

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

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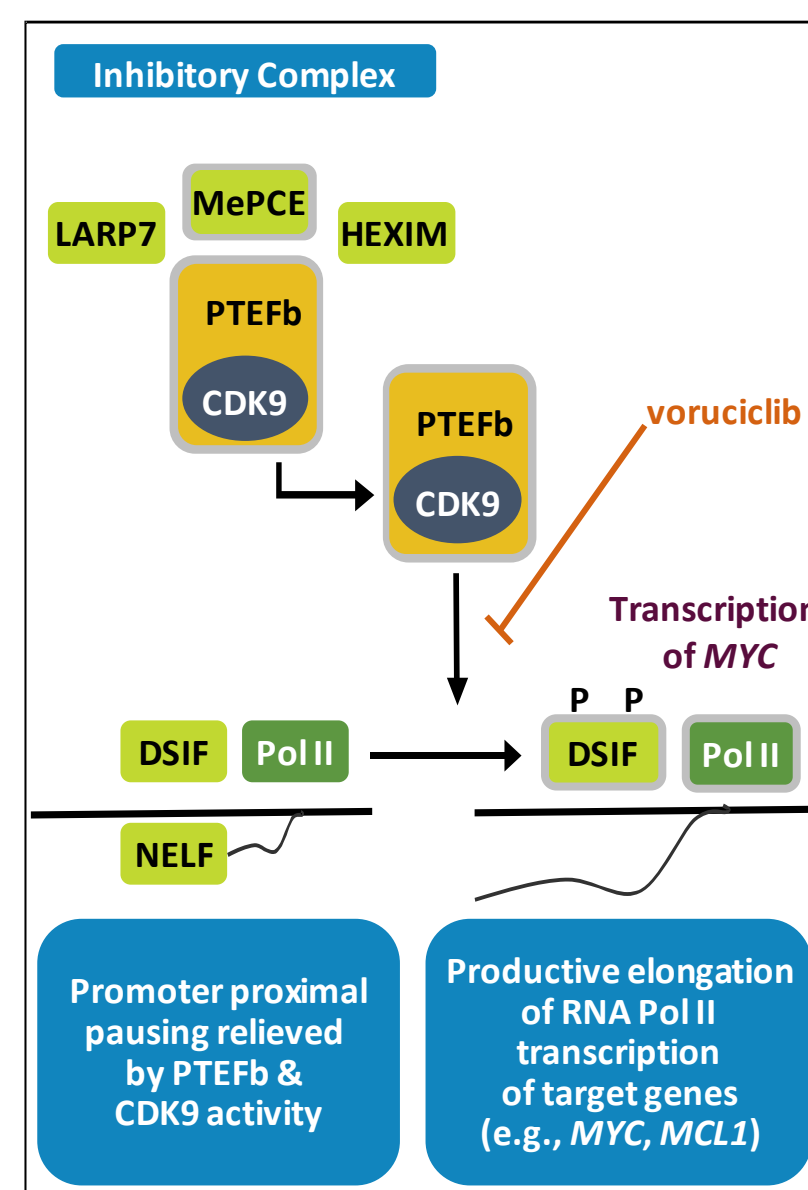
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ABSTRACT

