ABSTRACTS

POSTER SESSION B

B01
Active YAP as a Functional Marker of Drug-Tolerant Persister Cells in EGFR-Mutant and ALK Fusion-Positive NSCLC

F. Haderk,1 C. Fernández-Méndez,2 K.N. Shah,1 W. Wu,1 J. Guan,1 J. Rotow,3 D. Allegakoen,1 V. Olivas,3 S. Bandyopadhyay,1 C. Kuo,4 F. Haderk,1 C. Fernández-Méndez,2 K.N. Shah,1 W. Wu,1 J. Guan,1 J. Rotow,3 D. Allegakoen,1 V. Olivas,3 S. Bandyopadhyay,1 C. Kuo,4 T. Bivona1 Targeted therapies against clinically actionable oncogenic drivers in lung adenocarcinoma have significantly improved survival of cancer patients, but durable responses are limited due to the emergence of drug resistance. Resistance development is often characterized by the retention of a small subpopulation of cancer cells under drug treatment and their evolution from non-/low-proliferative residual disease to an aggressively growing resistant tumor. Most importantly, drug-tolerant persister cells have been identified as a reservoir for a multitude of drug resistance mechanisms and thus, their characterization and the development of rational combinatorial treatment may delay or prevent resistance development and improve treatment outcome for cancer patients. Using a multitude of in vitro models such as cell culture models and patient-derived organoids, we characterized signaling and transcriptional changes in drug-tolerant persisters. We identified YAP nuclear relocalization and its increased transcriptional activity as a key marker of persisters derived from EGFR-mutant and EML4-ALK fusion-positive specimens under third-generation TKI treatment. Image analysis of cells genetically engineered via CRISPR-Cas9 to express endogenously labeled YAP-mNeonGreen validated these results. Moreover, we were able to prove the functional relevance of YAP activation in drug persistence by overexpressing active mutants of YAP that are lacking inhibitory Hippo phosphorylation sites. The latter resulted in increased nuclear levels and transcriptional activity of YAP and mediated significantly reduced cell death under high-dose drug treatment in different cell line models. Using RNA sequencing, we show a clear evolutionary path from drug-sensitive parental cells to drug-tolerant persisters and long-term derived drug-acquired resistant cells. We are currently profiling vulnerabilities of drug-tolerant EGFR-mutant and EML4-ALK fusion persisters using genetic and pharmacologic approaches. In conclusion, YAP activation is a functional marker of EGFR-mutant and EML4-ALK fusion persisters derived under high-dose drug treatment with third-generation TKIs. Targeting YAP activation either on the level of upstream signaling input, its relocalization between cytoplasm and nucleus, or its action as transcriptional coactivator may represent a promising combinatorial treatment approach to limit resistance development and improve patient survival in lung adenocarcinoma.

B02
The GSK3 Signaling Axis Regulates Adaptive Glutamine Metabolism in Lung Squamous Cell Carcinoma

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B03
JNJ-61186372, an Fc Effector Enhanced EGFR/cMet Bispecific Antibody, Induces EGFR/cMet Downmodulation and Efficacy Through Monocyte and Macrophage Trogocytosis


Small-molecule tyrosine kinase inhibitors (TKIs) have become standard of care in EGFR-mutated NSCLC, but acquired resistance invariably develops due to new mutations in EGFR and activation of compensatory pathways such as cMet. JNJ-61186372 (JNJ-372) is an anti-EGFR and cMet bispecific low-fucose antibody (huIgG1) with enhanced Fc function designed to target tumors with activated EGFR and cMet signaling through a novel mechanism of action. An ongoing first-in-human study to assess the safety and efficacy of JNJ-372 in patients with advanced, treatment-refractory NSCLC revealed JNJ-372 to have clinical activity in patients with diverse EGFR-mutated NSCLC, including tumors with EGFR mutations (Exon20, T790M, C797S) resistant to TKIs and those resistant due to MET amplification. Despite observing potent antitumor activity of JNJ-372 in EGFR mutant xenograft models, only modest antiproliferative effects were observed in NSCLC cell lines in vitro. We also found that the Fc inactive version (IgG2sigma) of the EGFR/cMet antibody was significantly impaired in its ability to inhibit tumor growth in mice compared to the Fc enhanced JNJ-372. The IgG2sigma variant also reduced the ability of the bispecific antibody to mediate down-regulation of EGFR and cMet signaling. These observations suggested that the interaction of the Fc domain of the antibody with the Fcgamma receptors on innate immune cells may play a crucial role in the mechanism of action of JNJ-372. We performed a comprehensive assessment of the Fc effector functions of JNJ-372, including effects on EGFR and cMet levels, downstream signal transduction, and role in mediating antitumor activity. Using cancer cell lines in vitro, the
addition of isolated human immune cells (PBMCs) significantly enhanced JN-372-mediated EGFR and cMet downregulation, and dose-dependent tumor cell killing. Through depletion or enrichment of specific immune cell types, we demonstrated that monocytes and/or macrophages are necessary and sufficient for JN-372 Fc interaction-mediated EGFR/cMet downmodulation and that macrophages are required for in vivo efficacy. Finally, through imaging studies tracking labeled JN-372, we visualized monocyte/macrophage-mediated trogocytosis. Collectively, these data demonstrate a novel Fc-dependent mechanism of action of JN-372 and support the continued clinical development in patients with aberrant EGFR and cMet signaling.

