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 oncogene in lung cancers, and KRAS^{G12C} 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^{G12C} 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^{G12C} inhibitor may also recondition 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^{G12C} inhibitor-triggered efficacy and immune modulation are poorly understood.

Here we established several murine KRAS^{G12C} mutant lung cancer cell lines with co-alterations of STK11/LKB1 loss (K^{G12C}L), TP53^{R172H} oncogenic mutant (K^{G12C}P), and sgRNA-induced TP53 loss (K^{G12C}sgP), investigated the efficacies and immune modulations of KRAS^{G12C} inhibitor sotorasib on these major K^{G12C} NSCLC subtypes.

Methods

We derived several murine KRAS^{G12C} lung cancer cell lines by delivering Adeno-Cre to K^{G12C}L (*Kras*$^{LSL\text{-}G12C/+}$;*Stk11*$^{flox/flox}$), K^{G12C}P (*Kras*$^{LSL\text{-}G12C/+}$; *Trp53*$^{LSL\text{-}R172H/+}$), or delivering Lenti-Cre-Cas9-sgRNA(*Trp53*) to K^{G12C} (*Kras*$^{LSL\text{-}G12C/+}$) 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^{G12C}L, K^{G12C}P, and K^{G12C}sgP 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

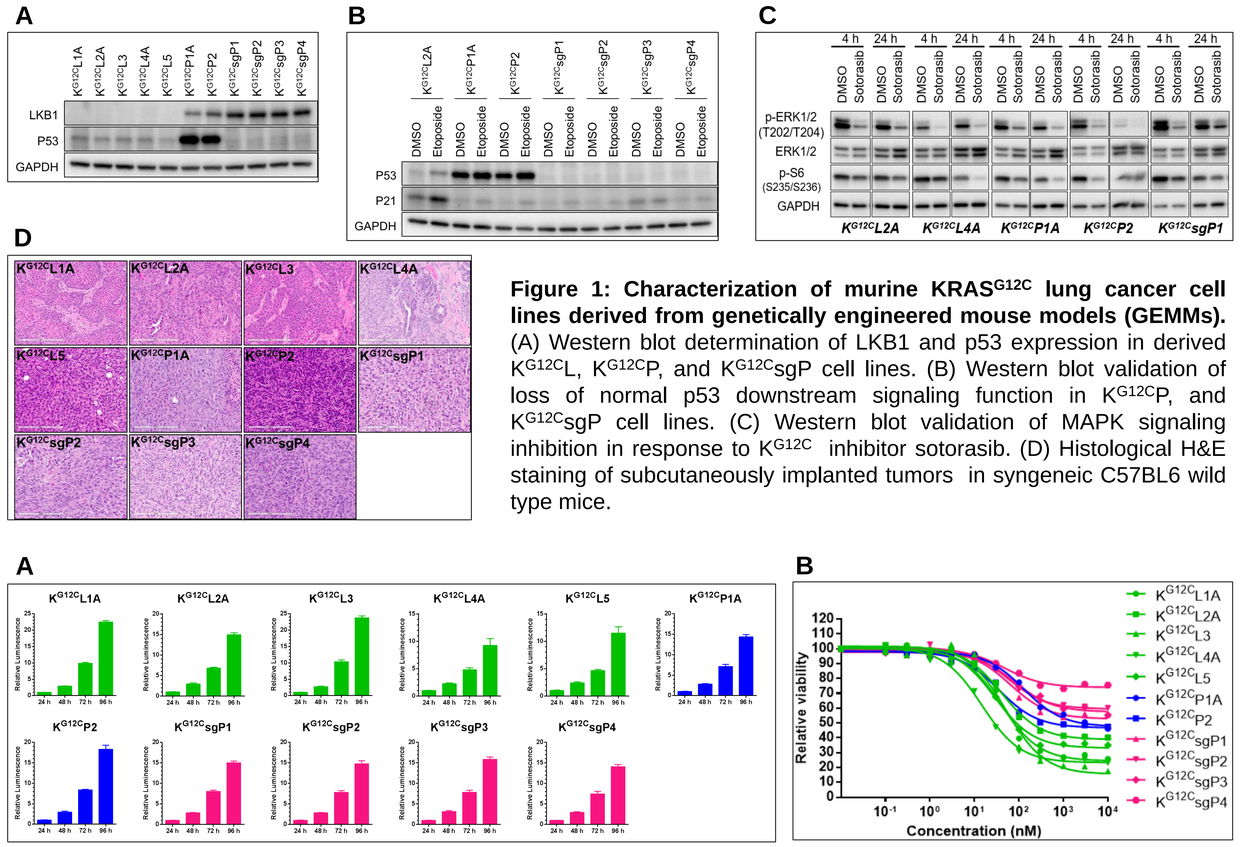

Figure 1: Characterization of murine KRAS^{G12C} lung cancer cell lines derived from genetically engineered mouse models (GEMMs). (A) Western blot determination of LKB1 and p53 expression in derived K^{G12C}L, K^{G12C}P, and K^{G12C}sgP cell lines. (B) Western blot validation of loss of normal p53 downstream signaling function in K^{G12C}P, and K^{G12C}sgP cell lines. (C) Western blot validation of MAPK signaling inhibition in response to K^{G12C} 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.

