Poster: # 1962

Voruciclib, a CDK9 inhibitor, downregulates MYC and inhibits proliferation of *KRAS* mutant cancers in preclinical models

Sandra Wiley*, Yongwei Su, Yubin Ge****

*MEI Pharma Inc, 11455 El Camino Real, Suite 250, San Diego, CA , 92130

**Department of Oncology and Molecular Therapeutics Program, Karmanos Cancer Institute, Wayne State University School of Medicine.

ABSTRACT

Mutations in *KRAS* at G12, G13, and Q61 are oncogenic drivers in many cancers, including lung, colorectal, pancreatic, bone marrow, and endometrial carcinomas.[1] *KRAS* mutations are frequently accompanied by stabilization of the MYC oncoprotein through increased *MYC* transcription and decreased protein degradation that is mediated by phosphorylation of MYC on Ser 62 by ERK and CDK9 kinases.[2,3] Voruciclib is a novel oral inhibitor of CDKs 9, 4, 6, and 1 that is currently being tested in Phase 1B clinical trials (NCT03547115) for B-cell malignancies and acute myeloid leukemia.[4] Voruciclib inhibition of CDK9 leads to decreased expression of transcriptional targets of RNA Pol II, such as *MCL1* and *MYC*.[5] Phosphoproteomics analysis revealed that voruciclib treatment resulted in a reduction in phosphorylation of proteins that regulate Pol II. To investigate MYC protein stability, MIA PaCa-2 (*KRAS* G12C) cells were treated with voruciclib, followed by Western Blot analysis with α-MYC and α-pSer62-MYC antibodies. Voruciclib treatment resulted in a reduction in phosphorylation of MYC on Ser 62. A 60% decrease in pSer62 was observed after 5 min that reached 80% by 60 min. In contrast, there was no decrease in total MYC protein at either 5 or 15 min. A 10% reduction in total MYC was observed at 60 min that reached 50% at 240 min. To test if voruciclib could be effective in cancers driven by dysregulated KRAS-MYC signaling, 22 cancer cell lines with *KRAS* mutations (G12A, G12C, G12D, G12S, G12V, G13C, G13D, Q61H) were treated in preclinical studies with voruciclib *in vitro*. Voruciclib decreased viability in all cell lines tested and inhibited tumor growth *in vivo* in murine xenograft models using *KRAS* mutant human cancer cells: HCT-116 (CRC, *KRAS* G13D), SW-480 (CRC, *KRAS* G12V), and H-460 (NSCLC, *KRAS* Q61H). Voruciclib also demonstrated synergy *in vitro* with the KRAS G12C inhibitors sotorasib (AMG 510) and adagrasib (MRTX849) in cell lines from multiple indications and *in vivo* within a MIA PaCa-2 murine xenograft model. Collectively, these data demonstrate that voruciclib inhibition of CDK9 leads to reduced phosphorylation of MYC on Ser62 followed by a decrease in total MYC protein in MIA PaCa-2 cells, and inhibition of growth in multiple *KRAS* mutant cancer cell lines *in vivo* and *in vitro*. This suggests that voruciclib could be an attractive therapeutic option for cancers driven by KRAS-MYC, possibly in combination with KRAS G12C inhibitors.