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

A. Transcription of MYC



B. MYC protein stability

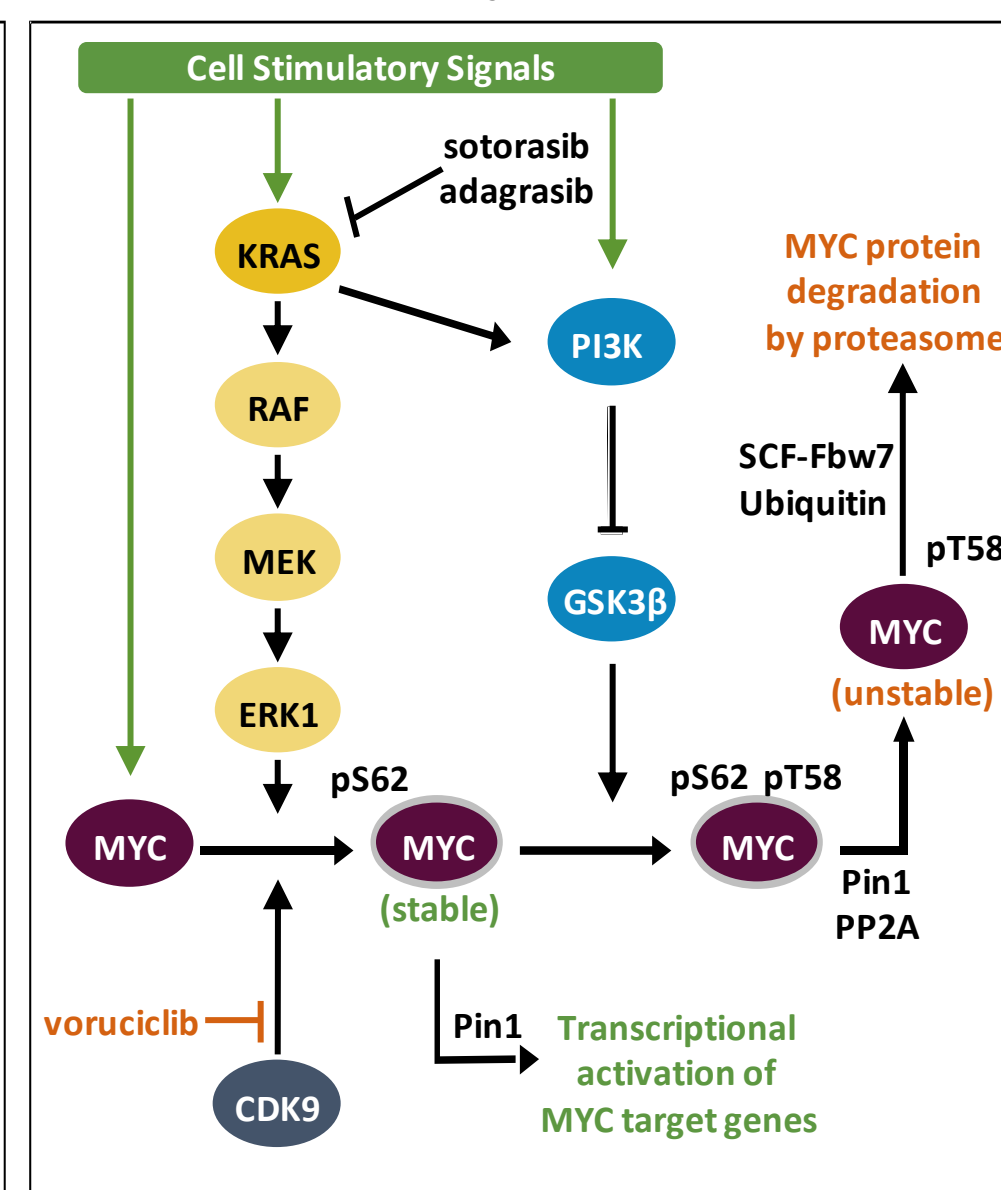


Figure 1. Schematic illustrating (A) P-TEFb regulation of RNA Pol II driven transcription of *MYC* and (B) *KRAS*-ERK1 signaling pathway and regulation of MYC protein stability by phosphorylation of Ser 62. Proteins with decreased phosphorylation after voruciclib treatment are circled in light gray.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

