Efficacy and immune modulation of KRAS G12C inhibitor sotorasib in murine KRAS G12C mutant non-small cell lung cancers with major co-occurring genomic alterations

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Background
KRAS is the most frequently mutated oncoprotein in lung cancers, and KRAS<sup>G12C</sup> is the most frequent mutant isoform in non-small cell lung cancers (NSCLCs). Sotorasib (AMG510) has been approved by FDA in 2021 as the first potent and selective KRAS<sup>G12C</sup> inhibitor. However, the clinical efficacy of inhibitor monotherapy is curtailed by molecular adaptation and characterized by broad heterogeneity in the depth and duration of individual responses. In addition to their tumor cell-autonomous effects, KRAS<sup>G12C</sup> inhibitor may also recondense the tumor immune microenvironment (TIME) and synergize with anti-PD-1 therapy to promote tumor regressions and T cell memory.

The contributions of major co-occurring genomic alterations to KRAS<sup>G12C</sup> inhibitor-triggered efficacy and immune modulation are poorly understood.

Here we established several murine KRAS<sup>G12C</sup> mutant lung cancer cell lines with co-alterations of STK11/LKB1 loss (K<sup>G12C-L</sup>), TP53<sup>T172H</sup> oncogenic mutant (K<sup>G12C-P</sup>), and sgRNA-induced TP53 loss (K<sup>G12C-sgp</sup>), investigated the efficacies and immune modulations of KRAS<sup>G12C</sup> inhibitor sotorasib on these major K<sup>G12C</sup> NSCLC subtypes.

Methods
We derived several murine KRAS<sup>G12C</sup> lung cancer cell lines by delivering Adeno-Cre to K<sup>G12C-L</sup> (Kras<sup>SL-SL-12C<sup>lox/lox</sup>Stk11<sup>11<sub>ex2</sub>-<sup>lox/lox</sup></sup>Tp53<sup>T172H<sup>+</sup></sup></sup>), or delivering Lenti-Cre-Cas9-sgRNA(Tp53<sup>-/+</sup>) to K<sup>G12C</sup> (Kras<sup>SL-SL-12C<sup>lox/lox</sup>G<sup>−/−</sup></sup>) genetically engineered mouse models (GEMMs).

We validated the LKB1 expression and p53 function by western blot, characterized the histological tumor types by H&E staining of subcutaneously implanted tumors, assessed the cell growth capability by CellTiterGlo luminescence assays.

We further determined the dose-dependent sensitivity in response to sotorasib treatment in vitro by CellTiterGlo luminescence assays, and the efficacy of sotorasib in vivo on different co-mutation cell lines in syngeneic C57BL6 wild type mice.

Finally, we investigated the immune modulation effect of sotorasib on K<sup>G12C-L</sup>, K<sup>G12C-P</sup>, and K<sup>G12C-sgp</sup> tumors by implanting cells subcutaneously in syngeneic C57BL6 wild type mice, treating mice with sotorasib for one week, and then collecting tumors for FACS-based immune profiling assays.

Results

Figure 1: Characterization of murine KRAS<sup>G12C</sup> lung cancer cell lines derived from genetically engineered mouse models (GEMMs).

(A) Western blot determination of LKB1 and p53 expression in derived K<sup>G12C-L</sup>, K<sup>G12C-P</sup>, and K<sup>G12C-sgp</sup> cell lines. (B) Western blot validation of loss of normal p53 downstream signaling function in K<sup>G12C-P</sup>, and K<sup>G12C-sgp</sup> cell lines. (C) Western blot validation of MAPK signaling inhibition in response to K<sup>G12C</sup> inhibitor sotorasib. (D) Histological H&E staining of subcutaneously implanted tumors in syngeneic C57BL6 wild type mice.

Figure 2: In vitro cell growth and sotorasib sensitivity assays. (A) Cell growth were recorded as CellTiterGlo luminescence and normalized relative to 24 h post cell seeding. (B) Cells were seeded in 96-well non-transparent plate, after 24 h, sotorasib or DMSO vehicle at different concentrations were added, and incubated with cells for another 72 h before subjected to CellTiterGlo luminescence determination.

Figure 3: In vivo efficacy study of sotorasib on K<sup>G12C-L</sup>, K<sup>G12C-P</sup>, and K<sup>G12C-sgp</sup> cell lines. 2 × 10<sup>6</sup> cells of each indicated cell line was implanted subcutaneously in syngeneic C57BL6 wild type mice. For each mouse, consecutive vehicle or sotorasib treatment (100 mg/kg, q.d.) was started when the tumor volume reached 200-250 mm<sup>3</sup>. Tumor volume was measured every day until it reached endpoint. Tumor volume and time to tumor doubling curves were plotted to show the efficacies.

Conclusions
Sotorasib has significant inhibitory effects on K<sup>G12C-L</sup>, K<sup>G12C-P</sup>, and K<sup>G12C-sgp</sup> cell lines in vitro, with K<sup>G12C-P</sup> be the most sensitive. Sotorasib shows significant initial inhibition of tumor growth in syngeneic models, while resistance and re-growth finally occur in all lines. K<sup>G12C-L</sup>, K<sup>G12C-P</sup> and K<sup>G12C-sgp</sup> tumors have different compositions of infiltrated immune cells. Sotorasib triggers a significant immune sensitization on K<sup>G12C-L</sup> and K<sup>G12C-P</sup> but not K<sup>G12C-sgp</sup> tumors.

The characterization of immune microenvironment modulation induced by sotorasib may contribute to design of combination strategies.

References