CDK9 REGULATES TRANSCRIPTION OF *MYC* BY RNA POL II AND MYC PROTEIN STABILITY

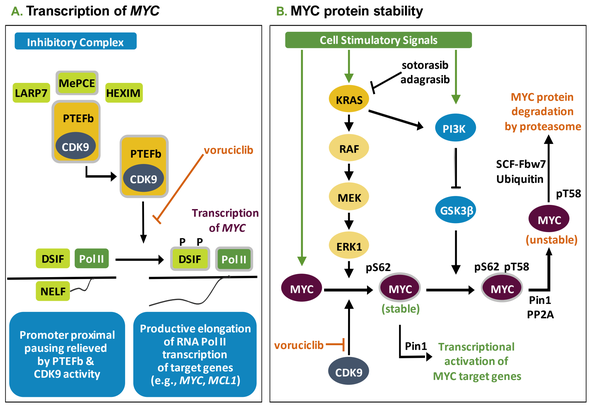


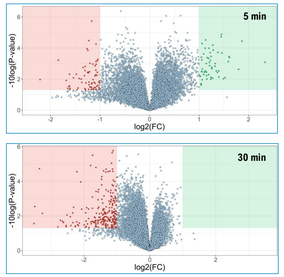
Figure 1. Schematic illustrating (**A**) P-TEFb regulation of RNA Pol II driven transcription of *MYC* and (**B**) KRAS-ERK1 signaling pathway and regulation of MYC protein stability by phosphorylation of Ser 62. Proteins with decreased phosphorylation after voruciclib treatment are circled in light gray. Points of CDK9 inhibition by voruciclib are noted.

VORUCICLIB INDUCES RAPID DOWN REGULATION OF RNA POL II ASSOCIATED PROTEINS THAT CONTROL *MYC* TRANSCRIPTION

A.

Summary of Phosphoproteomics Analysis	
Total Peptides	98,140
Total Proteins	5,753
Phosphoproteins (n)	**4,806**
Phosphosites (n)	**21,384**

B.



C.

Voruciclib (min)	Downreg Phospho-peptides (n)	Downreg Phospho-proteins (n)
5	72	67
15	33	28
30	237	174
60	159	117

D.

Database ID	Gene Symbol	Role in RNA Pol II Regulation
P24928	POLR2A	RNA Pol II complex
Q96ST2	IWS1	
O60885	BRD4	pTEFb complex
Q03111	MLLT1	
Q7L2J0	MEPCE	pTEFb regulation
O00267-2	SUPT5H	RNA Pol II regulation
Q15648	MED1	
P23193	TCEA1	
Q9UHB7	AFF4	

Figure 2. Landscape of the voruciclib-sensitive phosphoproteome in MIA Paca-2 cells reveals rapid downregulation of phosphoproteins controlling transcription of *MYC*. Cells were treated with voruciclib (4 μM) for 5, 15, 30, 60 min, followed by lysis, 16-plex TMT labelling, IMAC phosphopeptide enrichment, and analysis by LC-MS/MS. (**A**) Summary of total and phosphopeptide quantification for combined samples after MS. (**B**) Volcano plots of phosphosites (log2 fold change vs –log10 p-value). Significantly downregulated phosphosites are shown in red. Significantly upregulated phosphosites are shown in green ($p \leq 0.05$. Fold change ≥2.0). (**C**) Summary of significantly down-regulated phosphoproteins and phosphopeptides over time. (**D**) Downregulated phosphoproteins with a role in regulation of RNA Pol II activity. UniProt database ID and gene symbols noted.

VORUCICLIB CAUSES RAPID INHIBITION OF MYC pSer62 PHOSPHORYLATION AND REDUCES MYC PROTEIN LEVELS

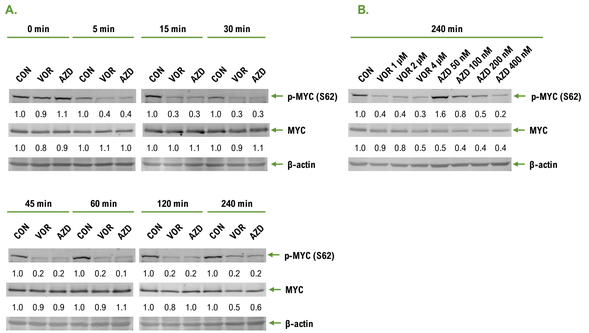


Figure 3. Immunoblot analyses of c-MYC, phospho-c-MYC (Ser62), and actin in MIA Paca-2 *KRAS* G12C mutant PDAC cells. (**A**) Cells were treated with vehicle control, voruciclib (VOR, 4 μM), or AZD4573 (AZD, a CDK9 inhibitor, 400 nM) for the indicated times. (**B**) Cells were treated with various concentrations of voruciclib or AZD4573 for 4 hours. Relative densitometry values are indicated.

VORUCICLIB INHIBITS PROLIFERATION OF *KRAS* MUTANT CANCER CELL LINES *IN VITRO* AND *IN VIVO*

A.

