The RAF/MEK clamp VS-6766 for treatment of cutaneous melanoma harboring BRAF, NRAS, NF1 or RAF1 (CRAF) alterations

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BACKGROUND

VS-6766 is a unique RAF/MEK clamp which blocks MEK activity without the compensatory MEK activation that limits the efficacy of MEK-only inhibitors (MEKi) (Figure 1) (1, 2). VS-6766 produced clinical responses as a single agent in gynecological cancers and KRAS mutant (mt) non-small cell lung cancer (NSCLC) (3). Clinical responses were also observed with VS-6766 in combination with the focal adhesion kinase (FAK) inhibitor defactinib in patients with low-grade serous ovarian cancer and KRAS mt NSCLC (4, 5).

In patients with advanced cutaneous melanoma, mutations in the RAS/RAF/MEK/ERK (MAPK) pathway occur mainly in BRAF (41%), NRAS (27%), NF1 (25%) and RAF1 (CRAF) (2.6%) (AACR Genie v10). Although several selective BRAF V600 inhibitors (BRAFi) are FDA-approved alone or in combination with MEKi for melanomas with BRAF V600E/K, there is still a need for agents to improve response rate, duration of response, and tolerability. There are no targeted therapy options for melanoma patients carrying NRAS or NF1 mt following progression on immune checkpoint inhibitors.

Here, using low passage cell lines derived from patients with metastatic melanoma and extensively profiled for genomic alterations and commercially available immortalized human melanoma cell lines, we tested the anti-proliferative activity of VS-6766 alone or in combination with other agents including the mTOR inhibitor (mTORi) everolimus and the CDK4/6 inhibitor (CDK4/6i) abemaciclib. We also tested the anti-proliferative activity of VS-6766 vs. RAF inhibitors in patient-derived melanoma cells harboring BRAF V600E, NRAS or RAF1 (CRAF) alterations.

