The RAF/MEK clamp avutometinib (VS-6766) enhances antitumor efficacy of KRAS G12C and G12D inhibitors through vertical inhibition of RAS, RAF and MEK

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BACKGROUND

KRAS G12C is the most prevalent KRAS mutation in non-small cell lung cancer (NSCLC) (~13%) (1). Although the KRAS G12C inhibitors (G12Ci) sotorasib and adagrasib have demonstrated antitumor activity in patients with KRAS G12C NSCLC (2, 3) and are now FDA approved, simultaneous targeting of multiple nodes in the MAPK pathway may be optimal for deeper and more durable responses (4, 5). Furthermore, acquired mutations in the MAPK pathway occur clinically upon progression on G12Ci (6-8) and MAPK pathway inhibition has been shown to activate parallel compensatory pathway including focal adhesion kinase (FAK) as adaptive resistance mechanisms (9, 10), altogether supporting the need for clinical combinations. Based on the clinical success of G12Ci, several KRAS G12D inhibitors (G12Di) are now being developed as G12D is the most prevalent KRAS mutation in pancreatic (~28%) and colorectal (~11%) cancers (1).

Avutometinib (VS-6766) is a novel RAF/MEK clamp that potently inhibits MEK kinase activity and induces dominant negative complexes of ARAF, BRAF and CRAF with MEK (11, 12) (Figure 1A, B). This unique mechanism allows avutometinib to block MEK signaling without the compensatory re-activation of MEK that appears to limit the efficacy of MEK-only inhibitors.

Here, we tested whether addition of avutometinib to G12Ci or G12Di could improve MAPK pathway blockade and anti-tumor efficacy through vertical inhibition of RAS, RAF and MEK (Figure 1C).

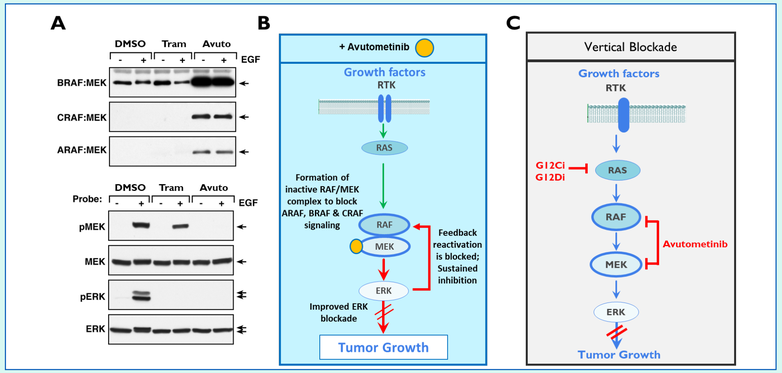


Figure 1. (A) Western blot analyses in serum-starved HeLa cells treated with 1 μM avutometinib (Avuto) or 1 μM trametinib (Tram) for 3 hours and with EGF for 5 minutes. (B) Schematic showing that avutometinib is a unique RAF/MEK clamp that induces inactive complexes of MEK with ARAF, BRAF and CRAF. (C) Addition of avutometinib to G12Ci or G12Di might improve MAPK pathway blockade and anti-tumor efficacy through vertical inhibition of RAS, RAF and MEK.

CONCLUSIONS

- The combination of avutometinib + sotorasib showed stronger inhibition of cell cycle/proliferation markers and stronger activation of pro-apoptotic markers than avutometinib or sotorasib alone.
- Avutometinib enhanced efficacy of sotorasib in KRAS G12C NSCLC models *in vivo* with strong tumor regressions in mice treated with the combination.
- Avutometinib potently inhibited proliferation of cells bearing acquired resistance mutations that have been shown to confer resistance to G12C inhibitors in patients progressing on sotorasib or adagrasib.
- In a sotorasib-resistant model expressing KRAS G12C/Y96D, avutometinib inhibited tumor growth & triple combination of avutometinib + FAK inhibitor + sotorasib induced maximal depth and duration of response.
- Addition of avutometinib + FAK inhibitor renews anti-tumor activity after progression on sotorasib monotherapy in a KRAS G12C NSCLC GEMM model.
- Avutometinib enhanced efficacy of the G12D inhibitor MRTX1133 in KRAS G12D PDX models.
- These results support ongoing clinical studies of avutometinib in combination with sotorasib (NCT05074810) or adagrasib (NCT05375994) for patients with KRAS G12C NSCLC and suggest that addition of a FAK inhibitor might further improve anti-tumor activity. Additionally, these data support combination of avutometinib with a G12D inhibitor for patients with KRAS G12D mutant cancers.