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

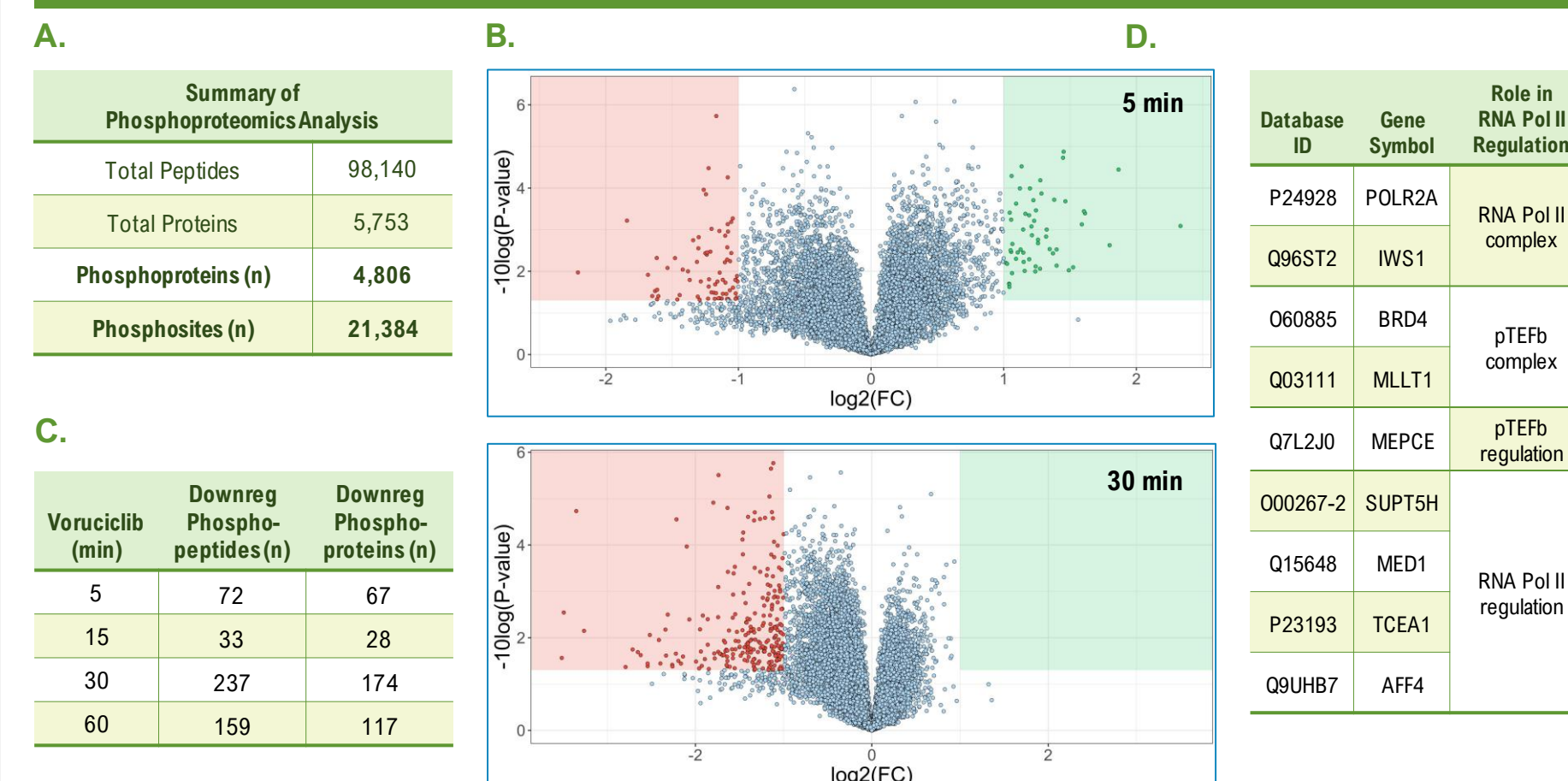


Figure 2. Landscape of the voruciclib-sensitive phosphoproteome in MIA PaCa-2 cells reveals rapid downregulation of phosphoproteins controlling transcription of *MYC*. Cells were treated with voruciclib (4 μ M) for 5, 15, 30, 60 min, followed by lysis, 16-plex TMT labeling, IMAC phosphopeptide enrichment, and analysis by LC-MS/MS. (A) Summary of total and phosphopeptide quantification for combined samples after MS. (B) Volcano plots of phosphosites (\log_2 fold change vs $-\log_{10}$ 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

