reactivation would be efficacious in this disease remains unknown. To model Rb pathway reactivation as a treatment strategy in lung adenocarcinoma and to shed light on its role in this disease, we established an RbXTR allele that enables Cre-dependent inactivation of Rb in developing tumors and allows Flp recombinase-inducible reactivation of Rb after tumors are established. In the Kraslox-stop-lox-G12D/+;p53fl/fl (KP) mouse model of lung adenocarcinoma, we show that Rb inactivation facilitates the bypass of two molecularly distinct barriers to tumor progression and dramatically accelerates malignant conversion and the development of metastatic disease. Although in the presence of Rb, malignant conversion requires amplification of the Raf/Mek/Erk (MAPK) signaling pathway beyond that normally activated by the Kras oncogene, we find that this requirement is abrogated when Rb is inactivated. Mechanistically, we identified Cdk2 as an important effector downstream of amplified MAPK signaling and that this activity suppresses Rb’s ability to limit the adenoma-to-carcinoma transition. Importantly, inactivation of Cdk2 reduces cell proliferation in Rb wild-type cells and confers sensitivity to Cdk4/6 inhibition in both human and mouse lung adenocarcinoma cell lines that were intrinsically resistant. Acquiring metastatic competency in Rb wild-type tumors is causally linked to epigenetic changes resulting in loss of lung lineage cell fate-determining transcription factors and concomitant derepression of factors normally restricted to embryonic cell types. However, inactivation of Rb uncouples the onset of metastatic competency from the loss of lung lineage factors, facilitates the early derepression of prometastatic factors, and significantly enhances metastatic proclivity. Finally, we demonstrate that reactivation of Rb in metastatic disease settings reprograms these tumors toward a less aggressive cell state and improves overall survival. Our study highlights an unappreciated role for Rb in regulating metastasis-promoting programs, and the potential of Rb restorative therapies to treat lung adenocarcinoma. Further, we suggest a renewed investment in the development of specific Cdk2 inhibitors may be necessary for Rb pathway reactivation in certain cancer types.

IA27
Restoring Capicua (CIC) Expression to Limit Lung Cancer Metastasis

R.A. Okimoto,1 Y. Lin,1 R. Ponce,1 W. Wu,‡ F. Breitenbucher,‡ M. Schuler,‡ T. Bivona∗1 University of California San Francisco, San Francisco, CA/US,∗2West German Cancer Center, Essen/DE

Metastasis accounts for >90% of cancer-related death, yet the molecular effectors that promote tumor dissemination remain poorly defined. Through development of an in vivo spontaneous lung cancer metastasis model, we recently revealed that genetic inactivation of the transcriptional repressor, Capicua (CIC), through genomic deletion or loss-of-function mutations can de-repress prometastatic effectors, ETV4 and MMP24, which is necessary and sufficient for metastasis. Beyond genetic inactivation, we find that hyperactive MAPK-ERK signaling leads to functional suppression of CIC through rapid protein degradation. Collectively, these data indicate that hyperactivation of MAPK signaling may enhance metastatic potential via ERK-driven suppression of CIC that promotes ETV4-MMP24-mediated metastasis. Hyperactive ERK signaling, a hallmark of lung adenocarcinoma, can lead to rapid CIC protein degradation, which may in part explain the high rate of metastatic recurrence and poor survival in early-stage lung adenocarcinoma patients who undergo curative intent surgery. Thus, decreased CIC protein expression in the context of hyperactive ERK signaling can potentially identify a subset of patients who may benefit from more aggressive antimetastatic therapeutic strategies. To explore this, we are testing MEK-ERK blockade as a pharmacologic strategy to restore CIC protein expression, thus limiting metastatic progression by dampening the ETV4-MMP24 prometastatic axis in cancers with genetically intact CIC. Collectively, through our studies we aim to repurpose anti-MEK and anti-ERK therapeutics to restore CIC expression to block lung cancer metastasis as a prelude to clinical trials.

IA30
Investigating and Overcoming Primary Resistance of EGFR and HER2 (ERBB2) Exon 20 Mutant NSCLC

J.P. Robichaux,1 Y.Y. Elamin,1 R.S.K. Vijayan,1 J. He,1 L. Hu,1 F. Zhang,2 A. Foteete,1 M. Pisegna,1 M.B. Nilsson,2 H. Sun,1 M.V. Negrao,1 X. Le,1 V.M. Raymond,2 R.B. Lanman,2 G.M. Frampton,1 V.A. Miller,1 A.B. Schrock,1 J.B. Cross,1 K. Wong,1 J.V. Heymach1 1The University of Texas MD Anderson Cancer Center, Houston, TX/US,2Guardant Health, Redwood City, CA/US,3Foundation Medicine, Cambridge, MA/US,4NYU Langone, New York, NY/US

