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 Rb<sup>xTR</sup> 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 Kras<sup>lox/lox-Stop-Lox-G12D/+;p53<sup>xTR/lox</sup> (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 tumors toward a less aggressive cell state and improves overall survival. 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**

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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 therapies due to 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 (N¼212,000) 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 (N¼20) and HER2 (N¼6) exon 20 insertion models with IC50 values of 1.5mM and 2.5mM, 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.