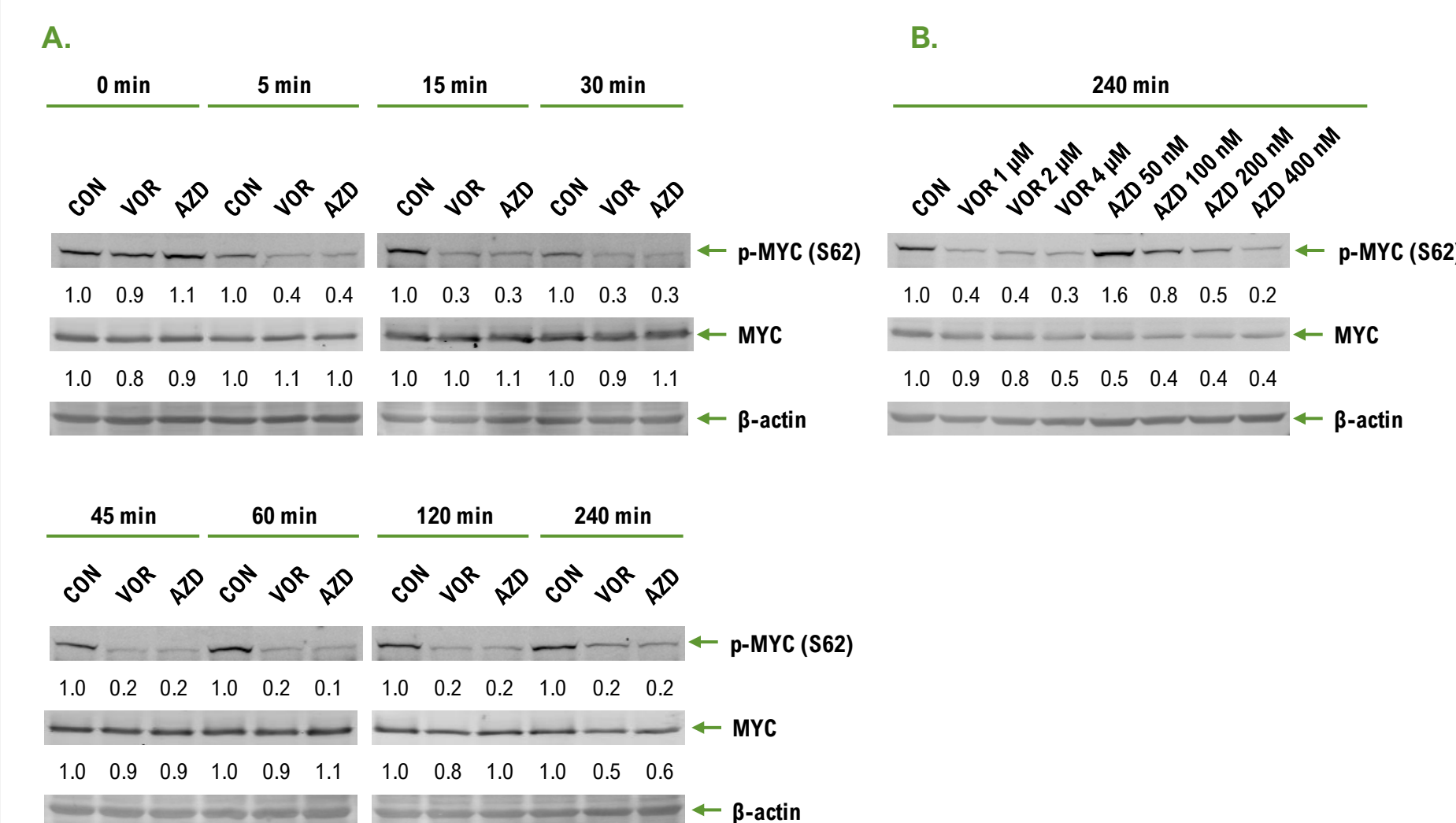


Figure 3. Immunoblot analyses of c-MYC, phospho-c-MYC (Ser62), and actin in MIA PaCa-2 *KRAS* G12C mutant PDAC cells. (A) Cells were treated with vehicle control, voruciclib (VOR, 4 μ M), or AZD4573 (AZD, a CDK9 inhibitor, 400 nM) for the indicated times. (B) Cells were treated with various concentrations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

