type 2 AEC hyperplasia. Histologic examination 6 months following LPS administration revealed sustained influx on monocyte-macrophages and early adenocarcinoma formation at 9 months of age in β1rtTA mice. Since the NF-kB pathway serves as a pro-survival mechanism, we treated ex vivo lung slices with an NF-kB inhibitor and LPS. In β1rtTA lung slices, NF-kB inhibition alone potentiated tissue remodeling and exacerbated AEC proliferation, suggesting that upregulated NF-kB plays a compensatory prosurvival role in the presence of chronically inflamed type 2 AECs. β1rtTA mice crossed to the CCR2 null background, which lack monocyte-macrophage recruitment, were protected from adenocarcinoma formation with age and following LPS challenge. Conclusions: These findings suggest that β1 integrin relies on the prosurvival properties of the NF-kB pathway to regulate AEC proliferation during homeostasis and dysregulation of β1-mediated inflammation post injury increases cancer susceptibility.

A20
Estrogen Metabolism in Patients with EGFR-Mutated and ALK-Mutated Non-Small Cell Lung Cancer (NSCLC)

Prior studies have shown that the human lung can extensively metabolize estrogen to reactive catechols. Of greatest concern is 4-hydroxyestrogen (4-OHE), a putative carcinogen, produced by cytochrome P450 1B1 (CYP1B1). In contrast, CYP1A1 metabolizes parent estrogens to 2-hydroxyestrogen (2-OHE), which are less reactive and converted to derivatives that may be antiproliferative. Data from this study group strongly suggest that 4-OHE contributes to lung tumorigenesis, though its role in driver-mutated NSCLC has not been investigated. This study assessed estrogen metabolite profiles in EGFR-mutated and ALK-mutated NSCLC patients as compared to cancer-free subjects. Advanced-stage NSCLC patients with tumors that possessed either an EGFR (n = 14) or ALK (n = 8) mutation and cancer-free subjects (n = 17) were recruited from Fox Chase Cancer Center. Tumor mutation status of NSCLC patients was determined by tissue biopsy. All study participants were 50 years of age or older to circumvent any confounding influence of young age or premenopausal status on estrogen levels. NSCLC patients included 14 females and 8 males. Cancer-free subjects serving as controls were never-smoking women. Urine specimens were collected from study participants and urinary estrogen