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 evaluable patients. While the HER2 cohort is still ongoing, in the testing poziotinib in patients with EGFR or HER2 exon 20 mutated poziotinib, a phase II investigator-initiated trial (NCT03066206) warranted.

Testing poziotinib alone and in combination with antibody-drug conjugates may have increased EGFR and HER2 exon 20 mutant NSCLC and that poziotinib in combination with drug-antibody 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

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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

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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 NEUR0D1, INSM1, 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 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 NEUR0D1, INSM1, 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
IA34
The YAP/FOXM1 Axis Regulates EMT-Associated EGFR Tyrosine Kinase Inhibitor Resistance and Increased Expression of Spindle Assembly Checkpoint Components

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While EGFR mutant NSCLC patients are initially responsive to EGFR targeted therapies, resistant disease inevitably emerges. In nearly half of resistance cases, tumors lack secondary EGFR mutations such as T790M and are refractory to 2nd- and 3rd-generation EGFR tyrosine kinase inhibitors (TKI). We and others have also observed that EGFR-independent resistant tumor cells may undergo a histologic and functional transformation through epithelial-to-mesenchymal transition (EMT) (Byers et al., 2013; Chung et al., 2011; Uramoto et al., 2010; Zhang et al., 2012), which can occur concurrently with other genomic alterations. The lack of treatment regimens with efficacy against EGFR-independent EGFR TKI resistance remains a major clinical challenge. We investigated transcriptomic and proteomic alterations that occur in NSCLC cells with acquired resistance to EGFR TKIs that occurs independent of EGFR and c-Met and screened >1,300 compounds to identify targetable vulnerabilities. T790M-negative EGFR TKI resistance was associated with evidence of a mesenchymal transition along with increased activation of the YAP/FOXM1 transcriptional program and a broad-spectrum multidrug resistance phenotype. EGFR TKI resistant cells displayed increased expression of spindle assembly checkpoint (SAC) proteins PLK1, Aurora kinases, survivin, and KSP, and expression of these proteins was dependent on the YAP/FOXM1 axis. Consistent with recent reports (Bertran-Alamillo et al., 2019; Shah et al., 2019), EGFR TKI resistant cells were found to be sensitive to aurora kinase inhibitors. We further determined that EGFR TKI resistant cells were likewise highly sensitive to inhibitors of components of the spindle assembly checkpoint (SAC) pathway including PLK1, KSP, and survivin, and treatment with these agents resulted in the accumulation of cells in the G2/M phase of the cell cycle and mitotic catastrophe. Using a patient-derived model of T790M negative EGFR TKI resistance, we observed that treatment with SAC component inhibitors, alisertib, ispinesib, or volasertib significantly inhibited tumor growth compared with vehicle-treated tumors. Analysis of NSCLC clinical data revealed that FOXM1 expression correlated with expression of SAC components including PLK1, Aurora kinases, KSP, and survivin. Moreover, in EGFR mutant NSCLC patients, high FOXM1 expression was associated with a worse clinical outcome compared to EGFR mutant NSCLC patients with low expression of FOXM1. In resistant models, targeting of YAP reduced FOXM1 expression and expression of SAC components. In conclusion, we provide novel insights into the molecular alterations associated with EGFR TKI resistance and demonstrate that upregulation of SAC components in EGFR TKI resistant cells occurs through the activation of the YAP/FOX1 pathway. These results support the future targeting of these pathways in NSCLC patients with EGFR-independent resistance to EGFR-targeted agents.