**Background**

- Lung adenocarcinoma (LUAD) represents the most common subtype of lung cancer. Patients with KRAS-mutant LUADs (KM-LUADs) common to smokers display dismal clinical outcomes and resistance to targeted therapies.

- Our group recently identified alveolar intermediary cells (AICs) that arise early on post-tobacco carcinogen exposure in vivo and during AT2-mediated repair mechanisms. Notably, and prior to tumor onset, a subset of AICs harbored driver KRAS\(^{G12D}\) mutations which were later found in the resultant LUADs, and which comprise the same variants found in human smokers with KM-LUADs. These findings support a role for AICs as precursors for KM-LUAD pathogenesis (Fig 1a). Our ongoing studies are studying AICs at high resolution to identify targets for early prevention and/or interception of KM-LUAD pathogenesis, including specifically targeting KRAS\(^{G12D}\) mutation (Fig 1b).

- Mutated KRAS has been an elusive target prior to the discovery of a conserved switch II binding pocket among all KRAS proteins. A structure-based drug design identified MRTX 1133 as a potent and selective KRAS\(^{G12D}\) inhibitor in both in vitro and in vivo models (Fig 2). While previous testing utilized patient-derived xenograft tumor models and orthotopic pancreatic cancer models, we investigate the effectiveness of MRTX 1133 in murine lung cancer cells as a precursor to potential clinical prevention of KM-LUAD in vivo.

**Objectives**

- Confirm the ability of MRTX 1133 to specifically downregulate downstream products of KRAS\(^{G12D}\) in a murine LUAD cell line (mF471).

- Study the pharmacokinetics of murine KRAS\(^{G12D}\) inhibition by MRTX 1133 to determine the onset of action and minimal inhibitory concentration (MIC).

**Methods**

- **MTT Proliferation Assay:** mF471 cells were plated on day 1, treated with MRTX 1133 (0nM, 1nM, 10nM, and 100nM) and Epidermal Growth Factor (50 ng/mL) on day 2, and MTT proliferation assay measured on day 4 (Fig 3a). Each well contains 1000 cells.

- **Western Blot:** mF471 cells were plated on day 1, treated with MRTX 1133 (0 nM, 1nM, 10nM, and 100nM) and Epidermal Growth Factor (50 ng/mL) on day 3 for 1 and 3 hours before analysis (Fig 3b). 200 ug protein was loaded per lane.

**Results**

- Increasing concentrations of MRTX 1133 result in statistically significant decreases in cell viability when compared to control.

- MRTX1133 shows dose-dependent inhibition of Kras signaling at all time points tested. A significant reduction in key KRAS pathway signaling molecules were observed as early as 3 hours and with concentration as little as 1 nM.

- These results suggest that MRTX 1133 is a potent KRAS\(^{G12D}\) inhibitor which has the potential to inhibit murine Kras mutant lung cancer cell lines.

- Further experiments are warranted to assess the role of inhibiting KRAS\(^{G12D}\) during the early stages of LUAD pathogenesis in an in vivo model (Figure 4). Our group previously showed that mice with knockout of Gprc5a (Gprc5a\(^{-/-}\)) develop pre-malignant lesions (PMLs) and LUADs which were markedly accelerated with exposure to nicotine-specific nitrosamine ketone (NNK), a tobacco carcinogen that is casually linked to lung cancer in humans. Hence, we will investigate the anti-tumor effects (including preventive) of MRTX 1133 in a mouse model of tobacco-associated KM-LUAD (Gprc5a\(^{-/-}\)) and in 3D organoid models of tumor precursors from the same mice.

**Conclusions & Future Directions**

- Increasing concentrations of MRTX 1133 result in statistically significant decreases in cell viability when compared to control.

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**References & Support**


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