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

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Abstract #402

BACKGROUND

KRAS is one of the most frequently mutated oncogenes in cancer, stimulating tumor growth through activation of the RAS/RAF/MEK/ERK (MAPK) pathway. KRAS G12C mutation is present in ~13% non-small cell lung cancer (NSCLC) and 2.9% colorectal cancer (CRC) (1).

KRAS G12C inhibitors (G12Ci), sotorasib and adagrasib, have demonstrated antitumor activity in patients with KRAS G12C NSCLC (2,3), and sotorasib has recently received FDA approval. However, it has been shown that simultaneous targeting of multiple nodes in the MAPK pathway may be optimal for durable response (4). Furthermore, acquired mutations in the MAPK pathway occur clinically upon progression on G12Ci (5,6,7). Therefore, clinical combinations with G12Ci are needed to improve depth and duration of MAPK pathway inhibition.

VS-6766 is a unique RAF/MEK clamp (Figure 1). In contrast to MEK-only inhibitors (MEKi), VS-6766 is a potent allosteric inhibitor of MEK kinase activity and induces a dominant negative RAF/MEK complex preventing phosphorylation of MEK by ARAF, BRAF and CRAF. (8,9) The combination of VS-6766 with the focal adhesion kinase (FAK) inhibitor defactinib with an intermittent schedule has shown clinical activity for patients with KRAS G12V and KRAS G12C NSCLC and low-grade serous ovarian cancer with a manageable safety profile relative to MEKi (10,11).

Here, we tested whether combination of G12Ci with VS-6766 for vertical blockade of RAS, RAF and MEK might yield superior pathway blockade and antitumor efficacy.

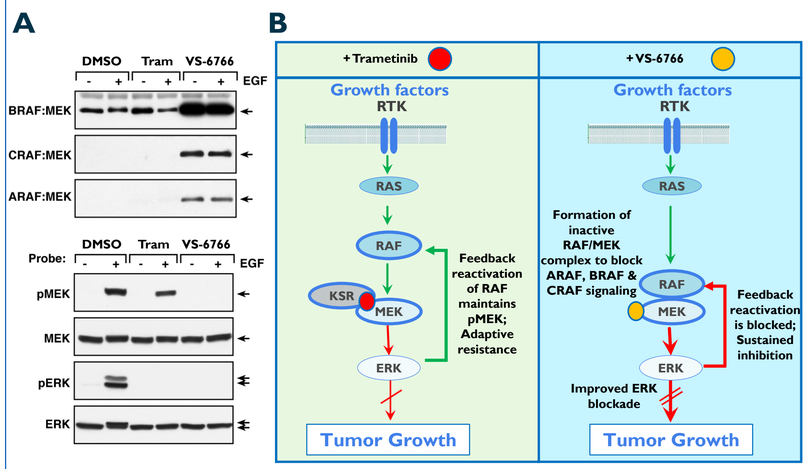


Figure 1. (A) Western blot analyses in serum-starved HeLa cells treated with 1 μM VS-6766 or 1 μM trametinib (Tram) for 3 hours and with EGF for 5 minutes. (B) Schematic showing that in contrast to MEKi (e.g. trametinib), VS-6766 is a unique RAF/MEK clamp that induces inactive complexes of MEK with ARAF, BRAF and CRAF.

CONCLUSIONS

- VS-6766 was synergistic with both sotorasib and adagrasib in reducing viability of a panel of KRAS G12C cancer cell lines, and the combination of VS-6766 + G12Ci showed improved depth and duration of MAPK pathway inhibition relative to G12Ci alone.
- RPPA analysis showed stronger inhibition of cell cycle/proliferation markers and stronger activation of pro-apoptotic markers with the combination than with VS-6766 or G12Ci alone.
- VS-6766 potently inhibited proliferation of cells bearing acquired resistance mutations that have been shown to inactivate G12Ci in patients progressing on sotorasib or adagrasib.
- Both VS-6766 & FAKi enhance efficacy of sotorasib in KRAS G12C NSCLC xenograft models. Triple combination of G12Ci + VS-6766 + FAKi yields ≥30% tumor regression in all mice in both models which may warrant future clinical investigation of this triplet regimen.
- VS-6766 potentiates G12Ci efficacy in a KRAS(+/G12C);p53null NSCLC GEMM model with VS-6766 + G12Ci combination inducing complete response in 25% of the mice.
- These results support the imminent clinical evaluation of VS-6766 in combination with a G12C inhibitor for treatment of KRAS G12C NSCLC in both G12Ci naïve patients and patients progressing on G12Ci treatment (NCT05074810; AACR 2022 abstract #CT204).