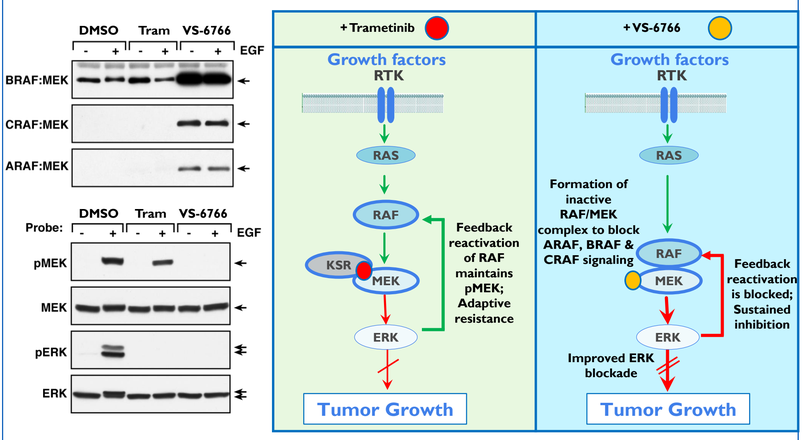


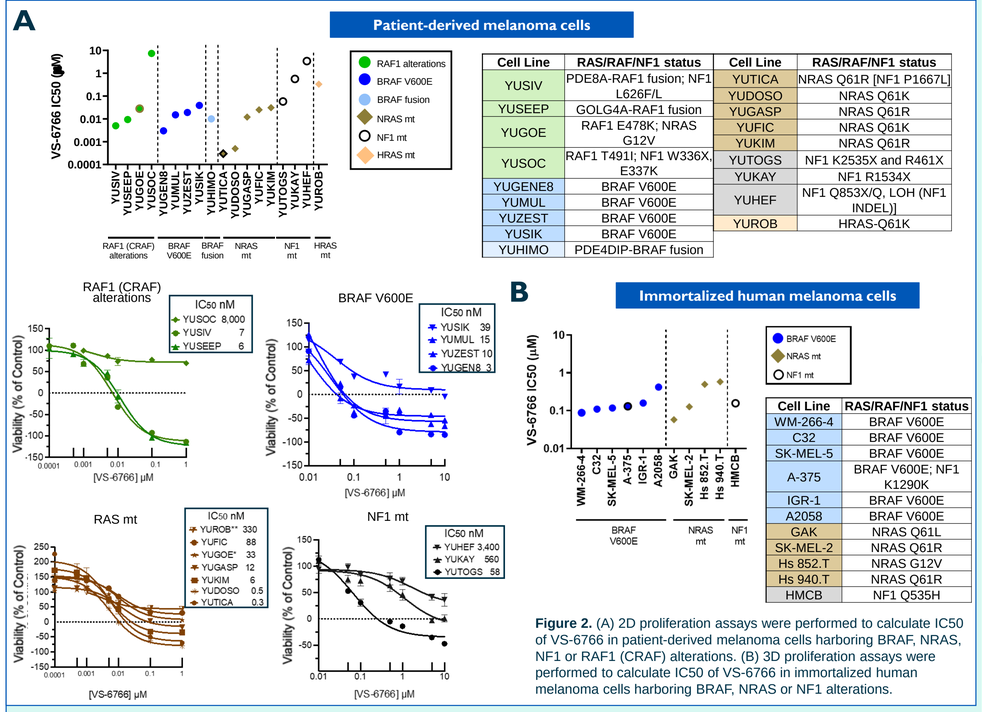
Figure 1. (A) Western blot analyses of serum-starved HeLa cells treated with 1 µM VS-6766 or 1 µM trametinib (Tram) for 3 hours and with EGF for 5 minutes. (B) Schematic showing that in contrast to MEKi (e.g. trametinib), VS-6766 is a unique RAF/MEK clamp that induces inactive complexes of MEK with ARAF,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

REFERENCES

1. Ishii et al., Cancer Res, 2013
2. Lito et al., Cancer Cell, 2014
3. Guo et al., Lancet Oncology 2020
4. Krebs et al., AACR 2021
5. Banerjee et al., ESMO 2021

RESULTS

VS-6766 potently inhibits proliferation of patient-derived melanoma cells and immortalized human melanoma cells harboring BRAF, NRAS, NF1 or CRAF alterations



Cell Line	RAS/RAF/NF1 status
YUSIV	PDE8A-RAF1 fusion; NF1 L626F/L
YUSEEP	GOLG4A-RAF1 fusion
YUGOE	RAF1 E478K; NRAS G12V
YUSOC	RAF1 T491I; NF1 W336X, E337K
YUGENE8	BRAF V600E
YUMUL	BRAF V600E
YUZEST	BRAF V600E
YUSIK	BRAF V600E
YUHIMO	PDE4DIP-BRAF fusion

Cell Line	RAS/RAF/NF1 status
YUTICA	NRAS Q61R [NF1 P1667L]
YUDOSO	NRAS Q61K
YUGASP	NRAS Q61R
YUFIC	NRAS Q61K
YUKIM	NRAS Q61R
YUTOGS	NF1 K2535X and R461X
YUKAY	NF1 R1534X
YUHEF	NF1 Q853X/Q, LOH (NF1 INDEL)]
YUROB	HRAS-Q61K

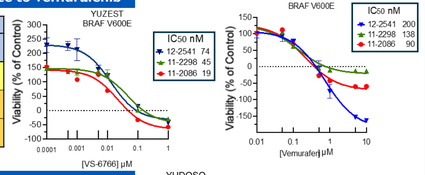
Cell Line	RAS/RAF/NF1 status
WM-266-4	BRAF V600E
C32	BRAF V600E
SK-MEL-5	BRAF V600E
A-375	BRAF V600E; NF1 K1290K
IGR-1	BRAF V600E
A2058	BRAF V600E
GAK	NRAS Q61L
SK-MEL-2	NRAS Q61R
Hs 852.T	NRAS G12V
Hs 940.T	NRAS Q61R
HMCB	NF1 Q535H

Figure 2. (A) 2D proliferation assays were performed to calculate IC50 of VS-6766 in patient-derived melanoma cells harboring BRAF, NRAS, NF1 or RAF1 (CRAF) alterations. (B) 3D proliferation assays were performed to calculate IC50 of VS-6766 in immortalized human melanoma cells harboring BRAF, NRAS or NF1 alterations.

