VS-7375, a non-covalent dual ON/OFF KRAS^{G12D} inhibitor, displays superior activity to ON-only KRAS^{G12D} inhibitors in preclinical models of pancreatic cancer

#LB197

Brandon L. Mouery[1], Wen-Hsuan Chang[1], Clint A. Stalnecker[1,2], Adrienne D. Cox[1,3,4], Silvia Coma[5], Jonathan A. Pachter[5], Channing J. Der[1,3]

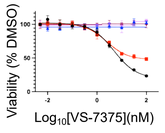
[1]Lineberger Comprehensive Cancer Center, [2]Department of Biochemistry & Biophysics, [3]Department of Pharmacology, [4]Department of Radiation Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599; [5]Verastem Oncology, Needham, MA 02494

Abstract

The FDA approval of two selective OFF-state KRAS^{G12C} inhibitors has stimulated comprehensive development of mechanistically distinct inhibitors of additional KRAS mutations. In particular, approximately 20 selective KRAS^{G12D} inhibitors are currently in clinical evaluation. Their mechanisms of action include both ON- and OFF-state inhibitors and both covalent and non-covalent inhibitors. Here we compared the activity of VS-7375 (GFH375), a non-covalent dual ON/OFF KRAS^{G12D} inhibitor, with the covalent ON-only KRAS^{G12D} inhibitor zoldonrasib (RMC-9805). We found that VS-7375 exhibited greater anti-proliferative potency than RMC-9805 in a panel of KRAS^{G12D}-mutant pancreatic ductal adenocarcinoma (PDAC) cell lines. VS-7375 was 40-fold less potent in the KRAS^{G12C} cell line MIA PaCa-2, indicating high selectivity for KRAS^{G12D}. Furthermore, VS-7375 showed near complete inhibition of phosphorylated ERK (pERK) at concentrations as low as 1 nM by 4 hours, which persisted for up to 48 hours, whereas incomplete suppression of pERK was observed with as high as 30 nM of RMC-9805 by 4 hours. RMC-9805 suppressed pERK by 24 hours but required concentrations 10-30x higher than VS-7375 and rebounded by 48 hours. Similarly, we found that VS-7375 exhibited greater potency than RMC-9805 in suppressing phosphorylated AKT and S6, and MYC expression levels. To further compare the durability of inhibition, we performed washout experiments monitoring signaling inhibition after removal of drug. We found that the non-covalent inhibitor VS-7375 exhibited more prolonged signaling inhibition compared with the covalent inhibitor RMC-9805. pERK reduction persisted for up to 48 hours after washout of VS-7375. In contrast, following washout of RMC-9805, pERK rebound was seen by 8 hours with near complete rebound by 48 hours despite retention of covalently modified KRAS. This durable effect of VS-7375 may be explained by the long residence time of VS-7375 (18-24 hours). To delineate mechanisms of resistance to VS-7375, we cultured cells at 100-fold the GI$_{50}$ concentration until subpopulations arose with acquired resistance. Initial evaluation of the resistant cells revealed heterogenous mechanism of resistance; some lines displayed cross-resistance to other mechanistically distinct RAS inhibitors including the tricomplex inhibitors RMC-9805 and RMC-6236, suggesting RAS-independent growth, whereas other lines retained sensitivity to MAPK-inhibition. Ongoing studies are aimed at extending our comparison of VS-7375 to other ON-only RAS inhibitors (e.g., RMC-6236). In summary, we reveal superior preclinical activity of VS-7375 compared to RMC-9805. These results suggest that targeting both the ON- and OFF-states of mutant KRAS may provide more clinical benefit than covalent modification of the ON-state only.

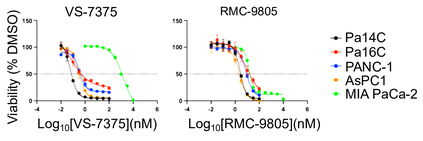
VS-7375 displays more potent and durable activity than RAS ON-only inhibitors in *in vitro* and *in* vivo models of PDAC

VS-7375 suppresses growth of KRAS G12D but not G12V mutant PDAC organoids



Model	KRAS
hM1A	G12D
hT105	G12D
PT3	G12V
PT6	G12V
PT8	G12V

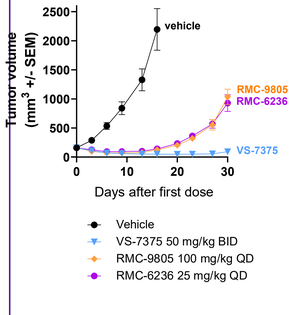
The noncovalent dual ON/OFF-state inhibitor VS-7375 is more potent (5- to 48-fold) and more selective than the covalent ON-state inhibitor RMC-9805 in KRAS^{G12D}- versus KRAS^{G12C}-mutant PDAC cell lines