EGFR and HER2 (ERBB2) exon 20 mutations occur in approximately 3.6% of NSCLC, and patients with tumors harboring these mutations have historically experienced poor response rates to clinically available clinical trials. In poor clinical responses in these patient populations, a deeper understanding of the effect of exon 20 mutations on the drug-binding pocket, sensitivity to available TKIs, and the genomic landscape of exon 20 mutations is greatly needed. We hypothesized that while exon 20 mutations are prevalent in NSCLC, these mutations also occur in other cancer types and alter the drug-binding pocket, resulting in de novo drug resistance across cancers. To test these hypotheses, we performed an analysis of eleven databases (N=212,000) to determine the prevalence of exon 20 mutations across cancer types and utilized in silico, in vitro, and in vivo models to investigate structural alterations induced by exon 20 mutations and identify effective inhibitors. Through this analysis we found that EGFR and HER2 exon 20 mutations occur in 28 different types of cancers, and that exon 20 mutations comprise 0.6% of all cancers, amounting to approximately 16,000 patients per year in the United States. Molecular modeling and molecular dynamics simulations showed that exon 20 insertions in both EGFR and HER2 reduced the overall volume of the drug-binding pocket, which correlated with decreased sensitivity to TKIs. Through in vitro screening using more than 14 EGFR TKIs, we found that poziotinib was the most potent inhibitor tested in EGFR (N=20) and HER2 (N=6) exon 20 insertion models with IC50 values of 1.5nM and 2.5nM, respectively. In our extensive panel of Ba/F3 cells engineered to express various EGFR/HER2 mutations, poziotinib was found to be the most selective TKI for the majority of EGFR and HER2 exon 20 mutants compared to WT EGFR (Mutant/WT IC50 ratio = 0.5). In vivo, poziotinib caused 70% and 85% reduction in tumor burden in PDX models of EGFR exon 20 mutant NSCLC models harboring EGFR S768dupSVD and EGFR H773insNPH mutations after 10 days of treatment. Using genetically engineered mouse models (GEMMs) of EGFR exon 20 mutant NSCLC, poziotinib reduced tumor volume in EGFR (D770insNPG) and HER2 (Y772dupYVMA) mutant tumors by 80% and 60%, respectively, after 4 weeks of treatment. In addition, we observed that low-dose poziotinib caused an upregulation in cell surface expression of HER2 exon 20 mutants and sensitized HER2 exon 20 mutant-expressing cells to T-DM1 treatment. To exploit this, we tested the combination of low-dose poziotinib (2.5mg/kg) and a single dose of T-DM1 (10mg/kg) in an HER2 mutant NSCLC PDX model (HER2 Y772dupYVMA). We observed complete tumor regression in 20/20 mice, compared to 2/9 mice receiving T-DM1 alone or 0/12 mice receiving low-dose poziotinib by day 15 (p<0.0001). Median progression-free survival (mPFS, tumor doubling from best response) was 3 days, 15 days, and 27 days in vehicle control,
low-dose poziotinib, and T-DM1 treated groups, whereas the mPFS had not been reached by day 45 in the combination-treated group. To validate these findings in an additional model of HER2 exon 20 mutant NSCLC, we tested low-dose poziotinib, T-DM1, and the combination in a GEMM of NSCLC harboring Y772dupYVMA. Recapitulating results seen in the PDX model, mice receiving either poziotinib or T-DM1 had on average of an 11% increase in tumor growth, whereas mice receiving the combination of low-dose poziotinib and T-DM1 had an average 47% reduction in tumor burden after four weeks. Lastly, to validate the activity of poziotinib, a phase II investigator-initiated trial (NCT03066206) testing poziotinib in patients with EGFR or HER2 exon 20 mutated NSCLC was opened. In the EGFR cohort, there was an objective response rate (ORR) of 43% and mPFS of 5.5 months in 44 evaluable patients. While the HER2 cohort is still ongoing, in the first twelve evaluable patients, there was an ORR of 42% and a mPFS of 5.6 months. Taken together, these data demonstrate that poziotinib is an effective and clinically active inhibitor for both EGFR and HER2 exon 20 mutant NSCLC and that poziotinib in combination with drug-antibody conjugates may have increased efficacy. Further, these studies demonstrate that clinical studies testing poziotinib alone and in combination with antibody-drug conjugates in other EGFR and HER2 exon 20 mutant cancers are warranted.

IA31

Genetic Contributors to Tumor Progression and Drug Resistance in EGFR Mutant Lung Cancer