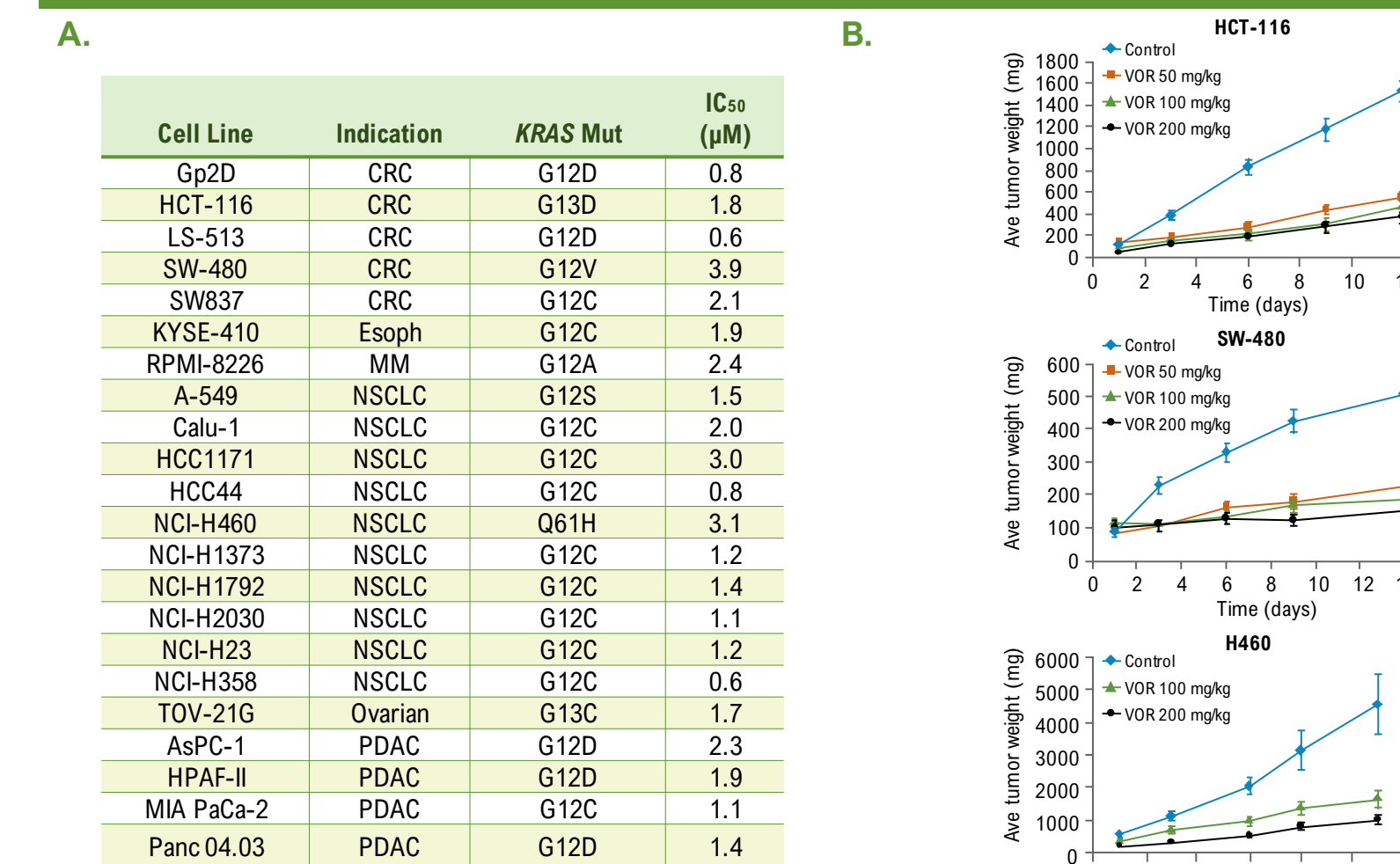


Figure 4. (A) Voruciclib IC₅₀ values in multiple cell lines with *KRAS* mutations. (B) Murine xenograft experiment showing tumor growth over time in mice bearing HCT-116 (CRC, *KRAS* G13D), SW-480 (CRC, *KRAS* G12V) or H-460 (NSCLC, *KRAS* Q61H) tumors after treatment with voruciclib (VOR) at various doses (QD, p.o.) for 11-14 days.