RESULTS

Synergy of VS-6766 + G12C inhibitors observed in KRAS G12C NSCLC, CRC and pancreatic cancer cell lines; Addition of VS-6766 to sotorasib increases depth & duration of inhibition of p-ERK across a panel of KRAS G12C NSCLC cell lines

A

Cell line	Indication	Sensitivity to G12C inhibitors	Combined Synergy Score: VS-6766 + sotorasib	Combined Synergy Score: VS-6766 + adagrasib
H2122	NSCLC	Moderately sensitive	44.7	44.6
H1373	NSCLC	Sensitive	10.0	3.4
SW1573	NSCLC	Insensitive	8.6	12.0
H358	NSCLC	Sensitive	6.9	5.4
H2030	NSCLC	Moderately sensitive	5.1	ND
SW837	CRC	Sensitive	16.1	18.5
MIAPACA2	Panc	Sensitive	2.3	5.3

ND: not determined

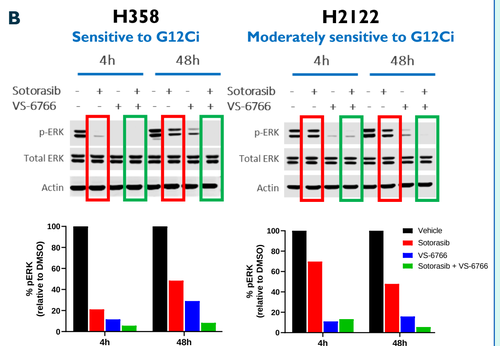


Figure 2. (A) 3D proliferation with VS-6766 + sotorasib or adagrasib in KRAS G12C NSCLC, CRC and pancreatic cancer (Panc) cell lines. Bliss, Loewe, HSA and ZIP synergy analyses were performed to generate a composite synergy score. (B) Western blot analyses of pERK and total ERK in KRAS G12C NSCLC cells treated for 4 or 48 hours with 100 nM VS-6766 or 100 nM sotorasib as single agents or in combination.

RAS, RAF & MEK blockade with VS-6766 + sotorasib confers anti-proliferative & pro-apoptotic signaling

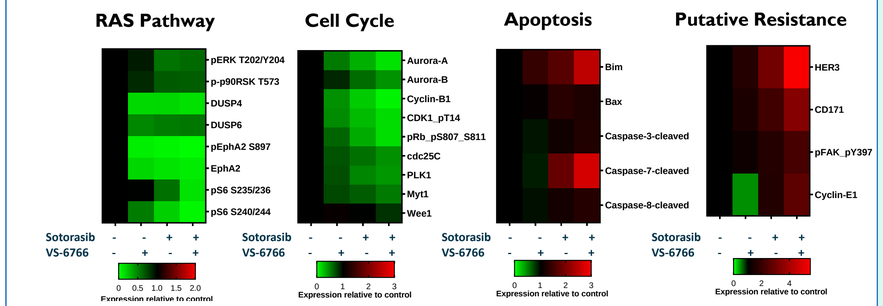
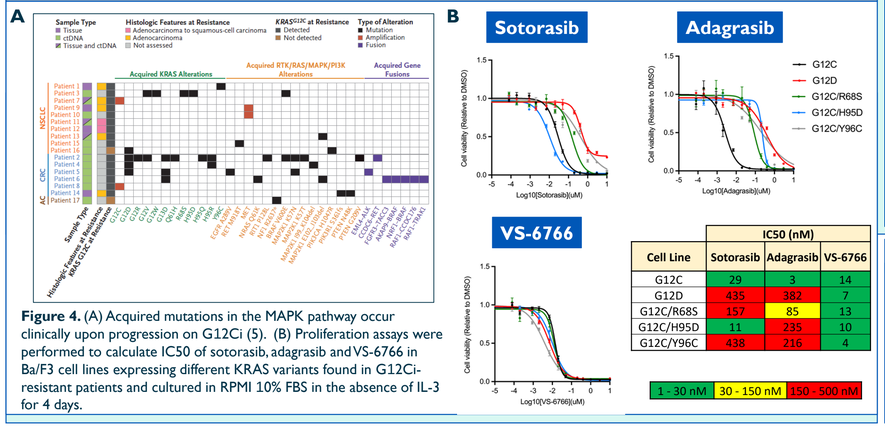


Figure 3. Reverse phase protein array (RPPA) analysis in H358 KRAS G12C NSCLC cells treated for 48 hours with 100 nM VS-6766 or 100 nM sotorasib as single agents or in combination. Putative adaptive resistance markers may include HER3 and pFAK.