VS-6766 is more potent than RAF inhibitors in patient-derived melanoma cells harboring BRAF V600E, NRAS or CRAF alterations

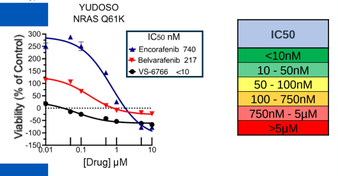
BRAF V600E melanoma cells derived from a patient with acquired resistance to vemurafenib

Cell Line	Mutation status (RAS. RAF, RAF1, NF1)	IC50 (nM) VS-6766 (RAF/MEK clamp)	IC50 (nM) Vemurafenib (BRAFi)
YUZEST pre vemurafenib (11-2086)	BRAF V600E	19	90
YUZEST 3 weeks vemurafenib (11-2298)	BRAF V600E	45	138
YUZEST 6 months vemurafenib (12-2541)	BRAF V600E	74	200



Melanoma cells with NRAS mt

Cell Line	Mutation status (RAS. RAF, RAF1, NF1)	IC50 (nM) VS-6766 (RAF/MEK clamp)	IC50 (nM) Encorafenib (BRAFi)	IC50 (nM) Belvarafenib (pan-RAFi)
YUDOSO	NRAS Q61K	0.5	740	217
YUFIC	NRAS Q61K	25	>10 µM	3,000
YUKIM	NRAS Q61R	31	2,500	900
YUGASP	NRAS Q61R	14	2,800	100
YUGOE	NRAS G12V; RAF1 E478K	24	1,200	650



IC50
<10nM
10 - 50nM
50 - 100nM
100 - 750nM
750nM - 5µM
>5µM

Melanoma cells with RAF1 (CRAF) alterations

Cell Line	Mutation status (RAS. RAF, RAF1, NF1)	IC50 (nM) VS-6766 (RAF/MEK clamp)	IC50 (nM) Encorafenib (BRAFi)	IC50 (nM) Vemurafenib (BRAFi)	IC50 (nM) Belvarafenib (pan-RAFi)	IC50 (nM) Naporafenib (pan-RAFi)
YUGOE	RAF1 E478K; NRAS G12V	24	1,200	>10 µM	650	500
YUSEEP	GOLG4A-RAF1 fusion	9	>10 µM	>10 µM	60	210
YUSIV	PDE8A-RAF1 fusion; NF1 L626F/L	5	5,000	>10 µM	130	160

Figure 3. 2D proliferation assays were performed to calculate IC50 of VS-6766, BRAFi (encorafenib, vemurafenib) and pan-RAFi (belvarafenib, naporafenib) in patient-derived melanoma cells harboring BRAF V600E (top panel), NRAS (middle panel) or RAF1 (CRAF) (bottom panel) alterations.

Abemaciclib (CDK4/6i) enhances the potency of VS-6766 in patient-derived NRAS mt melanoma cells

VS-6766 + CDK4/6i (abemaciclib)

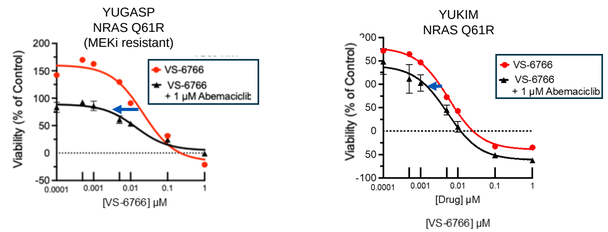
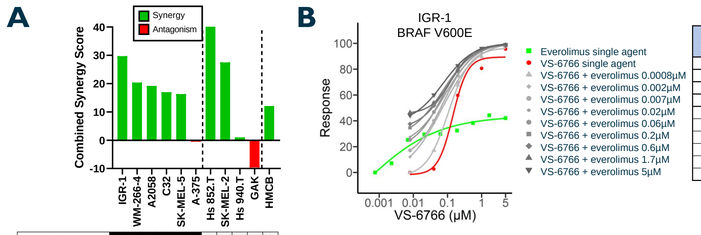


