AMG510 and MRTX849 have clinical activity in patients harboring a *KRAS*^{G12C} mutation, however, the durability of response is attenuated by the onset of multiple resistance mechanisms. Feedback-mediated activation of upstream receptor tyrosine kinases (RTK) in concert with the co-activation of the PI3K/AKT signaling pathway is proposed to bypass MAPK pathway dependence. SRC/FAK signaling promotes tumor cell survival by activation of the PI3K/AKT survival pathway. In addition, oncogenic KRAS induces secretion of various cytokines and growth factors by tumor cells leading to a tumor promoting microenvironment. Tumor cell cytokine secretion is driven, in part, though JAK2/STAT3 signaling. Therefore, combination of KRAS-G12C inhibitors with agents that simultaneously inhibit SRC, FAK, and JAK2 could suppress drug resistance as well as attenuate oncogenic stromal remodeling.

Material and methods: *In vitro* and *in vivo* models of KRAS^{G12C} cancer are used to evaluate KRAS-G12C inhibitor combinations with repotrectinib, a next generation ROS1/TRK/ALK inhibitor with SRC/FAK/JAK2 inhibitory potencies at clinically relevant exposures.

Results: The combination of repotrectinib and AMG510 potently inhibits activation of SRC, FAK, STAT3, AKT, and ERK in KRAS^{G12C} non-small cell lung cancer (NSCLC) cells and a xenograft tumor model at clinically relevant exposures. Repotrectinib/AMG510 suppresses more KRAS signaling nodes than AMG510 combinations with dasatinib, defactinib, or ruxolitinib and therefore affects a broader signaling network. Repotrectinib/AMG510 synergistically decreases KRAS^{G12C} NSCLC cell viability and induces apoptosis more robustly relative to either single agent treatments or AMG510 combinations with inhibitors that have a subset of repotrectinib activities. A rapid adaptive RTK feedback reactivation occurs in response to AMG510 which is suppressed by repotrectinib. Tumor cell cytokine secretion is suppressed by repotrectinib implicating tumor extrinsic effects. In the H358 KRAS^{G12C} xenograft tumor model, repotrectinib has single agent activity and enhances AMG510 efficacy in combination. Significant combination activity is observed in the KRAS-G12C inhibitor resistant LU11693 PDX tumor model. In a survival study using the H2122 xenograft tumor model, repotrectinib significantly prolongs survival for both moderate (10 mg/kg) and high dose (30 mg/kg) AMG510 treatment groups.

Conclusions: In preclinical studies, simultaneous inhibition of SRC/FAK/JAK2 by repotrectinib has a range of effects that synergize with KRAS-G12C inhibitor pharmacology and suppress mechanisms of resistance. Further investigation for the potential to increase the duration of response for KRAS-G12C inhibitor therapies is warranted.

Conflict of interest:
Ownership: All of the authors are employees of Turning Point Therapeutics.

148 Poster

Discovery proteomics detects expression trends associated with resistance to the most commonly used chemotherapies in esophageal adenocarcinoma

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Background: The rate of incidence of esophageal adenocarcinoma (EAC) is increasing faster than any cancer in the United States, and the survival rates have remained very low for decades, even in the current era of molecular medicine. Despite a dozen chemotherapeutic treatment options currently used for EAC, durable patient responses to anticancer therapies are hard to achieve in these cohorts. Indeed, a study in 2014 found no survival advantage for EAC patients treated with chemotherapy. A large-scale mass spectrometric experiment was designed to detect proteomic expression patterns that may contribute to the robust chemoresistance observed in EAC tumors.

Materials and Methods: Formalin fixed paraffin embedded (FFPE) tissue was cut at 10 microns with one 5μm section stained with H&E. Twenty EAC tumors and 20 normal esophageal samples were analyzed in this study. The serially sectioned unstained slides were microdissected with the guidance of a board-certified pathologist. Dissected cells were heated with proprietary buffers to break formalin cross-links, trypsinized, and reduced with DTT. All samples were analyzed by reverse-phase high-pressure liquid chromatography electrospray ionization tandem mass spectrometry (RP-HPLC-ESI-MS/MS) using a 6600 TripleTOF spectrometer. Overexpression was confirmed in a selected biomarker via immunofluorescence. Gene knockdown of the marker and subsequent cytotoxicity and proliferation (MTT) assays were completed using OE-33 cell lines.

