mechanism that emerges. Using RNA-seq data, we searched for epigenetic regulators that might be mediating the differentially expressed genes in the resistant cells. This analysis revealed that the chromatin remodeling protein SMARCA4/BRG1 is required for maintenance of the resistant phenotype in one of the models as knockdown of BRG1 sensitized cells to osimertinib. Further analysis revealed that SMARCA4 is stabilized in TKI-resistant cells, thus leading to TKI resistance. Finally, immunohistochemistry (IHC) examination of a collection of TKI-resistant patient-derived xenografts (PDXs) revealed higher levels of SMARCA4 expression in TKI-resistant tumors without on-target EGFR-dependent resistant mechanisms. To further elucidate the role of SMARCA4, we are currently performing ATAC-seq experiments that involve epigenetic changes in tumors are likely to be increasingly observed. Our studies offer insights into the mechanisms that underlie such resistance that could lead to new therapeutic possibilities for these tumors.

B31
Development of Multicell-Type Organoid Cultures for Preclinical Studies of Immunotherapeutics for Lung Cancer

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Introduction/Purpose of Study: Macrophages are key regulators of the immune landscape within the tumor microenvironment (TME). The plasticity of macrophage phenotypes in the TME has previously been correlated with prognosis within non-small cell lung cancer (NSCLC). Depending on their phenotype, macrophages in the TME can secrete protumor cytokine and chemokines, ultimately suppressing the function of other immune cells in the TME. The purpose of our study was to explore the ability of individual NSCLC preclinical models to alter macrophage phenotype in organoid cultures and to relate effects on macrophages to the molecular characteristics of different NSCLCs. We hypothesized that immune suppression occurs through tumor-secreted signaling molecules, and if blocked, macrophage suppression can be alleviated, resulting in a better antitumor immune response. Experimental Procedures: We developed an in vitro organoid coculture system (NSCLC tumor cells, human cancer-associated fibroblasts, CAFs, and mouse macrophages) to interrogate cancer cell features causing heterogeneity of macrophage phenotypes across a panel of NSCLCs. We measured (with 4-7 replicates for each NSCLC): mRNA expression in mouse macrophages with a panel of qPCR probes for important macrophage-related genes (Arg, Nos2, Il1b, Il-6, Chil3, Socs3), and in selected cases whole-genome RNAseq; and protein expression using cytokine arrays measuring expression of 40 inflammatory cytokines. Positive controls were stimulation with LPS and IL-4. Summary of New Data: Using our platform, we characterized 70 NSCLC patient-derived lines by their ability to alter mouse macrophage phenotype. We found: 1. the macrophage phenotypes induced by any one NSCLC were highly reproducible; 2. three major clusters of cancer polarized macrophage phenotypes: high Arg (immune suppressive), high IL-1beta (inflammatory) or high Socs3 (cGAS-STING pathway) expression; and 3. the major oncogenotypes (KRAS, TP53, STK11, EGFR, BRAF) have no correlation to the induced macrophage phenotype. We selected 7 NSCLC "exemplar" lines representing each of these 3 clusters for RNA sequencing (mouse genes) and cytokine array protein (human) profiling. Across all clusters we found: 1. suppression of macrophage endocytosis pathways and activation of scavenger receptor A (SRA) signaling (M2 immune suppressive phenotype); and 2. increased expression of human IL6, IL8, and MCP1 proteins, which have been implicated in suppressing innate immune tumor sensing. Analyses of differences between the 3 clusters is ongoing. Conclusions: Patient-derived NSCLC preclinical models have reproducible effects on altering macrophage phenotypes in organoid cultures. Three major classes of NSCLC initiated macrophage alteration, which are not linked to oncogenotype. Cytokines secreted by the NSCLCs appear responsible for these macrophage changes, and this system provides an experimental mechanism to systematically test each as potential therapeutic targets.

B32
Drug Sensitivity and Allele Specificity of First-Line Osimertinib Resistance EGFR Mutations

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Osimertinib, a mutant-specific third-generation EGFR TKI, is emerging as the preferred first-line therapy for EGFR mutant lung cancer. Despite initial responses in patients, however, resistance inevitably develops over time. In order to investigate mechanisms of resistance to first-line osimertinib, we modeled acquired resistance to this drug in transgenic mouse models of EGFRVII797S-induced lung adenocarcinoma and found that it is mediated largely through secondary mutations in EGFR—either C797S or L718V/Q. Analysis of circulating free DNA data from patients with EGFR mutant lung cancer revealed that L718Q/V mutations almost always arise in the context of an L858R driver mutation, and may occur at least as frequently as C797S in T790M-negative tumors. Therapeutic testing in mice revealed that both erlotinib and afatinib caused regression of osimertinib-resistant C797S-containing tumors, whereas only afatinib was effective in L718Q mutant tumors. Combination first-line osimertinib plus erlotinib treatment prevented the emergence of secondary mutations in EGFR. Finally, we report a patient with a tumor harboring both the L718V and L718Q mutations at resistance to first-line osimertinib who benefited from afatinib treatment. Our data identify specific secondary EGFR mutations as a major mechanism of acquired resistance to first-line osimertinib treatment and highlight potential strategies to overcome or prevent osimertinib resistance in vivo. Furthermore, these findings emphasize how knowledge of the specific characteristics of resistance mutations is important for determining potential subsequent treatment approaches.

B33
Short-Term Exposure to REV-5901 Decreases the Viability of Chemotherapy-Resistant Adherent Lung Cancer Cells and Floating Tumorspheres

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Toxicity to normal cells as well as specificity of the presence of highly resistant cancer cells, such as cancer stem-like cells (CS-LCs), are key factors that limit the efficacy of chemotherapy. In tumors, CS-LCs are often associated with chemoresistance and tumor relapse. In this study we used...