Figure 4. 2D proliferation assays were performed to calculate IC50 of VS-6766 ± CDK4/6i (abemaciclib) in NRAS mt patient-derived melanoma cells: YUGASP MEKi resistant (abemaciclib IC50 = 1 µM) (left panel), and YUKIM (abemaciclib IC50 = 2.8 µM) (right panel). To generate YUGASP MEKi resistant cells, YUGASP cells were treated with 5 µM selumetinib for 3 months *in vitro* to select resistant clones.

Anti-proliferative synergy of VS-6766 + everolimus (mTORi) in immortalized human melanoma cells

VS-6766 + mTORi (everolimus)



VS-6766 IC50 (nM)
166
120
71
58
40
39
26
25
16
14

	IGR-1	WM-266-4	A2058	C32	SK-MEL-5	A-375	Hs 852.T	SK-MEL-2	Hs 940.T	GAK	HMCB
BRAF V600E	Y	Y	Y	Y	Y	Y	N	N	N	N	N
NRAS mt	N	N	N	N	N	N	Y	Y	Y	Y	N
NF1 mt	N	N	N	N	N	Y	N	N	N	N	Y
PTEN mt	N	N	Y	N	N	N	N	N	N	N	N
PI3K family mt*	Y	N	Y	N	Y	Y	N	Y	N	Y	Y
PIK3C2G mt	N	N	N	Y	N	N	N	N	N	N	N
AKT1 mt	N	N	N	N	N	N	N	N	N	N	Y
RICTOR mt	N	N	N	N	Y	N	N	N	N	N	N
RAPTOR mt	N	Y	N	N	N	N	N	N	N	N	N
mTOR mt	N	N	N	Y	N	Y	N	N	N	Y	N

Figure 5. (A) 3D proliferation assays were performed to calculate synergy score between VS-6766 and everolimus in Immortalized human melanoma cells. Bliss, Loewe, HSA and ZIP synergy analysis were performed to generate a composite synergy score. Mutation status for BRAF, NRAS, NF1 and PAM (PI3K/AKT/mTOR) pathway are shown below. (B) Dose responses of VS-6766 ± everolimus in IGR-1 BRAF V600E melanoma cells as an example.
** PI3K family mt = PIK3C2A, PIK3C2B, PIK3C2G, PIK3CB, PIK3CG, PIK3R2, PIK3R5, PIK3R6*

CONCLUSIONS

- VS-6766 is a potent inhibitor of proliferation of human melanoma cells carrying BRAF, NRAS, NF1 or RAF1 (CRAF) alterations.
- VS-6766 was more potent than BRAFi or pan-RAFi in reducing proliferation of melanoma cells harboring CRAF or NRAS alterations.
- VS-6766 potently inhibited proliferation of BRAF V600E melanoma cells derived from a patient with acquired resistance to vemurafenib.
- In patient-derived NRAS mt melanoma cells, abemaciclib (CDK4/6i) enhanced the anti-proliferative potency of VS-6766.
- In immortalized melanoma cells harboring BRAF V600E, NRAS and/or NF1 mt, VS-6766 was synergistic with everolimus (mTORi) in reducing the viability of melanoma cells.
- These preclinical data support clinical testing of VS-6766 in rational combinations for treatment of cutaneous melanoma harboring BRAF, NRAS, NF1 or CRAF alterations. In clinical trials, a recommended phase 2 dose has been defined for the combination of VS-6766 with everolimus.