REFERENCES

1. TCGA PanCancer Atlas
2. Park, ESMO 2020
3. Janne, ENA 2020
4. Ryan, Clin Cancer Res 2019
5. Robert, N Engl J Med 2015
6. Awad, N Engl J Med 2021
7. Tanaka, Cancer Disc 2021
8. Zhao, Nature 2021
9. Hirata, Cancer Cell 2015
10. Chen, Mol Cancer Ther 2018
11. Ishii, Cancer Res, 2013
12. Lito, Cancer Cell, 2014

RESULTS

RAS, RAF & MEK blockade with avutometinib + sotorasib confers anti-proliferative & pro-apoptotic signaling

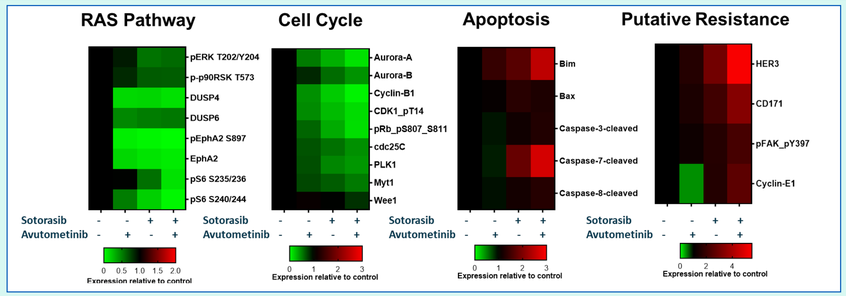


Figure 2. Reverse phase protein array (RPPA) analysis in H358 KRAS G12C NSCLC cells treated for 48 hours with 100 nM avutometinib or 100 nM sotorasib as single agents or in combination. Putative adaptive resistance markers may include HER3 and pFAK.

Avutometinib potentiates sotorasib efficacy in KRAS G12C NSCLC models *in vivo*; Strong tumor regression in mice treated with the combination

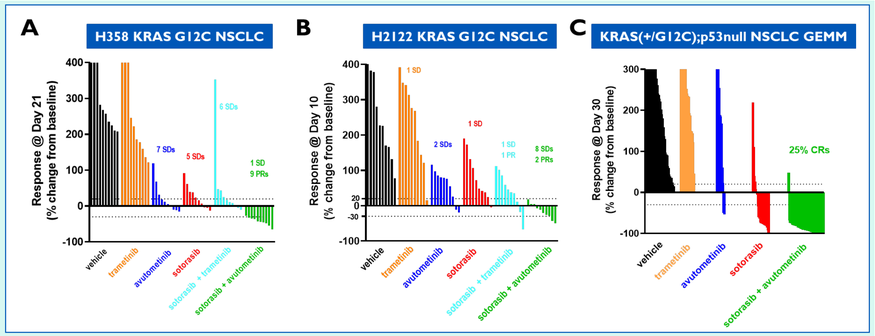
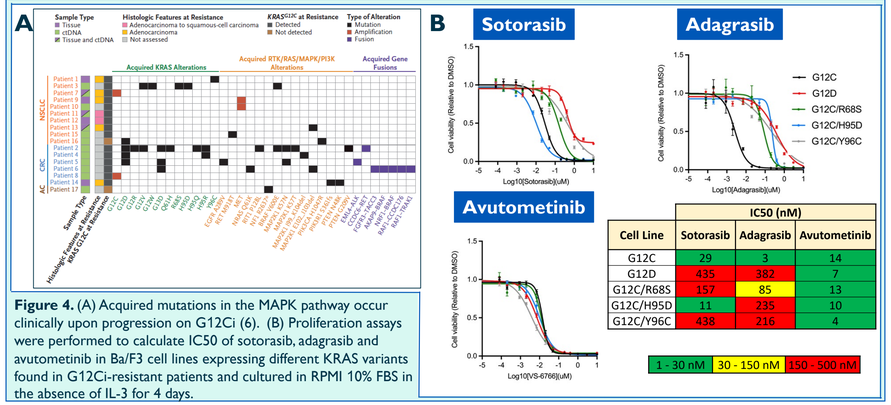


Figure 3. Changes in tumor volume in H358 (A) and H2122 (B) KRAS G12C NSCLC tumor-bearing mice treated with avutometinib (0.3 mg/kg PO QD) +/- sotorasib (10 mg/kg PO QD for H358; 30 mg/kg PO QD for H2122). Trametinib was tested at 0.3 mg/kg PO QD. N = 10 mice/group. SD: stable disease; PR: partial response. p value calculated using t test: ** = p<0.01; **** < p<0.0001. (C) Changes in tumor volume in KRAS(+/G12C);p53null NSCLC GEMM mice treated with avutometinib (0.1 mg/kg PO QD) +/- sotorasib (100 mg/kg PO QD). Trametinib was tested at 0.1 mg/kg PO QD. N = 5-15 mice/group. CR: Complete response. p value calculated using t test.