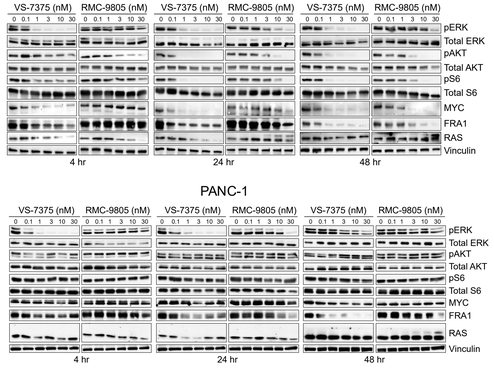
Cell line	KRAS	GI$_{50}$ (nM)		
		VS-7375	RMC-9805	RMC-9805/VS-7375
Pa14C	G12D	0.10	4.84	48.4
Pa16C	G12D	0.66	18.7	28.3
PANC-1	G12D	1.61	28.4	17.6
AsPC-1	G12D	0.60	2.21	3.7
MIA PaCa-2	G12C	386	15.4	0.04

VS-7375 causes more durable tumor regression versus ON-state mutant-selective and pan-RAS inhibitors

KRAS^{G12D}-mutant KP-4 PDAC cell line-derived xenograft (CDX) mouse tumors



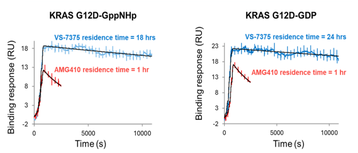
VS-7375 causes rapid and durable suppression of KRAS effector signaling and ERK activation



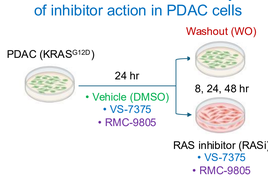
VS-7375 more durably suppresses pERK signaling following drug washout than the covalent KRAS^{G12D} inhibitor RMC-9805

VS-7375 has equivalent affinities for and prolonged association with the ON and OFF states of KRAS

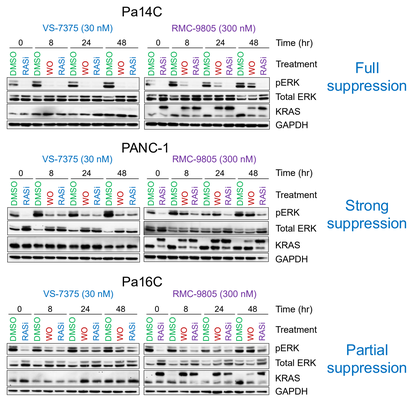
VS-7375 binding affinity (SPR assay)	
Bound nucleotide	Kd (pM)
GppNHp (ON-state)	18
GDP (OFF-state)	12



Protocol to evaluate durability of inhibitor action in PDAC cells

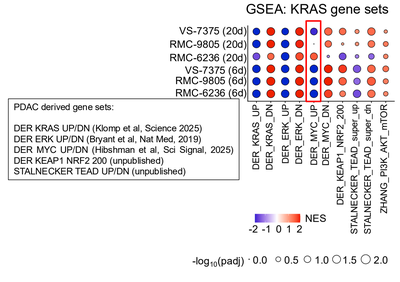


VS-7375 causes prolonged suppression of pERK comparable or superior to the covalent inhibitor, RMC-9805

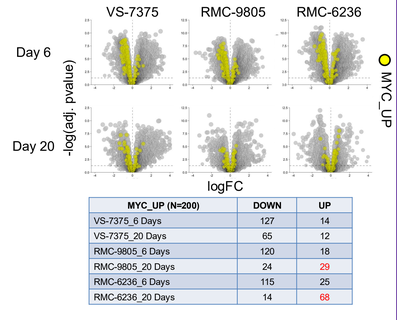


VS-7375 causes more durable suppression of MYC and growth factor signaling relative to RAS ON-only inhibitors in an *in vivo* model of PDAC

VS-7375 causes prolonged inhibition of KRAS-regulated gene transcription

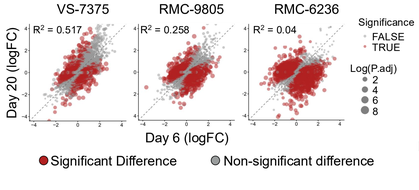


VS-7375 causes more prolonged suppression of MYC-driven gene transcription than ON-only inhibitors

