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 (PDx) 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 will offer insights into chromatin accessibility mediated by the protein in the resistant cells. In addition, we are assessing the protein levels of SMARCA4/BRG1 and the expression of SMARCA4 in TKI-resistant patient-derived NSCLC preclinical models have reproducible effects on altering macrophase phenotypes in organoid cultures. Three major classes 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 will offer insights into chromatin accessibility mediated by the protein in the resistant cells.

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 been previously correlated with prognosis within non-small cell lung cancer (NSCLC). Depending on their phenotype, macrophages in the TME can secrete protumor cytokines and chemokines, ultimately suppressing the function of other immune cells in the TME. The purpose of our study was to determine if individual NSCLC preclinical models are able to alter macrophase phenotype in organoid cultures and to relate effects on macrophages to molecular characteristics of different NSCLCs. We hypothesized that immune suppression occurs through tumor-secreted signaling molecules, and if blocked, macrophase 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 macrophase-related genes (Arg, Nos2, Il1beta, Il-6, Chil-3, 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 macrophase phenotype. We found: 1. the macrophase phenotypes induced by any one NSCLC were highly reproducible; 2. three major clusters of cancer polarized macrophase phenotypes: high Arg (immune suppressive), high Il1beta (inflammatory) or high Socs3 (cGas-STING pathway expression); and 3. the major oncogenotypes (Kras, Tp53, STK11, Egfr, Braf) have no correlation to the induced macrophase 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 macrophase 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 macrophase phenotypes in organoid cultures. Three major classes of NSCLC initiated macrophase alteration, which are not linked to oncogene gain. Cytokines secreted by the NSCLCs appear responsible for these macrophase 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 EGFR L858R-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/V 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
two models of highly resistant lung cancer cells: 1) Adherent cells (anchorage-dependent) growing under prolonged periods of serum starvation (PPSS) and 2) cells growing as floating (anchorage-independent) tumorspheres (FTs) to evaluate the effect of REV 5901. Cell viability was determined by the MTT or the CCK assay for adherent cells and FTs, respectively. Protein levels were determined by Western blots. Compared to cells growing under routine culture conditions (RCCs), cells growing under PPSS or as FTs were highly sensitive to REV. REV was able to selectively and irreversibly decrease the viability of cells growing under PPSS or as FTs within 24 h. Recovery experiments exposing cells to REV for 24 h followed by incubation in drug-free media for 48 h demonstrated that while PPSS as well as FTs cells were unable to recover, the noncancerous cell line Beas-2B growing under RCCs was not only less sensitive to REV but was also able to recover significantly. At the molecular level, REV induced significant changes in the expression of key proteins of the Wnt signaling pathway. Our data demonstrate that short treatment with REV can eliminate highly resistant cancer cells and that the Wnt signaling pathway may play a central role.

B34
Combination Therapy with Wnt Pathway Modulators to Override Chemoresistance in Human Lung Cancer Cells
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Background: The serum levels of DKK1, a negative regulator of the Wnt signaling pathway, have been reported to be elevated in cancer patients. DKK1 expression and association to chemoresistance has not been extensively investigated in cancer stem-like cells. In this study, by using cancer cell lines growing under anchorage-dependent conditions (Adherent cells; chemosensitive phenotype) as well as cells growing under anchorage-independent conditions (Floating Spheroids (FSs); chemoresistant phenotype), we evaluated a) the expression of DKK1 and the downstream effector of the Wnt signaling pathway β-catenin and b) the effect of iCRT-14 (a β-catenin inhibitor) and WAY-262611 (a DKK1 inhibitor) on the viability of cancer cells. Methods: FSs were grown in ultra-low attachment plates for 7 days. Cell viability were determined by the MTT or the CCK assay for adherent cells and FTs, respectively. Protein levels were determined by Western blots. Results: A549 and H460 adherent cells were sensitive to both iCRT-14 and WAY-262611. FSs generated from these cell lines were resistant to WAY-262611 but still sensitive to iCRT-14. FSs prepared from H460 cells were more sensitive to iCRT compared to FSs prepared from A549 cells. Western blot analysis from protein lysates prepared from H460 cells showed that iCRT-14 decreased the expression of β-catenin.
Conclusions and Future Directions: Our data demonstrate that a DKK-1 inhibitor in combination with a β-catenin inhibitor has the potential to eliminate lung cancer cells displaying varying degrees of chemoresistance. We are currently characterizing the mechanism by which this combination modulates the Wnt signaling pathway.