K. Politi
Yale University, New Haven, CT/US

Targeted therapies have transformed the landscape for the diagnosis and treatment of metastatic lung cancer. These tumors are now routinely tested for the presence of mutations or rearrangements in specific oncogenic drivers that, if present, predict sensitivity to targeted therapies directed to the genomic alterations present. Genotype-directed therapies have improved outcomes in specific subsets of patients with metastatic lung cancer. Despite this success, targeted therapies are not curative and acquired resistance is a major impediment to cures for patients treated with these therapies. Moreover, there is heterogeneity in the durability and depth of responses between patients. A paradigm for the success of targeted therapies in lung cancer comes from Epidermal Growth Factor Receptor (EGFR) mutant lung cancer. Mutations in exons encoding the tyrosine kinase domain of EGFR confer sensitivity to tyrosine kinase inhibitors (TKIs), and several are currently approved for the first-line treatment of EGFR mutant lung cancer. Most recently, the third-generation TKI osimertinib was approved and is increasingly being used in the first line. However, we have very limited knowledge of the mechanisms of resistance to osimertinib given its recent adoption in the clinic. Without knowledge about resistance mechanisms, optimal post-osimertinib treatment strategies remain to be defined. We modeled acquired resistance to first-line osimertinib treatment in transgenic mouse models of EGFR/L858R-induced lung adenocarcinoma and found that it is mediated largely through secondary mutations in EGFR and identified therapeutic strategies to treat these tumors and prevent their emergence. Moreover, since EGFR mutant tumors in patients harbor additional genetic alterations beyond EGFR, many of them in tumor suppressor genes, we tested how the presence of co-occurring genetic alterations in tumor suppressor genes contributes to the progression and osimertinib sensitivity of the tumors in the mouse models of EGFR mutant lung cancer. Collectively, our findings highlight how genetically engineered mouse models of lung cancer, including those with complex genotypes, can be leveraged to study tumor progression and drug resistance in vivo.

IA33

Mechanisms of Small-Cell Lineage Transformation in Resistance to Targeted Therapies

Y. Inoue, W. Lockwood
BC Cancer, Vancouver, BC/CA

EGFR tyrosine kinase inhibitors (TKIs) are highly effective for tumors with EGFR mutations. However, resistance to these compounds remains a major issue, with the most frequent mechanism including the acquisition of a secondary mutation in EGFR (T790M) (1), followed by amplification of the hepatocyte growth factor receptor (MET) gene (2) and mutations in BRAF and PIK3CA genes (3,4). Epithelial–mesenchymal transition (EMT) and lineage transformation are less frequent but also prevalent, with up to 15% of cases with acquired resistance to first- and second-generation EGFR TKIs demonstrating histologic change from lung adenocarcinoma (LUAD) to small-cell lung cancer (SCLC) (4). Histologic plasticity as a mechanism of resistance is becoming increasingly prominent as other resistant mechanisms can now be successfully targeted (5). Currently, as with de novo SCLC, conventional platinum doublet chemotherapy is the standard of care for patients with treatment-induced SCLC. Unfortunately, this treatment often produces an incomplete and nondurable response followed by inevitable relapse within months, leading to poor patient outcomes (6). Thus, this mechanism of resistance will represent a major barrier towards the success of third-generation TKIs, and new strategies to prevent this lineage shift or to treat SCLC transformed tumors are urgently needed. Despite the increasing clinical importance, the biologic pathways regulating LUAD to SCLC transformation are poorly understood. Assessment of clinical samples has revealed that EGFR-mutant tumors universally lose EGFR protein expression upon SCLC transformation, despite still harboring EGFR mutation (7). Furthermore, the mutation spectrum of these transformed cases includes inactivation of the tumor suppressors RB and p53 in nearly all cases, mirroring de novo SCLC (7). However, accumulating experimental evidence has demonstrated that while necessary, dual inactivation of RB and p53 is not sufficient to cause SCLC lineage transformation in EGFR-mutated LUAD, suggesting that additional factors are required (7). MYC amplification and PIK3CA mutation have been proposed to potentially cooperate with RB/p53 loss to facilitate transformation (8), and specific epigenetic regulators may also provide the appropriate context for lineage reprogramming to occur. Despite this, no in vitro or in vivo models of SCLC transformation in EGFR TKI resistance have been developed, making it difficult to comprehensively explore the molecular events driving this lineage shift. Interestingly, there are clear differences between LUAD and SCLC regarding EGFR expression and gene alterations in MAPK pathway including EGFR/KRAS mutations: EGFR is usually not expressed (9) and EGFR/KRAS mutations are extremely rare in SCLC (10); in contrast, EGFR/KRAS play crucial roles in LUAD biology, including regulating differentiation in addition to proliferation (11). To date, however, no clear explanation has been given for these differences. We have recently shown that activation of MAPK signaling in SCLC leads to suppression of the neuroendocrine phenotype, including downregulation of the transcription factors NEUROD1, INS1M1, BRN2, and ASCL1—and transformation to an NSCLC-like state (12). Using this model system, we have begun to elucidate the key transcription factors and epigenetic changes that drive SCLC to NSCLC transformation in the hope that the same processes will also be involved in the clinically relevant scenario: SCLC transformation from EGFR mutant LUAD during TKI resistance. We suggest that only EGFR-mutant LUADs that do not reactivate MAPK signaling through secondary EGFR mutations or alterations in parallel kinase pathways (i.e., MET) during development of TKI resistance will be able to undergo SCLC lineage transformation, and that RB/p53 loss and epigenetic plasticity provide the permissive context in which this transformation can occur. Greater understanding of lineage transformation in LUAD will provide important insights in terms of