Avutometinib is effective against acquired mutations in the MAPK pathway that occur clinically upon progression on G12C inhibitors



Cell Line	IC50 (nM) Sotorasib	Adagrasib	Avutometinib
G12C	29	3	14
G12D	435	382	7
G12C/R68S	157	85	13
G12C/H95D	11	235	10
G12C/Y96C	438	216	4

1 - 30 nM | 30 - 150 nM | 150 - 500 nM

Figure 4. (A) Acquired mutations in the MAPK pathway occur clinically upon progression on G12Ci (6). (B) Proliferation assays were performed to calculate IC50 of sotorasib, adagrasib and avutometinib in Ba/F3 cell lines expressing different KRAS variants found in G12Ci-resistant patients and cultured in RPMI 10% FBS in the absence of IL-3 for 4 days.

Addition of avutometinib + FAK Inhibitor to sotorasib increases depth and duration of tumor growth inhibition in the sotorasib-resistant KRAS G12C/Y96D model *in vivo*

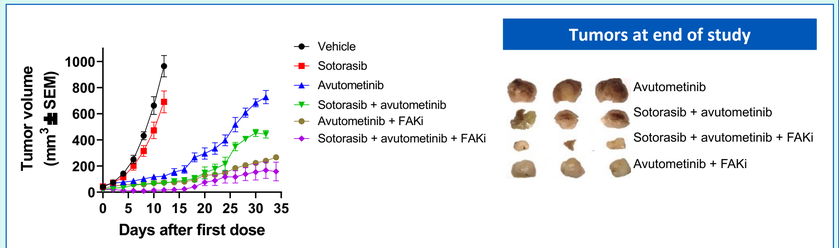


Figure 5. Changes in tumor volume in KRAS G12C/Y96D G12Ci-resistant NSCLC tumor-bearing mice treated with sotorasib (10 mg/kg PO QD) +/- avutometinib (0.3 mg/kg PO QD) +/- FAKi (50 mg/kg PO BID). N = 10 mice/group.

Addition of avutometinib + FAKi renews anti-tumor activity after progression on sotorasib monotherapy in a KRAS G12C NSCLC GEMM model

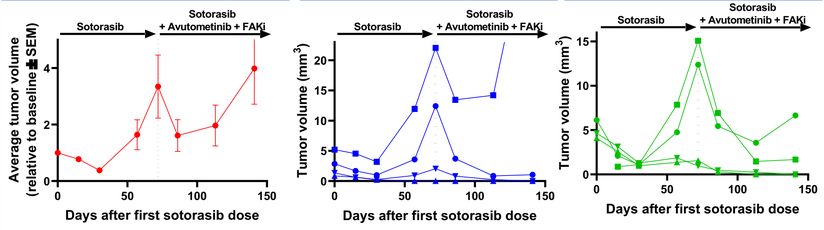


Figure 6. Changes in tumor volume in KRAS(+/G12C);p53null NSCLC GEMM mice treated with sotorasib (100 mg/kg PO QD) until tumor progression and with avutometinib (0.3 mg/kg PO QD), FAK inhibitor (50 mg/kg PO BID) and sotorasib (10 mg/kg PO QD) afterwards. Individual tumors in 6 mice are shown.

Avutometinib potentiates G12D inhibitor (MRTX1133) efficacy in KRAS G12D PDX models

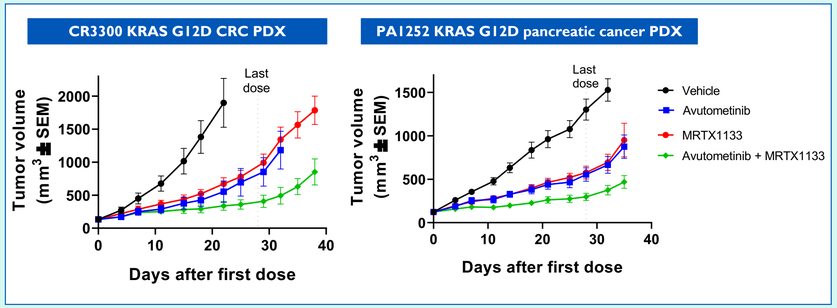


Figure 7. Changes in tumor volume in KRAS G12D CR3300 CRC and PA1252 pancreatic cancer PDX tumor-bearing mice treated with avutometinib (0.3 mg/kg PO QD) +/- MRTX1133 (30 mg/kg IP twice per week; collaboration with Mirati). N = 10 mice/group.