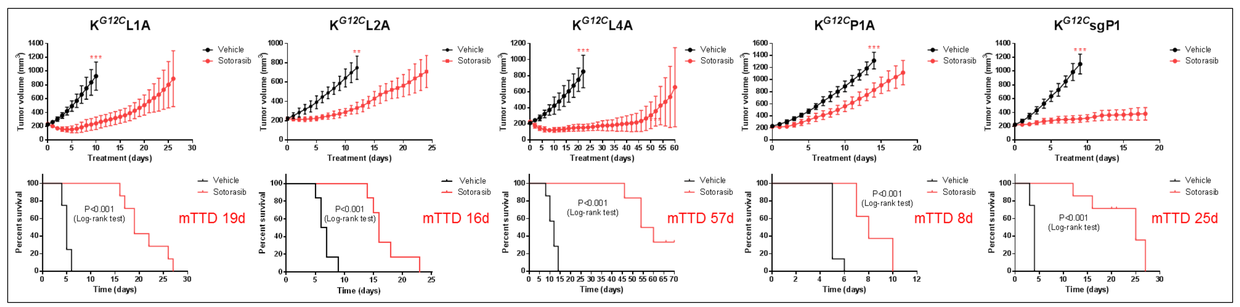

Figure 3: In vivo efficacy study of sotorasib on K^{G12C}L, K^{G12C}P, and K^{G12C}sgP cell lines. 2×10^6 cells of each indicated cell line was implanted subcutaneously in syngeneic C57BL6 wild type mice. For each mouse, consecutive vehicle or sotorasib treatment (100 mg/kg, q.d.) was started when the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

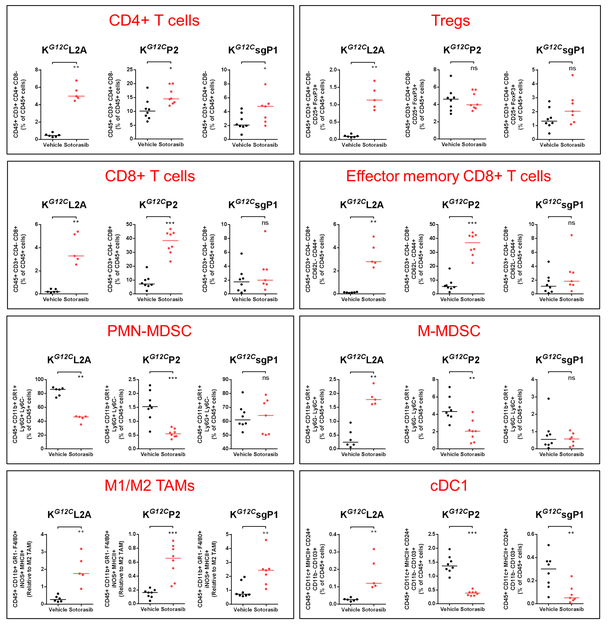

Figure 4: Immune modulation of sotorasib on K^{G12C}L, K^{G12C}P, and K^{G12C}sgP tumors. Cells were implanted as in Figure 3 and treated with vehicle or sotorasib (100 mg/kg, q.d.) for one week before subjected to FACS-based immune profiling. To allow having enough sample, mice were treated with a starting tumor volume 300-500 mm^3.

Conclusions

Sotorasib has significant inhibitory effects on K^{G12C}L, K^{G12C}P, and K^{G12C}sgP cell lines in vitro, with K^{G12C}L 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^{G12C}L, K^{G12C}P and K^{G12C}sgP tumors have different compositions of infiltrated immune cells. Sotorasib triggers a significant immune sensitization on K^{G12C}L and K^{G12C}P but not K^{G12C}sgP tumors.

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

References

1. Sotorasib for Lung Cancers with KRAS p.G12C Mutation. N Engl J Med 2021; 384:2371-2381
2. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nat Rev Cancer 2019; 19(9):495-509