RTK Signaling and WT RAS Activity as Vulnerabilities in Tumors with Acquired Resistance to GDP-state selective KRAS^{G12C} Inhibitors in NSCLC Preclinical Models

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Introduction

- Sotorasib (AMG-510) and adagrasib (MRTX849) have demonstrated clinical benefits in lung cancer patients harboring an oncogenic KRAS^{G12C} mutation.
- Both the depth/rate of initial response as well as durability of monotherapy benefit are limited by the onset of resistance.
- Recently published data in preclinical models and clinical samples identified additional oncogenic alteration in RAS isoforms or other bypass genomic lesions as potential mechanisms of acquired resistance. However, features associated with resistance in more than half of patients were not defined by genomic sequencing of available on-treatment samples.
- In this study, through multi-modal genomic, transcriptomic and mass spectrometry-based phosphoproteomics analyses of several preclinical sotorasib and adagrasib resistance models, we identified rewiring of signaling networks as a non-genomic mechanism of tumor escape.
- We further identified RAS(ON) multi-selective inhibitor and combinations thereof as therapeutic approaches that overcome resistance to this mechanism.

Methodology

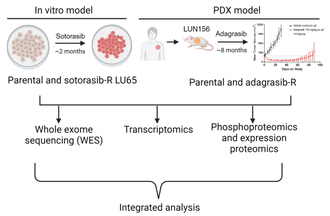


Figure 1. Workflow followed to understand acquired resistance to KRAS^{G12C} inhibitors

Results

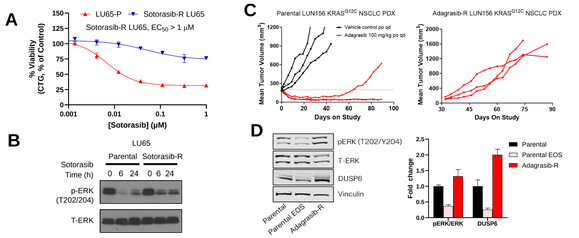


Figure 2. Characterization of resistant models. A) Antiproliferative activity assessed by CellTiter-Glo (CTG) after 96 hours treatment with sotorasib in resistant model of LU65 (Sotorasib-R LU65), compared with its parental counterpart (LU65-P). **B)** Immunoblot for pERK suppression in LU65-P and sotorasib-R LU65, treated with sotorasib (1µM) **C)** Tumor growth curve of adagrasib in parental and adagrasib-R LUN156 NSCLC model with daily adagrasib 100 mg/kg treatment. **D)** Immunoblot for pERK and DUSP6 from the parental, parental tumors treated with adagrasib for 28 days (Parental EOS; collected 6-hours post last dose), and adagrasib-R LUN156 NSCLC tumors.

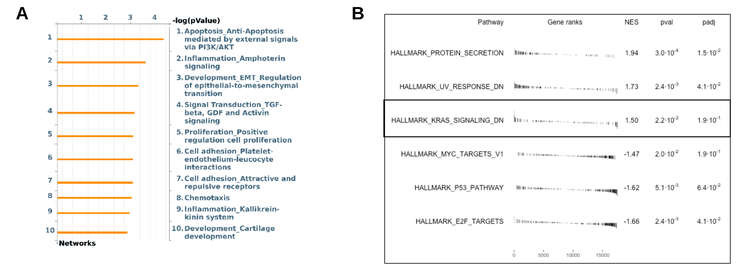


Figure 3. Transcriptomics analysis revealed enrichment of anti-apoptosis pathways associated with RAS and PI3K/AKT signaling in resistant cells. A) Metacore pathway analysis performed with differentially expressed upregulated genes in sotorasib-R LU65 cells compared to parental. **B)** GSEA analysis performed with differentially expressed genes in adagrasib-R LUN156 tumors.

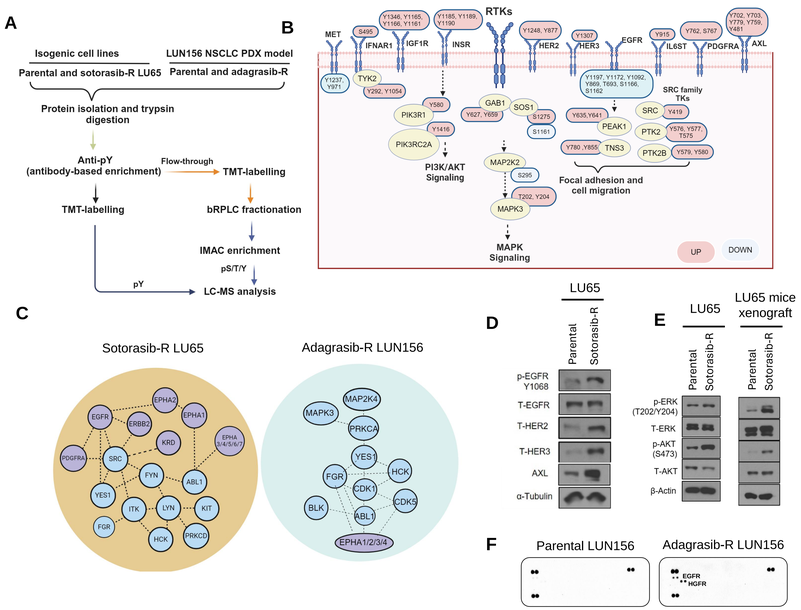


Figure 4. Phosphoproteomics using Mass-spectrometry identified enhanced activation of multiple RTKs in resistant cells. A) The workflow followed to perform phosphoproteomic (pY and pS/T/Y) and proteomic analysis in biological triplicates. **B)** Depiction of manually curated signaling network in sotorasib-R LU65 cells based on experimentally affirmed signaling information available in literature for differentially perturbed phosphosites (log2 fold change > 1.5 [or <0.67] and P < 0.05). **C)** Kinase-Substrate Enrichment Analysis (KSEA). **D)** Western blot validation of RTKs expression, and phosphorylation; and **(E)** MAPK and PI3K pathway proteins in sotorasib-R LU65 in vitro and in vivo. **F)** phospho-RTK array showed upregulated EGFR and HGFR in adagrasib-R tumor compared to parental LUN156 PDX tumor.

