clinical outcomes. Different detection assays for METex14 using various platforms have yielded mixed results across studies. It is imperative to utilize reliable and validated molecular assays to identify pts to be treated with METL. RNA-based detection of METex14 is considered the gold standard, since this assay measures the direct result of deletion of exon 14 event regardless of underlying genomic events. DNA-based next-generation sequencing (NGS) must detect genomic alterations within MET exon 14 and adjacent intronic regions that alter a splicing site or delete the whole MET exon 14. **Methods:** The GEOMETRY mono-1 study evaluated the efficacy and safety of capmatinib in pts with EGFR-wt, ALK-neg, NSCLC harboring METex14. This retrospective analysis compared DNA-based NGS with RNA-based RT-PCR in detecting METex14 in the GEOMETRY mono-1 study. Eligible METex14-mutated pts confirmed by RT-PCR qualitative assay using RNA extracted from baseline formalin-fixed, paraffin-dipped (FFPE) tissue samples were assigned to cohorts 4 (4; previously treated) or 5b (5b; treatment-naïve), independent of MET amplification status. Retrospectively, METex14 positive and prescreen failed negative baseline FFPE tissue samples were tested using a hybrid capture DNA-based NGS assay (FoundationOne®). The METex14 positive pts by DNA NGS were defined as having MET alterations that are predicted to lead to MET exon 14 skipping. **Results:** Of the 97 enrolled pts from the METex14-mutated cohorts C4 (n=69) and C5b (n=28) of the GEOMETRY mono-1 study, 73 pts had baseline tumor biopsy samples (C4, n=53; C5b, n=20) that met the requirements for the FoundationOne® NGS assay (minimum requirements: tissue volume ≥0.1 mm³, DNA yield ≥22 ng, percent tumor nuclei ≥10%). The FoundationOne® NGS assay identified METex14 in 72 of 73 positive pts, with a concordance of 99% to the qualitative RT-PCR test used previously for testing. The variants detected included 41 unique canonical alterations that are predicted to lead to METex14. 1 pt with only a noncanonical METex14 rearrangement was not included in the concordance analysis and reported stable disease. None of the RT-PCR negative patients were reported as positive by NGS. **Conclusions:** Detection of MET exon 14 skipping events can be achieved by sequencing DNA or RT-PCR. A very high concordance was observed between DNA-based hybrid-capture NGS and RNA-based RT-PCR in the detection of METex14 in FFPE tumor tissue from advanced NSCLC pts. NGS enables parallel detection of actionable alterations without sequential testing by single gene. Furthermore, this technique provides a comprehensive genomic profile to inform treatment plan and any potential mechanisms of resistance.

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**B13**

**Selectively Targeting Lung Cancer with a Novel Small Molecule that Induces Lethality Through Dual Inhibition of Disulphide Reductases**

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Lung cancer (LC) is the leading cause of cancer-related deaths worldwide, mainly due to the lack of effective therapies. Through a screen of 189,290 small molecules, the compound LC Screen 3 (LCS3) that inhibits the growth of LC cells but not normal cells was identified. LCS3 is structurally unique and its mechanism of action is unknown. Twenty-six lung adenocarcinoma cell lines were screened, and all but two were found to be sensitive to LCS3 (IC50>5μM). Transcriptome and proteome profiling by microarray and SILAC, respectively, suggest that LCS3 strongly induces redox imbalance. The top four predicted upstream transcriptional regulators of LCS3-induced RNA expression changes all have key functions in the response to oxidative stress (NRF2, MAKP, CEBPB, and BACH1). We confirmed LCS3 induces NRF2 activation through Western blot and flow cytometry analyses using a stably expressed antioxidant response element GFP reporter. In addition, flow cytometry with oxidative stress sensor H2DCFDA detected reactive oxygen species (ROS) induction by LCS3 only in sensitive cell lines. Notably, the most resistant LC cell line, NCI-H1648, has biallelic functional loss of KEAP1, which negatively regulates NRF2-mediated cytoprotective gene expression. We confirmed that NCI-H1648 has low basal ROS and high basal expression of genes that support redox balance. KEAP1 silencing and antioxidants including N-acetylcysteine partially rescued LCS3-induced cytoxicity, which further implicates oxidative stress in the mechanism of LCS3-induced cell death. To elucidate the molecular targets of LCS3, we applied thermal proteome profiling (TPP), which identifies thermally stabilized protein binders with proteome-wide coverage, and identified 47 proteins that are putative binders of LCS3. Of the 47 TPP hits, 8 are enzymes that function in redox homeostasis. Through in vitro enzymatic assays of the top TPP hits, we discovered that LCS3 inhibits glutathione disulfide reductase (GSR) and thioredoxin reductase 1 (TXNRD1) through reversible, uncompetitive inhibition at low micromolar IC50s. In silico molecular docking suggests LCS3 interacts with the GSR homodimer interface, and our structure-activity relationship studies have identified the putative functional moiety on LCS3 necessary for both enzymatic inhibition and cellular toxicity. We found that Luperox, a direct-acting hydroperoxide source of ROS, sensitizes nonresponsive cells to LCS3, thus implicating ROS as a requirement for LCS3-mediated toxicity. We are currently investigating why nonresponsive cells are less dependent on the glutathione and thioredoxin pathways and how oncogenic transformation, and the inherent oxidative stress that coincides, confers sensitivity to dual disulfide reductase inhibition. Through this work, we