Cell Line	Indication	*KRAS* Mut	IC_{50} (μM)
Gp2D	CRC	G12D	0.8
HCT-116	CRC	G13D	1.8
LS-513	CRC	G12D	0.6
SW-480	CRC	G12V	3.9
SW837	CRC	G12C	2.1
KYSE-410	Esoph	G12C	1.9
RPMI-8226	MM	G12A	2.4
A-549	NSCLC	G12S	1.5
Calu-1	NSCLC	G12C	2.0
HCC1171	NSCLC	G12C	3.0
HCC44	NSCLC	G12C	0.8
NCI-H460	NSCLC	Q61H	3.1
NCI-H1373	NSCLC	G12C	1.2
NCI-H1792	NSCLC	G12C	1.4
NCI-H2030	NSCLC	G12C	1.1
NCI-H23	NSCLC	G12C	1.2
NCI-H358	NSCLC	G12C	0.6
TOV-21G	Ovarian	G13C	1.7
AsPC-1	PDAC	G12D	2.3
HPAF-II	PDAC	G12D	1.9
MIA PaCa-2	PDAC	G12C	1.1
Panc 04.03	PDAC	G12D	1.4

B.

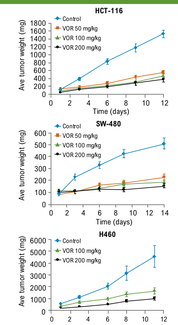


Figure 4. (**A**) Voruciclib IC_{50} values in multiple cell lines with *KRAS* mutations. (**B**) Murine xenograft experiment showing tumor growth over time in mice bearing HCT-116 (CRC, *KRAS* G13D), SW-480 (CRC, *KRAS* G12V) or H-460 (NSCLC, *KRAS* Q61H) tumors after treatment with voruciclib (VOR) at various doses (QD, p.o.) for 11-14 days.

VORUCICLIB SYNERGIZES WITH *KRAS* G12C INHIBITORS *IN VITRO*

Cell Line	*KRAS* mut	Sensitivity to G12C Inhibitors	Synergy Scores: Voruciclib + Sotorasib	Synergy Scores: Voruciclib + Adagrasib
NCI-H23	G12C	High		
HCC1171	G12C	High		
MIA Paca-2	G12C	High		
SW837	G12C	Moderate - High		
NCI-H2030	G12C	High		
Calu-1	G12C	Moderate - High		
HCC-44	G12C	Moderate - High		
NCI-H1373	G12C	Moderate - High		
NCI-H358	G12C	High		
NCI-H1792	G12C	Moderate - High		
KYSE-410	G12C	Low - High		
Panc 04.03	G12D	Low		
Gp2D	G12D	Low		
LS-513	G12D	Low - Moderate		
AsPC-1	G12D	Low		
HPAF-II	G12D	Low		
TOV-21G	G13C	Low		

Non-small cell lung cancer cell lines
Pancreatic adenocarcinoma cell lines
Colorectal cancer cell lines
Esophageal cell line
Ovarian cell line

low Moderate high

Figure 5. Heatmap of combination activity of voruciclib with KRAS G12C inhibitors in cancer cell lines after 72 hours. Cell lines are ranked by synergy score of voruciclib in combination with either sotorasib or adagrasib. HSA, Bliss, and Loewe analyses were performed to generate the synergy scores using Chalice Analyzer. High synergy scores are represented as dark green. Moderate synergy scores are represented in shades of green. Low to moderate synergy scores are represented in white. Cell sensitivity to KRAS G12C inhibitors are ranked by EC_{50} scores. High (<0.1 μM), Moderate (> 0.1 μM), low (>1 μM). Where sensitivities to the two inhibitors differ, a range of responses is given.