Results

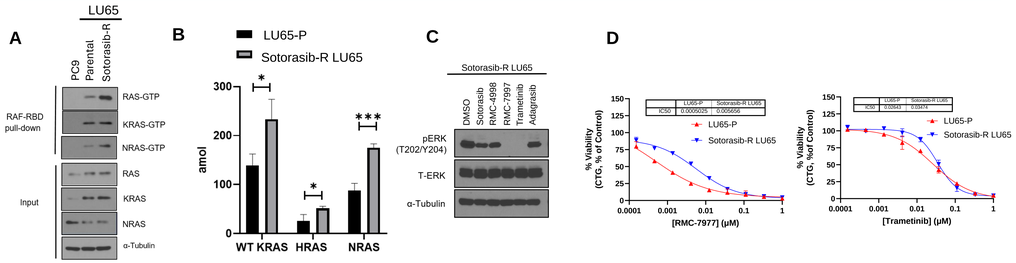
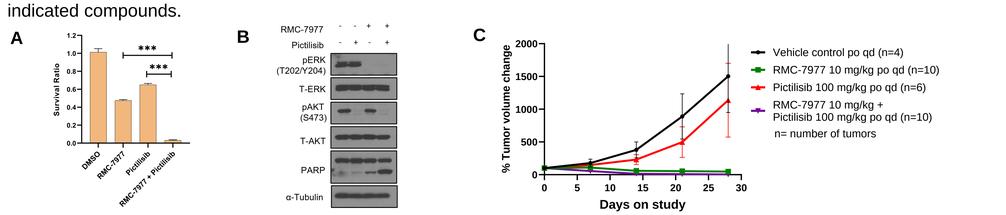


Figure 5. Sotorasib-R LU65 cells exhibit enhanced WT RAS activation, and MAPK signaling is fully suppressed by RAS(ON) multi-selective inhibitor RMC-7977. A) RAF-RBD pull down assay. **B)** LC-MRM based quantification of WT RAS. **C)** Immunoblot depicting effects of sotorasib (200nM), RAS(ON) G12C-selective inhibitor (RMC-4998, 30nM), RMC-7977 (30nM), trametinib (50nM) and adagrasib (200nM) on pERK expression. **D)** Dose response curves of RMC-7977 and trametinib in parental and sotorasib-R LU65 cells. Cell viability was assessed by CellTiter-Glo after 96 hours treatment with indicated compounds.



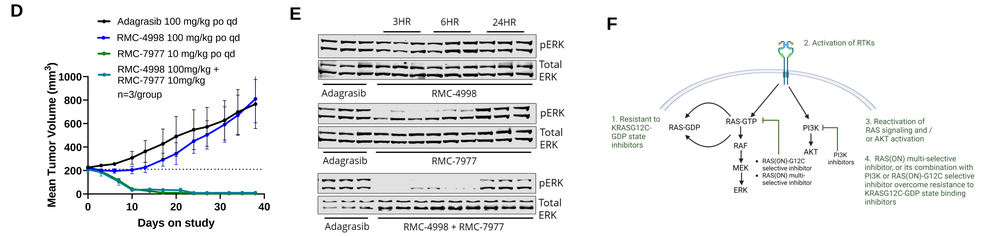


Figure 6. RMC-7977 monotherapy and its combination with pictilisib or RAS(ON) G12C-selective inhibitor RMC-4998 completely regressed tumor growth in models resistant to KRAS^{G12C}-GDP state binding inhibitors. A) Cell viability and **B)** immunoblotting-based signaling analysis with RMC-7977 (30nM) alone and in combination with pictilisib (1µM) in sotorasib-R LU65 cells. **C-D)** In vivo response of RMC-7977 monotherapy and its combination with pictilisib in sotorasib-R LU65 mice xenograft and adagrasib-R LUN156. **E)** Immunoblot of pERK from adagrasib-R LUN156 tumors post single dose of RMC-4998 or RMC-7977 monotherapy and RMC-4998 plus RMC-7977 combination. **F)** Model depicting role of RTKs and WT RAS signaling in driving resistance to KRAS^{G12C}-GDP state binding inhibitors.

Summary

- Several novel models of on-treatment resistance to KRAS^{G12C}-GDP state binding inhibitors were developed and comprehensively evaluated, using NGS as well as proteomic / phosphoproteomic and functional analyses.
- WES revealed no novel acquired mutation in RAS and downstream signaling mediators suggesting that resistance developed via non-genomic mechanisms.
- Activation of RTKs maintains MAPK dependency and/or PI3K signaling in KRAS^{G12C}-GDP state binding inhibitors-resistant models.
- RMC-7977 as a single agent or in combination with pictilisib or RMC-4998 drove significant tumor regressions in sotorasib or adagrasib resistant models, respectively.

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