IA24

Targeting DLL3 in Small-Cell Lung Cancer with Novel Modalities

J.T. Poirier
New York University Langone Health, New York, NY/US

Delta-like ligand 3 (DLL3) is a single-pass transmembrane Notch ligand that interacts with full-length, unprocessed NOTCH1 in the Golgi apparatus, inhibiting the pathway in cis. DLL3 is selectively overexpressed in the subtype of small-cell lung cancer (SCLC) driven by the transcription factor ASCL1 (SCLC-A) that accounts for 70% percent of diagnoses (95% CI [60–79%]) (1). In one study immuneactivity was observed in 1,040/1,363 (70.4%) of SCLC specimens, consistent with this incidence (2). Overexpression of DLL3 leads to low-level cell surface expression of the protein on the order of 10,000 proteins per cell while expression in normal tissues is restricted to intracellular compartments: the same study demonstrated only low to moderate cytoplasmic or nuclear immunoreactivity in normal adult tissues (3). High expression of DLL3 has also been reported in low-grade glioma (4,5), neuroendocrine prostate (6), and occasionally in other cancer types when neuroendocrine features are present (7,8). The exclusively selective expression of surface DLL3 on cancer cells presents an excellent target for a variety of therapeutic strategies. Rovalpituzumab tesorine (Rova-T; SC16LD6.5) is an antibody-drug conjugate consisting of a monoclonal antibody targeting DLL3, a cathepsin-cleavable linker, and a pyrrolobenzodiazepine (PBD) warhead (4). The first-in-human clinical trial of Rova-T in recurrent SCLC demonstrated encouraging activity despite often severe side effects attributable to the PBD warhead (9); however, the phase 2 TRINITY study showed a disappointing 16% objective response rate while reporting a similar toxicity profile (NCT02674568). Subsequently, the phase 3 TAHOE study was halted due to shorter overall survival in the treatment arm. A phase 3 trial of Rova-T in the maintenance setting (MERU) was terminated at the interim analysis due to lack of survival benefit (NCT03033511). AbbVie has discontinued development of Rova-T. Other DLL3-targeting therapies under active investigation include the bispecific T-cell engager (BITE) AMG757 (NCT03319940) and a chimeric antigen receptor CAR-T AMG119 (NCT03392064). These agents have shown significant antitumor activity in preclinical models of SCLC; however, AMG119 required direct delivery of the engineered T cells for activity. AMG575 was therefore the more potent of the two molecules and may be better suited to overcome known barriers to CAR-T activity in solid tumors. Alternative strategies remain under exploration including the use of 89Zr-SC16, a PET radiotracer, for in vivo imaging and as a companion diagnostic to optimize the selection of patients for treatment with DLL3-directed therapeutic agents. 89Zr-labeled-SC16 antibody successfully delineated normal tissue from subcutaneous and orthotopic SCLC tumor xenografts. Radiotracer accumulation in tumors was directly correlated with the degree of DLL3 expression and also correlated with response to SC16LD6.5 therapy in SCLC patient-derived xenograft models. On the basis of these preclinical results, an investigator-initiated first-in-human phase 1/2 clinical trial of 89Zr-SC16 was recently opened to determine the safety and feasibility of immunePET imaging of DLL3 in patients with small-cell lung cancer. References: 1. Rudin CM et al. Molecular subtypes of small cell lung cancer: A synthesis of human and mouse model data. 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IA25

Adaptive Determinants of Metastatic Progression in Lung Adenocarcinoma

S. Adua, E. Wingrove, K. Patel, Z. Liu, D. Nguyen
Yale School of Medicine, New Haven, CT/US

The central nervous system (CNS) is a major site of treatment refractory metastases from lung cancers, yet deciphering the mechanisms of brain relapse remains a challenge because of the complexity of the brain tumor microenvironment (TME) and the perceived pharmacologic limitations of systemic therapies. To define the molecular landscape of brain metastases in situ, we developed a bulk RNA sequencing-based approach (BMX-seq), which leverages the transcriptome of tumor xenografts and effectively distinguishes tumor cell and stromal gene expression with increased accuracy and sensitivity. BMX-seq analysis was also integrated with single-cell profiling of distinct metastasis cell populations. In models of metastatic non-small cell lung cancer, we demonstrate that tumor cells in the brain exhibit an enhanced capacity for resistance to targeted therapies, despite strong brain penetration of drug. Accordingly, BMX-seq reveals shifts in cytoskeletal signaling, metabolic stress, and neuronal-like lineage programs in tumor cells as they adapt to the TME and the reciprocal neuroinflammatory response of the stroma. Several transcriptional hallmarks of metastasis are identified that are specific to particular regions of the brain and confirmed in syngeneic models and patient biopsies. Finally, certain epigenetic alterations can be reversed, while others are features of selected tumor cell populations. Despite recent improvements in the pharmacologic properties of targeted therapies, drug resistance in the CNS still develops. Our results suggest that adaptive epigenetic responses to the brain TME not only promote malignant outgrowth but also precondition disseminated tumor cells for subsequent therapeutic responses.

IA26

Stage-Specific Roles of RB Constrain Tumor Progression, Lineage Fidelity, and Metastasis

D.M. Feldser
University of Pennsylvania, Philadelphia, PA/US

Mutations in the Rb tumor suppressor pathway are a hallmark of cancer and a prevalent feature of lung adenocarcinoma. Additionally, recent clinical successes with cyclin-dependent kinase inhibitors have reinvigorated interest in reactivating the Retinoblastoma (Rb) pathway to treat lung adenocarcinoma and other tumor types. Remarkably, though, Rb’s role in suppressing lung adenocarcinoma remains unclear and whether Rb pathway
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>KTR</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<sub>Cre</sub>-Step-Lox-<sub>G12D</sub>/+; p53<sup>fl/fl</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 lung adenocarcinoma, can lead to rapid CIC protein degradation, which is mediated by ERK-driven suppression of CIC that promotes ETV4-MMP24 prometastatic axis in cancers with genetically intact CIC.

IA30

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

J.P. Robichaux,1 Y.Y. Elamín,1 R.S.K. Vijayan,1 J. He1, L. Hu1, F. Zhang2, A. Poteete1, M. Pisegna1, M.B. Nilsson2, H. Sun3, M.V. Negro4, X. Le4, V.M. Raymond4, R.B. Lannan4, G.M. Frampton4, V.A. Miller4, A.B. Schrock4, J.B. Cross4, K. Wong4, J.V. Heymach4

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 drugs. 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.