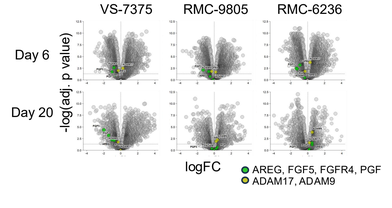


MYC_UP (N=200)	DOWN	UP
VS-7375_6 Days	127	14
VS-7375_20 Days	65	12
RMC-9805_6 Days	120	18
RMC-9805_20 Days	24	29
RMC-6236_6 Days	115	25
RMC-6236_20 Days	14	68

Pearson correlation analyses: VS-7375 causes prolonged suppression of gene transcription, whereas RMC-9805 and RMC-6236 treated tumors show gene reactivation



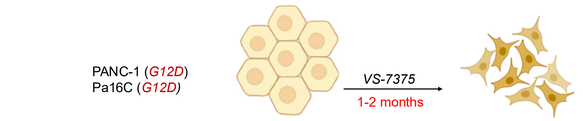
VS-7375 causes more prolonged suppression of growth factor-related genes than ON-only inhibitors



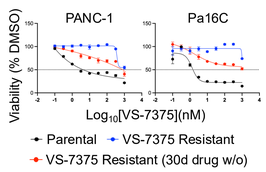
Methods: Bulk RNA-sequencing was done on KP-4 CDX tumors treated with the indicated RAS inhibitor for either 6 or 20 days to assess durability of KRAS inhibition, prior to Gene Set Enrichment Analysis (GSEA) and other analyses

PDAC cell lines selected for acquired resistance to VS-7375 show heterogenous dependency on MAPK signaling

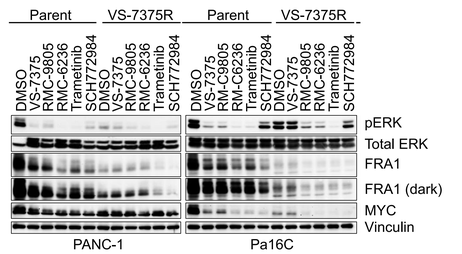
Protocol for isolation of PDAC cell line subpopulations with acquired resistance to VS-7375



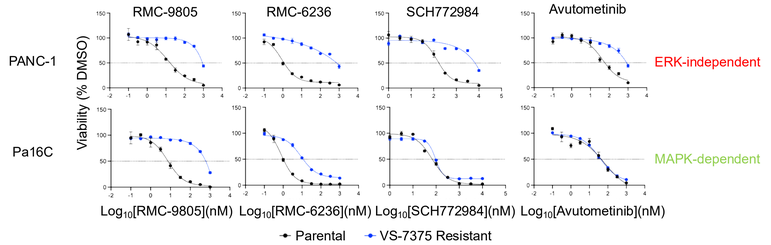
Resistance is partially reversible: drug withdrawal (30 days) partially restores VS-7375 sensitivity



MEK and ERK inhibitors block ERK signaling in VS-7375R PDAC cells: resistance is at the level of RAS, not ERK



Heterogeneity in mechanisms of resistance to VS-7375: RAF-MEK-ERK inhibitors can still suppress proliferation in Pa16C but not PANC-1 cells



Summary

- We observed more potent activity of the non-covalent, dual ON/OFF-state KRAS^{G12D} inhibitor VS-7375 in comparison to ON-state covalent tricomplex KRAS^{G12D} (RMC-9805) or ON-state noncovalent pan-RAS (RMC-6236) inhibitors in both *in vitro* and *in vivo* models of PDAC.
- The superior antiproliferative activity of VS-7375 *in vitro* is correlated with lower doses required for stable pERK suppression, as well as more durable pERK suppression following drug washout. The latter suggests VS-7375 could confer the benefits expected from a covalent inhibitor, despite being reversible.
- VS-7375 suppresses MYC-dependent gene expression more durably than RMC-9805 or RMC-6236 *in vivo*, correlating with sustained tumor growth inhibition.
- Collectively, our results highlight the benefits of targeting both the GTP- (ON) and GDP-bound (OFF) states of KRAS and support the ongoing evaluation of VS-7375 in clinical trials for patients with KRAS^{G12D}-mutant cancers including PDAC (NCT07020221).

Support

P50CA257911 (CJD, CAS)
R35CA232113 (CJD, ADC)
F31CA284869 (CAS)
PF-23-1072348-01-CDP (WHC)
22-WG-DERB (CJD)