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**B12**

**FOXA2 Promotes the Growth of KRAS-Mutant Lung Tumors but Suppresses the Growth of EGFR-Mutant Lung Tumors in Vivo**

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**Background:** Using GEMM (Genetically Engineered Mouse Models), we showed that a lung-lineage transcription factor NKK2-1 promotes the growth of EGFR-mutant lung tumors but suppresses the growth of KRAS-mutant lung tumors in vivo (Maeda et al., JCI 2012), suggesting that such transcription factors expressed in the lung act as a context-dependent tumor promoter or suppressor. Here, we report the roles of a pioneer transcription factor FOXA2 expressed in lung epithelium in KRAS-mutant or EGFR-mutant lung tumors in vivo. **Methods:** Using doxycycline-regulatable GEMM expressing mutant KRAS or mutant EGFR along with FOXA2 in lung epithelium (CCSP-rtTA; otet-KrasG12D; otet-Foxa2 or CCSP-rtTA; otet-EGFR/L858R; otet-Foxa2), we assessed whether FOXA2 influenced the growth of KRAS-mutant or EGFR-mutant lung tumors in vivo. The number and size of lung tumors were analyzed by microCT. The histology of the lung tumors was further analyzed by H&E and immunohistochemistry. **Results:** FOXA2 induced an increase in volume but not the number of KRAS-mutant lung tumors associated with lung adenocarcinoma while FOXA2 reduced the volume and number of EGFR-mutant lung tumors in vivo. Phosphohistone H3 was increased in KRAS-mutant lung tumors but decreased in EGFR-mutant lung tumors by FOXA2. Caspase-3 was not affected. These results indicate that FOXA2 differentially influences the initiation and progression of lung tumor growth depending on the type of driver oncogenes (mutant KRAS vs. mutant EGFR) in part through proliferation but not apoptosis. **Conclusion:** Transcription factors NKK2-1 and FOXA2 function as yin and yang to affect the growth of KRAS-mutant or EGFR-mutant lung tumors.
B15
COP1 E3 Ligase Modulates Response to Oncogenic MAPK Pathway Inhibition

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Oncogenic activation of the RAS-MAPK pathway drives several cancers, including a majority of non-small cell lung adenocarcinomas (LAD). RAS-MAPK pathway is activated in lung adenocarcinomas via diverse genetic alterations in upstream receptor tyrosine kinases such as EGFR and ALK as well as in RAS, BRAF, MEK, and the RAS GTPase activating protein (GAP) and tumor suppressor, NF1. Therapeutically targeting components of the RAS-MAPK pathway can lead to initial tumor responses in many patients. However, very few patients show complete responses despite harboring the targeted RAS-MAPK pathway activating genetic lesion in the tumor. Responses and hence patient survival can be improved by better characterizing the molecular basis of response and resistance to therapies targeting the RAS-MAPK pathway in lung adenocarcinomas. To identify modulators of response to MAPK pathway inhibition in lung adenocarcinomas, we conducted genetic screens in BRAF-driven human lung adenocarcinoma cells. This identified the E3 ubiquitin ligase COP1/RFWD2 as a previously unknown genetic modifier in lung adenocarcinomas. We found that depletion of COP1 and members of its protein complex, as well as proteasomal subunits, confers resistance to RAS-MAPK pathway inhibition in patient-derived lung adenocarcinoma cells with oncogenic RAS-MAPK signaling. Intriguingly, oncogenic targets of COP1 include critical MAPK pathway effectors such as ETV1. Hence, we tested if depletion of COP1 protects those MAPK pathway effectors from the impact of RAS-MAPK pathway inhibitors. COP1 depletion had a substantial impact on the levels of these effectors in the presence of RAS-MAPK small-molecule inhibitors in lung adenocarcinoma cells. Furthermore, we found that co-depletion of these transcription factors resensitized COP1-depleted cells to MAPK pathway inhibition. Upon analyzing the transcriptomic and signaling changes, we found that low levels of COP1 facilitate survival of lung adenocarcinoma cells upon inhibition of the RAS-MAPK pathway by buffering the cells from the impact of the MAPK pathway inhibitor and thereby sustaining prosurvival pathways. Additionally, depletion of COP1 in in vitro derived models of resistance also resensitized them to MAPK pathway inhibition. This study has furthered our understanding of the molecular basis of tumor cell resilience during initial treatment as well as of secondary treatment resistance. We are examining if COP1 also modulates response to MAPK pathway inhibition in vivo and if levels of COP1 could be a biomarker for predicting response to RAS-MAPK pathway inhibitor therapy in patients.

