Prevention of early stage *Kras*-mutant lung adenocarcinoma via targeted *Kras*^{G12D} inhibition

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Background

- Lung adenocarcinoma (LUAD) represents the most common subtype of lung cancer[1]. Patients with *KRAS*-mutant LUADs (KM-LUADs) common to smokers display dismal clinical outcomes and resistance to targeted therapies[3].

- 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).

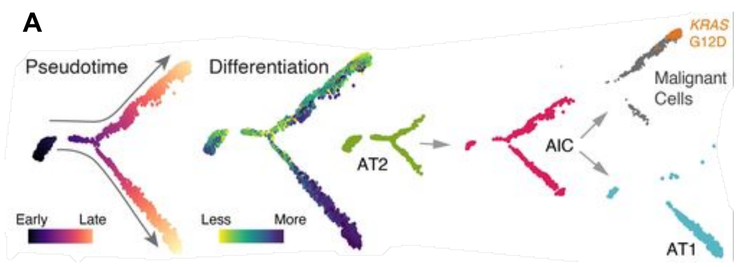

Figure 1. (a) Trajectory analysis of KACs and malignant cells implicated in human KM-LUAD development. Adapted from Han G (2022).

(b) Fraction of *Kras*G12D mutant cells (right) in KACs, malignant, AT1 and AT2 subsets. Absolute numbers of *Kras*G12D mutant cells are indicated on top of each bar. Figure and legend adapted from Han G, Sinjab A (2022).

- 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)[2]. While previous testing utilized patient-derived xenograft tumor models and orthotopic pancreatic cancer models[2], we investigate the effectiveness of MRTX 1133 in murine lung cancer cells as a precursor to potential clinical prevention of KM-LUAD *in vivo*.

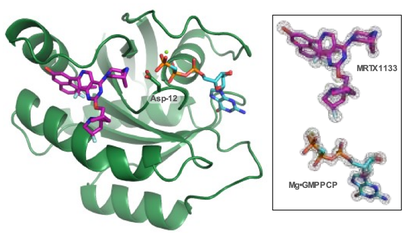

Figure 2. Crystal structure of KRAS^{G12D} bound to MRTX 1133 via the switch II binding pocket. Figure adapted from Christensen J (2022).

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, 1 nM, 10 nM, 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

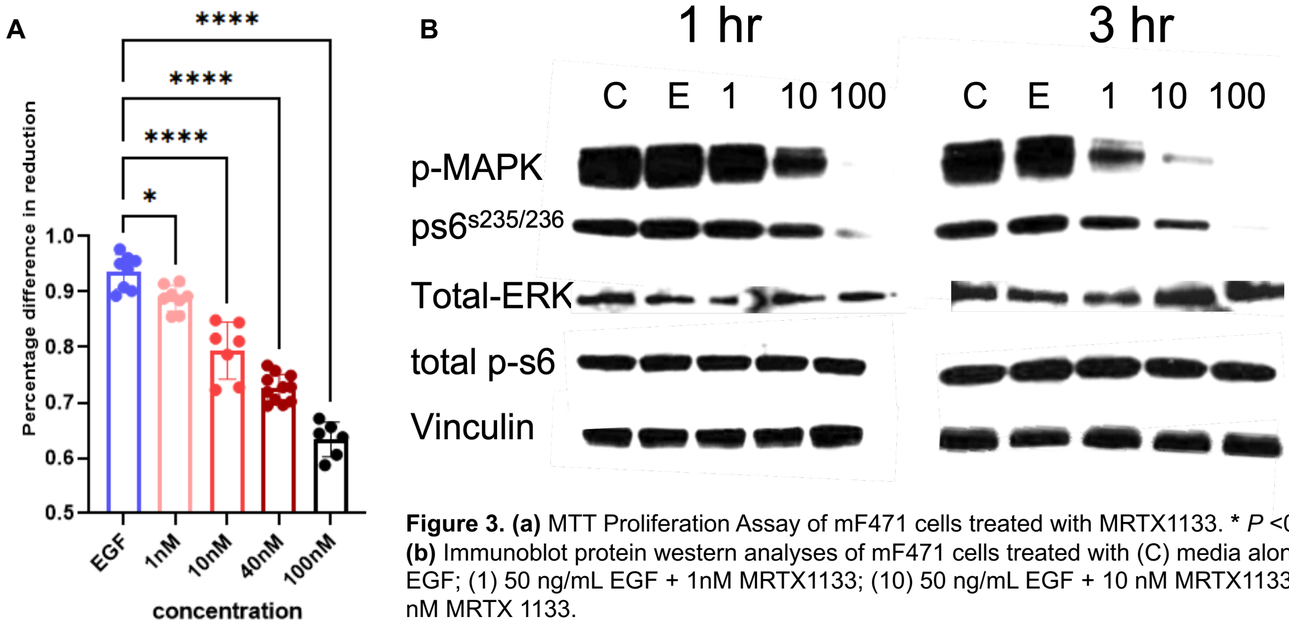

Figure 3. (a) MTT Proliferation Assay of mF471 cells treated with MRTX1133. * P <0.05, ** P <0.01, *** P <0.001. **(b)** Immunoblot protein western analyses of mF471 cells treated with (C) media alone for control; (E) 50 ng/mL EGF; (1) 50 ng/mL EGF + 1nM MRTX1133; (10) 50 ng/mL EGF + 10 nM MRTX1133; (100) 50 ng/ mL EGF + 100 nM MRTX 1133.

Conclusions & Future Directions

- 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.

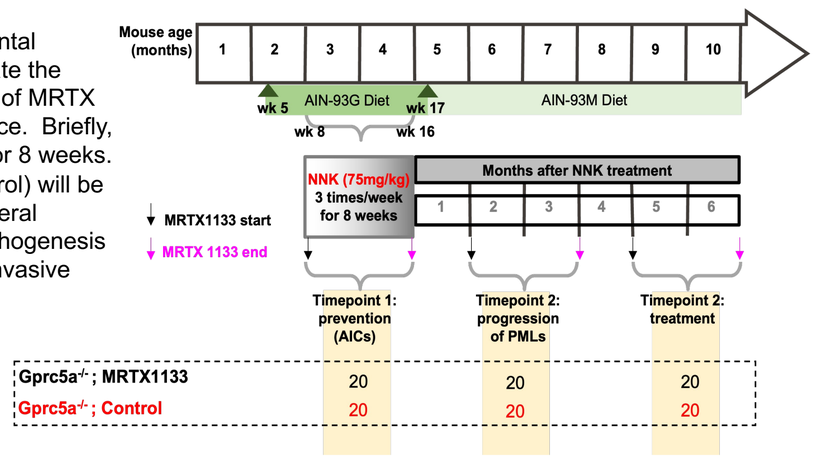

Figure 4. Experimental scheme to investigate the anti-tumor potential of MRTX 1133 in *Gprc5a*$^{-/-}$ mice. Briefly, NNK will be given for 8 weeks. MRTX1133 (or control) will be administered at several stages of LUAD pathogenesis (AICs, PMLs, and invasive LUAD).

References & Support

1) Han G, Sinjab A, et al. *BioRxiv*. 2022
2) Christensen J, et al. *Research Square*. 2022
3) Fujimoto J et al. *Int J Cancer.* 2017;141(8):1589-1599.

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