B04 Activity of Larotrectinib in Tropomyosin Receptor Kinase Fusion Lung Cancer

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Background: Tropomyosin receptor kinase (TRK) fusions involving neurotrophic receptor tyrosine kinase (NTRK)1, NTRK2, and NTRK3 genes occur in a range of tumor types. Larotrectinib, the first FDA-approved highly selective TRK inhibitor, has demonstrated an overall response rate (ORR) of 75% by independent central review across a broad spectrum of tumors that harbor NTRK gene fusions (Drilon et al., NEJM 2018;378:731–9). Here we report updated data on the lung cancer patients who have been treated with larotrectinib.

Methods: Patients with non-small cell lung cancer (NSCLC) from two clinical trials (NCT02122913 and NCT02576431) with TRK fusion cancer were included in this analysis. Larotrectinib (100 mg, twice daily) was administered on a continuous 28-day schedule until withdrawal, unacceptable toxicity, or disease progression. Here we report responses assessed by investigator (INV) per RECIST v1.1.

Results: As of February 19, 2019, 12 patients with metastatic lung adenocarcinoma were enrolled. Median age was 49 years (range 25–76). Nine patients had fusions involving NTRK1 and diverse fusion partners: TPM3 (n=2), SQSTM1 (n=1), IRF2BP2 (n=2), TPR (n=1), CD74 (n=1), and EPS15 (n=2). Three patients had fusions involving NTRK3 (fusion partner: SQSTM1 [n=2] and ETV6 [n=1]). Eleven patients had prior systemic therapy (six patients had three or more prior therapies) with best responses on last prior therapy being one partial response, four with stable disease, three progressive disease, and four unknown, unfavorable, or not applicable. All 12 patients were evaluated for response to larotrectinib as per INV assessment. ORR was reported in nine patients (75%) with one complete response, and eight partial responses with one partial response pending confirmation. The median time to response was 1.8 months. There were three patients with stable disease. Six of the 12 patients had brain metastases at the time of study enrollment, and the ORR in those six patients was 67%. The overall duration of response by INV ranged from 3.9+ months to 25.9+ months; the median duration of response not reached. One patient continued receiving treatment post-progression. Two patients discontinued treatment due to disease progression and one discontinued due to disease progression in nontarget lesion. Larotrectinib was well tolerated, with treatment-related adverse events being predominately grade 1–2.

Conclusions: Larotrectinib is highly active in patients with advanced lung cancer harboring NTRK gene fusions, including those with central nervous system metastases, with a favorable safety profile. These results support the use of larotrectinib in TRK fusion lung cancer.

B05 Identifying SCLC Vulnerabilities Using Phenotypic Chemical Screens

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Small-cell lung carcinoma (SCLC) is an aggressive neuroendocrine cancer in which few actionable mutations have been uncovered in the last 30 years. With the goal of identifying chemically tractable proteins essential for SCLC viability, our lab has performed a phenotypic high-throughput small-molecule screen (HTS) in collaboration with the UT Southwestern Core Facility using a library of 200,000 drug-like compounds. We used a SCLC cancer cell line derived from a p53;Rb1 genetically engineered mouse model that recapitulates cardinal features of the human disease. By counter-screening against a panel of murine cancer cell lines (NSCLC, papillary thyroid cancer, and rhabdomyosarcoma), we identified 51 SCLC-selective toxins exhibiting at least 5-fold selectivity for SCLC cancer cells compared to the panel of non-SCLC cell lines. We hypothesized that identifying the target of these molecules will allow the discovery of important vulnerabilities for SCLC. To uncover the mechanism of action of these 51 SCLC-selective toxins, we are using two orthogonal approaches. One strategy uses forward genetics to identify compound resistant alleles that impair compound-target interaction. We recently demonstrated that engineering mismatch repair (MMR) deficiency into murine SCLC cancer cells (using CRISPR/Cas9 to silence Msh2) led to hypermutation and enabled the acquisition of compound resistant alleles for three anti-cancer compounds with known mechanisms of action. We used these cells to identify compound resistant alleles that co-occur in multiple resistant clones that emerge following selections in cell culture of these anticancer compounds. We are also using medicinal chemistry as another approach to elucidate the mechanism of action of the SCLC-selective toxins. In collaboration with the De Brabander lab, three from the 51 SCLC-selective toxins were chosen for suitability for medicinal chemistry efforts due to their high selectivity (over 10-fold). We are performing structure-activity relationship studies to optimize potency and selectivity as well as target-ID studies. We also developed analogs harboring cross-linkable moieties with the goal of being able to efficiently enrich candidate proteins that can be identified by mass spectrometry.

B06 Time-Resolved RNA-Seq Identifies Transient Gene Expression Changes Following Initial Chemotherapy Challenge in Small-Cell Lung Cancer


Small-cell lung cancer (SCLC) comprises about 15% of all lung cancer and exhibits a remarkably aggressive clinical course, with early metastasis, rapid development of chemoresistance, and an overall survival of 6%. While standard-of-care combination chemotherapy with platinum-based agents and etoposide elicits dramatic responses following initial treatment, chemoresistant disease develops rapidly and contributes to the poor mortality rate in this disease. Transcriptional changes and underlying epigenetic changes have increasingly been recognized in the development of chemoresistance across different cancer types and in response to a variety of neoplastic agents. Indeed, a notable study in SCLC identified a role of the enhancer of