B16
The ROS1 Cancer Model Project: A Unique Patient-Driven Partnership to Accelerate Research

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Background: ROS1 rearrangements (ROS1+) are found in a wide variety of cancer types but are relatively uncommon, occurring in 1-3% of lung, gastric, and ovarian cancers, as well as melanoma, cholangiocarcinoma, glioblastoma, and other tumor types. ROS1 has been studied primarily in lung cancer, where there are now several FDA-approved drugs to treat advanced ROS1+ lung cancer. The rarity of ROS1 fusions makes studying them more challenging, as patients are too geographically dispersed to support a traditional clinical research study. To address this challenge, the ROS1ders joined forces with a leading lung cancer advocacy organization, an international research consortium, industry, and leading academic investigators to focus efforts on this rare molecular subset of cancer. Method: The ROS1 Cancer Model Project currently consists of two studies supported by the Addario Lung Cancer Medical Institute’s research infrastructure and remote study capabilities. Patients are empowered to contact the study team directly and do not have to be seen at a specific site to participate in the studies and donate samples for research. Due to the sparsity of research tools available to study ROS1+ cancer, the first study focuses on creation of patient-derived xenograft (PDX) models while the second study supports creation of cell lines. The ROS1ders and GO2 Foundation for Lung Cancer have effectively utilized social media to connect with ROS1+ patients across the globe to educate them about the opportunity to participate in these ongoing research efforts. Both studies are currently open to ROS1+ patients located in North America. Results: The ROS1 Cancer Model Project has successfully demonstrated the feasibility and power of patient-driven research and cross-sector collaboration to implement an innovative study motivated by patient need. Since its launch, the project has effectively mobilized the international ROS1+ patient population to create new cancer models for this rare molecular subset. To date, over 30 patients have been screened, with five patients referred to the PDX study and eight patients referred to the cell line study. Together, these studies have led to the successful development of new murine and cell-line research tools and have resulted in a doubling of the preclinical models now available for ROS1 research. Conclusion: Through unique partnerships, the ROS1ders have accelerated the creation of new cancer models that will further researchers’ understanding of this rare molecular subset. The success of this collaboration highlights the power of patients in driving research and has laid the foundation for similar efforts by other patient groups. This effort is part of the larger Global ROS1 Initiative, which is working to address the ongoing needs of the international ROS1+ patient community.

B18
Structural Insight into Sensitivity and Resistance of RET Mutants to Selpercatinib (LOXO-292)

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Selpercatinib (LOXO-292) is a RET-selective protein tyrosine kinase inhibitor (TKI) designated as breakthrough therapy by the United States Food and Drug Administration. However, structural detail of its binding to RET was elusive. Protein tyrosine kinase targeted therapies often encounter resistance due to on-target mutations. Knowledge of TKI binding and resistant mutants is important for continuous TKI pipeline development and disease management. We have identified a panel of selpercatinib-resistant RET mutants in a preclinical model and determined the co-crystal structure of RET-selpercatinib complex to 2.06-Å resolution. Unlike vandetanib or nintedanib that insert into the gate, selpercatinib anchors one end in the front cleft and wrap around the other b site to participate in the studies and donate samples for research. Due to the sparsity of research tools available to study ROS1+ cancer, the first study focuses on creation of patient-derived xenograft (PDX) models while the second study supports creation of cell lines. The ROS1ders and GO2 Foundation for Lung Cancer have effectively utilized social media to connect with ROS1+ patients across the globe to educate them about the opportunity to participate in these ongoing research efforts. Both studies are currently open to ROS1+ patients located in North America. Results: The ROS1 Cancer Model Project has successfully demonstrated the feasibility and power of patient-driven research and cross-sector collaboration to implement an innovative study motivated by patient need. Since its launch, the project has effectively mobilized the international ROS1+ patient population to create new cancer models for this rare molecular subset. To date, over 30 patents have been screened, with five patients referred to the PDX study and eight patients referred to the cell line study. Together, these studies have led to the successful development of new murine and cell-line research tools and have resulted in a doubling of the preclinical models now available for ROS1 research. Conclusion: Through unique partnerships, the ROS1ders have accelerated the creation of new cancer models that will further researchers’ understanding of this rare molecular subset. The success of this collaboration highlights the power of patients in driving research and has laid the foundation for similar efforts by other patient groups. This effort is part of the larger Global ROS1 Initiative, which is working to address the ongoing needs of the international ROS1+ patient community.

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