B35
Circulating Tumor-Associated Cells in Lung Cancers Are Resistance-Educated per Previous Chemotherapy Treatments
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Resistance to chemotherapy agents is frequently encountered in non-small cell lung cancers (NSCLC) and is largely undetected until symptomatic or radiologic detection of disease progression. Real-time monitoring of chemoresistance in NSCLC is an unmet clinical need. We describe a novel approach for real-time chemoresistance profiling (CRP) in NSCLC using peripheral blood circulating tumor-associated cells (CTACs), which are apoptosis-resistant cells of tumorigenic origin (EpCAM+, pan-Ck+, CD45±). Peripheral blood was collected from 145 patients with confirmed NSCLC including 102 therapy-naïve cases and 43 pretreated cases. Peripheral blood mononuclear cells (PBMCs) were harvested by centrifugation. CTACs were enriched using an epigenetically activated medium that eliminates normal (nontumorigenic) cells and confers survival privilege on apoptosis-resistant tumorigenic cells (CTACs). Surviving CTACs were confirmed by immunostaining (EpCAM, pan-Ck, CD45, TFF-1, Napsin-A). Harvested CTACs were treated in vitro with a panel of conventional cytotoxic agents and the fraction of surviving cells estimated to determine resistance profiles. Among the therapy-naïve NSCLC, innate chemoresistance towards any agent was observed in 51.7% of cases, which included resistance towards platinum agents in 37.8% of cases, microtubule targeting agents in 54.5% of cases, antimetabolites in 57.1% of cases, and topoisomerase inhibitor in 57.3% of cases. Among the pretreated NSCLC cases, resistance towards any agent was observed in 88.1% of cases, which included resistance towards platinum agents in 84.9% of cases, microtubule targeting agents in 85.1% of cases, antimetabolites in 96.7% of cases and topoisomerase inhibitor in 100% of cases, respectively. In vitro chemoresistance profiling of CTACs is a viable approach for real-time monitoring of innate and acquired chemoresistance. Higher chemoresistance in the pretreated population, as compared to the therapy-naïve population, indicates that CTACs are resistance-educated by prior treatments.

B36
Effects of Trifluoperazine and Its Analog on A549 Human Lung Cancer Cells
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Although there have been great advances in technology, molecular diagnosis, and therapeutics, lung cancer is still the leading cause of cancer-related mortality all over the world. Recently, some antipsychotic drugs have been shown to possess anticancer activity. Thus, the present study was designed to evaluate the anticancer effects of trifluoperazine (TFP), a commonly used antipsychotic drug, and its synthetic analogs on human lung cancer cell lines. To this end, effects of TFP and its selected analog on A549 cells were investigated in vitro as well as in vivo experiments. Synthetic TFP analogs were evaluated by the proliferation of A549 cells following drug treatment and compared to TFP. 3dc, a selected TFP analog, showed stronger anticancer effects in all the experiments than TFP. Further experiment showed that TFP and 3dc had activities to inhibit the anchorage dependent/independent colony formation, and migration of A549 cells. Western blot analysis revealed that 3dc affected the gene expression levels related to apoptosis and cell cycle. Flow cytometric analysis showed that 3dc induced sub-G1 and G1 population and reduced cell population in S and G2/M phase. Additionally, Annexin V/PI staining showed that 3dc increased apoptotic cell population. Moreover, 3dc increased DNA fragmentation. 3dc showed stronger anticancer effects in all the experiments than TFP. In addition, in vivo experimental models, 3dc also showed powerful anticancer effect in orthotopic lung cancer development than TFP. Thus, the present study demonstrates that a synthetic TFP analog has anti-lung cancer activity and provides a potential therapeutic candidate for lung cancer.