VORUCICLIB SYNERGIZES WITH SOTORASIB IN AN *IN VIVO* MIA PaCa-2 TUMOR MODEL

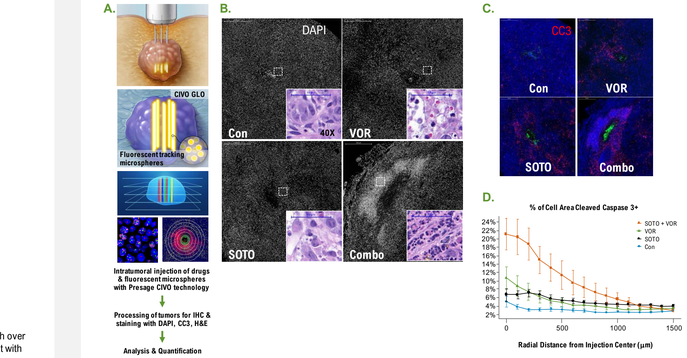


Figure 6. Voruciclib synergizes with sotorasib *in vivo*. (**A**) Presage CIVO technology[4,6] was used to inject MIA PaCa-2 cell tumors *in vivo* in a murine xenograft model. Tumors were injected with either vehicle (Con), voruciclib (VOR), sotorasib (SOTO), or voruciclib + sotorasib (Combo). Tumors were harvested and processed for IHC 24 hr after drug injection. (**B**) Representative IHC images of DAPI and H&E staining. (**C**) Representative IHC images of cleaved caspase-3 staining (CC3). (**D**) Analysis of cell area with cleaved caspase-3 staining for each treatment. (Mean ± SEM) (**C, E**) Data represents 5 tumors with duplicate combination and SOTO injection sites per tumor, single injection sites other conditions; 4 sections imaged per tumor. Similar results were obtained for VOR + adagrasib combination (data not shown).

CONCLUSIONS

- MYC is implicated in *KRAS* mutant tumors. CDK9 is a known regulator of *MYC* transcription and a modulator of MYC protein phosphorylation at Ser62. Treatment of *KRAS* G12C mutant MIA PaCa-2 pancreatic cancer cells with voruciclib, a potent inhibitor of CDKs 9/4/6/1, resulted in a rapid decrease in both phosphorylation of proteins that regulate transcription of *MYC*, and in phosphorylation of MYC protein on Ser62 that was followed by a reduction in total MYC protein.
- In *in vitro* and *in vivo* preclinical models, voruciclib demonstrated single agent efficacy against multiple *KRAS* mutant cancer cell lines harboring various G12, G13, and Q61 mutations.
- Voruciclib acted synergistically with KRAS G12C inhibitors in killing *KRAS* G12C mutant cancer cell lines, both *in vitro* and *in vivo*.
- Collectively, these experiments suggest that voruciclib could be an attractive therapeutic option for cancers driven by KRAS-MYC.

REFERENCES

1. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. *Cell.* 2017;170(1):17-33.
2. Blake DR, et al. Application of a MYC degradation screen identifies sensitivity to CDK9 inhibitors in KRAS-mutant pancreatic cancer. *Sci Signal.* 2019;12(590):eaav7259.
3. Kalkat M, et al. MYC deregulation in primary human cancers. *Genes (Basel).* 2017;8(6):151.
4. Dey J, et al. Voruciclib, a clinical stage oral CDK9 inhibitor, represses MCL-1 and sensitizes high-risk Diffuse Large B-cell Lymphoma to BCL2 inhibition. *Sci Rep.* 2017;7(1):18007.
5. Luedtke DA, et al. Inhibition of CDK9 by voruciclib synergistically enhances cell death induced by the Bcl-2 selective inhibitor venetoclax in preclinical models of acute myeloid leukemia. *Signal Transduct Target Ther.* 2020;5(1):17.
6. Klinghoffer RA, et al. A technology platform to assess multiple cancer agents simultaneously within a patient's tumor. *Sci Tranl Med.* 2015;7(284):1-12.

ACKNOWLEDGMENTS AND DISCLOSURES

SW – employee of MEI Pharma, Inc.
YS – nothing to disclose.
YG – research grant received from MEI Pharma, Inc.
This study was funded by MEI Pharma, Inc. Editorial support provided by BluPrint Oncology Concepts, LLC.

Presented at American Association for Cancer Research (AACR) 10 – 15 Apr 2021.