Results: The expression trends of seven markers that are associated with resistance to cisplatin were detected ($P < 0.0001$). Two markers, which were downregulated significantly when comparing EAC tissue to normal squamous epithelium, are associated with decreased sensitivity to taxanes ($P < 0.0001$). The upregulation of two other markers, which overexpression is associated with increased resistance to 5-fluorouracil, were also discovered ($P < 0.0001$). The expression pattern of a selected marker was confirmed with immunofluorescence, and knockdown of the associated gene yielded increased cytotoxicity and decreased proliferation in OE-33 esophageal cancer cells.

Conclusions: The three most common classes of drugs used for EAC are platinum-based (95%), anthracyclines (63%), and taxanes (37%). Here we have discovered a network of consistently downregulated or overexpressed biomarkers that may contribute to broad resistance against these chemotherapies during the course of treatment for EAC. These findings demonstrate a clinical need for actionable molecular diagnostics and new targeted therapy options for EAC patients. Additional studies are required to confirm these markers as major players in the low efficacy of chemotherapy regimens against solid tumors of the esophagus.

Conflict of interest:
Ownership: Stella Diagnostics, LLC.

149 Poster

Secondary resistance to the PI3K inhibitor copanlisib in marginal zone lymphoma

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Background: PI3K kinase has a prominent role in the B-cell receptor signaling. Copanlisib, a pan-PI3K inhibitor with predominant selectivity to PI3Kα and PI3Kδ, is Food and Drug Administration (FDA) approved for the treatment of patients with relapsed or refractory follicular lymphoma, and it is currently under clinical development in other indolent lymphomas including marginal zone lymphoma (MZL). However some patients might eventually relapse because of acquired resistance and so a better understanding of resistance mechanisms is needed. Thus we generated MZL cell lines resistant to copanlisib which could help to design improved therapies.

Materials and Methods: Cells from VL51 were treated with no drug (parental, PAR) or high concentrations of copanlisib (IC90) until acquisition of resistance (RES). Sensitivity to vincristine ruled out multi-drug resistance and MTT assay after 3-weeks of drug-free culture confirmed stable resistance. Transcriptome profiling (RNA-Seq) and immunophenotypic analysis were performed in PAR and RES cells.

Results: RES models from VL51 cell line exhibited over 50-fold times higher IC50 s than PAR counterparts. The mechanism observed here might drive resistance to others downstream B-cell receptor inhibitors since sensitivity to other PI3K inhibitors such as duvelisib (50-fold) and idelalisib (5-fold), to the BTK inhibitor ibrutinib (15-fold) was decreased in RES. Transcriptome analyses of RES revealed overexpression of negative regulators of apoptosis (CD44, JUN), cytokine signaling (IL1A, IL1B, CXCR4), NFkB (LTA, TNF), MAPK (RASGRP4, RASGRP2) and JAK-STAT (STAT3, JAK3) signaling pathways; while genes involved in cell adhesion (ITGA4, ITGB1), antigen presentation (HLAs) and IFN response (PARP12, GBP6) were repressed in RES. Accordingly to the elevated expression of anti-apoptotic signaling genes, RES cells were resistant to the BCL2-inhibitor venetoclax, either as a single as in combination with copanlisib. Paired with transcript expression, RES exhibited increased surface expression of CXCR4 and repression of CD49d, CD20 and CD81 by flow-cytometry. Finally, combination of copanlisib with a CXCR4 inhibitor overcame resistance.

Conclusions: We have developed and characterized a preclinical model of secondary resistance to the PI3K inhibitor copanlisib in splenic marginal zone lymphoma. We have also identified novel potential targets, including IL1 and CXCR4, that are worth of further investigations. The current work provides new insights into the mechanisms of resistance to copanlisib and can lead to novel therapeutic approaches to overcome the resistance.

No conflict of interest.