VS-6766 is effective against acquired mutations in the MAPK pathway that occur clinically upon progression on G12C inhibitors



Cell Line	IC50 (nM) Sotorasib	Adagrasib	VS-6766
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 (5). (B) Proliferation assays were performed to calculate IC50 of sotorasib, adagrasib and VS-6766 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.

VS-6766 & FAKi potentiate sotorasib efficacy in KRAS G12C NSCLC models *in vivo*. Tumor regression in all mice with triple combination

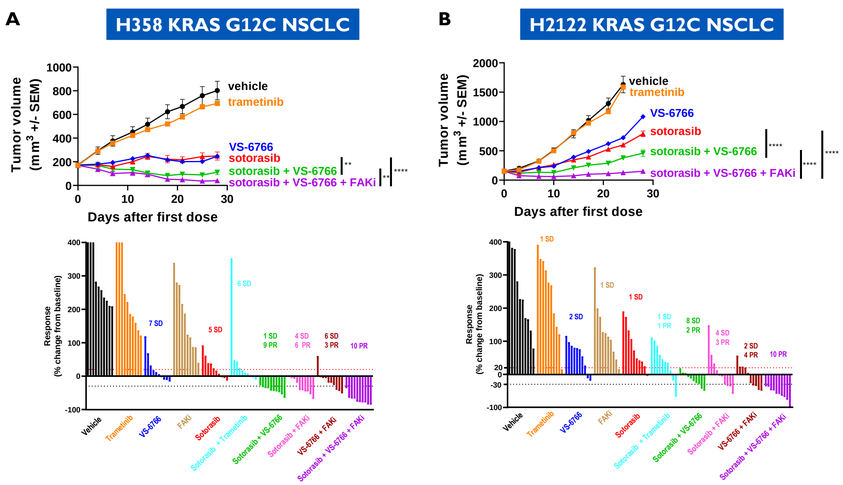


Figure 5. Changes in tumor volume in H358 (A) and H2122 (B) KRAS G12C NSCLC tumor-bearing mice treated with VS-6766 (0.3 mg/kg QD) +/- FAKi (50 mg/kg BID) +/- sotorasib (10 mg/kg QD for H358 and 30 mg/kg QD for H2122). Trametinib was tested at 0.3 mg/kg QD. N = 10 mice/group. SD: stable disease; PR: partial response. p value calculated using t test: ** = p<0.01; **** < p<0.0001

VS-6766 potentiates sotorasib efficacy in a KRAS(+/G12C);p53null NSCLC GEMM model. 25% complete responses with VS-6766 + sotorasib

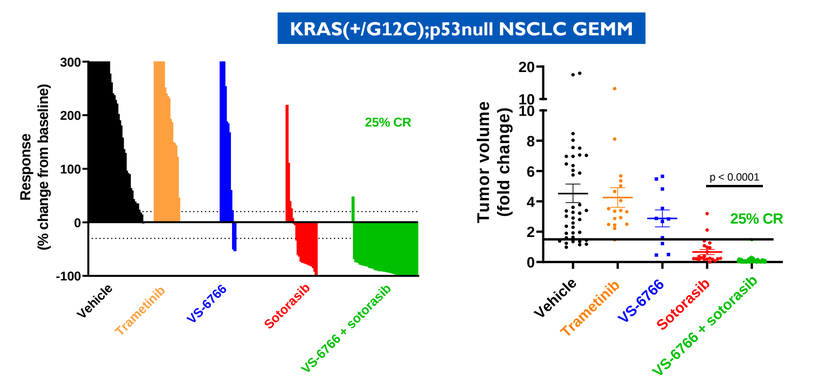


Figure 6. Changes in tumor volume in KRAS(+/G12C);p53null NSCLC GEMM mice treated with VS-6766 (0.1 mg/kg QD) +/- sotorasib (100 mg/kg QD). Trametinib was tested at 0.1 mg/kg QD. N = 5-15 mice/group. CR: Complete response. p value calculated using t test.

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