VORUCICLIB SYNERGIZES WITH KRAS G12C INHIBITORS IN VITRO

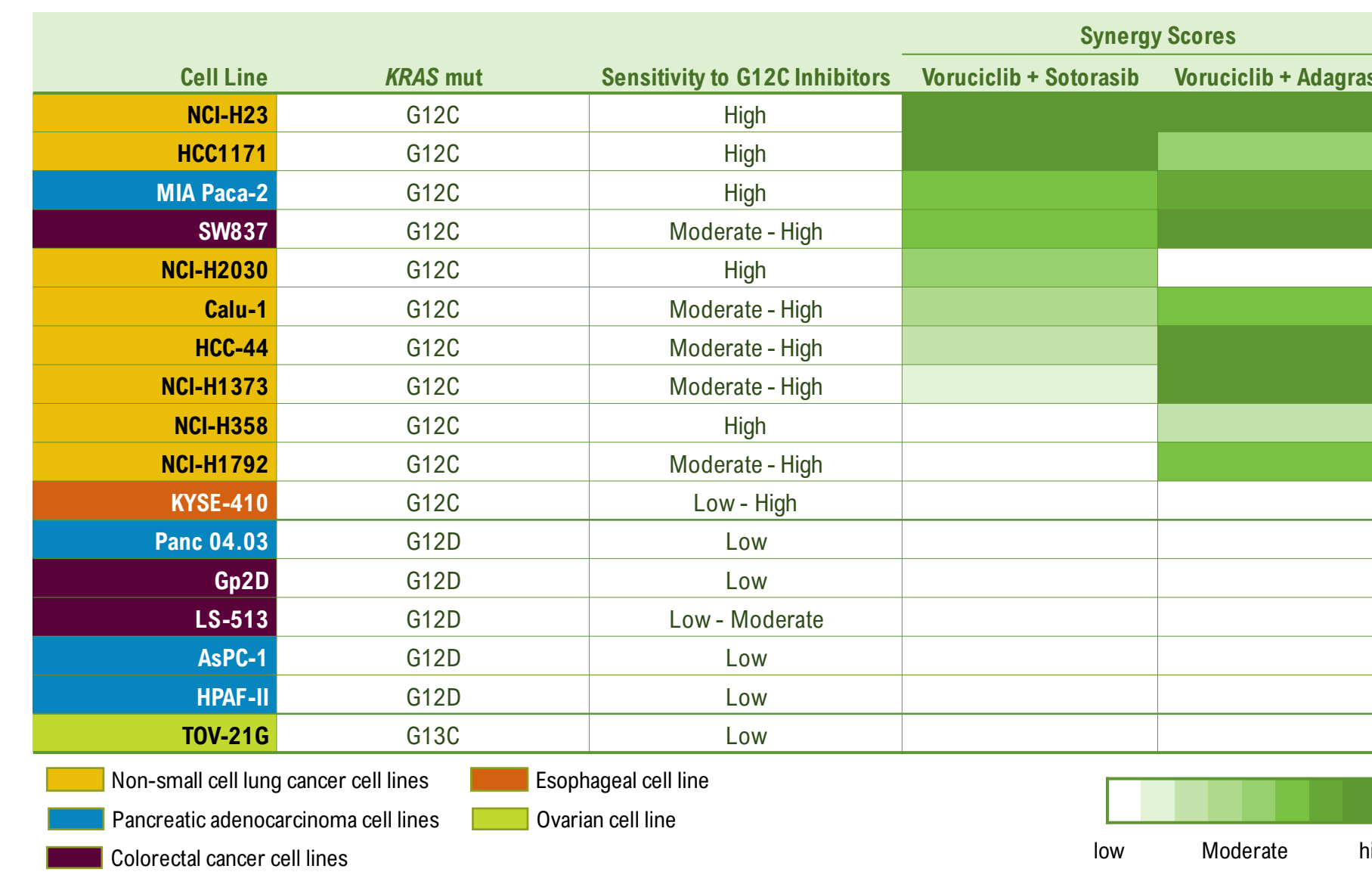


Figure 5. Heatmap of combination activity of voruciclib with *KRAS* G12C inhibitors in cancer cell lines after 72 hours. Cell lines are ranked by synergy score of voruciclib in combination with either sotorasib or adagrasib. HSA, Bliss, and Loewe analyses were performed to generate the synergy scores 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 EC₅₀ scores. High (<0.1 μ M), Moderate (> 0.1 μ M), low (> 1 μ M). 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

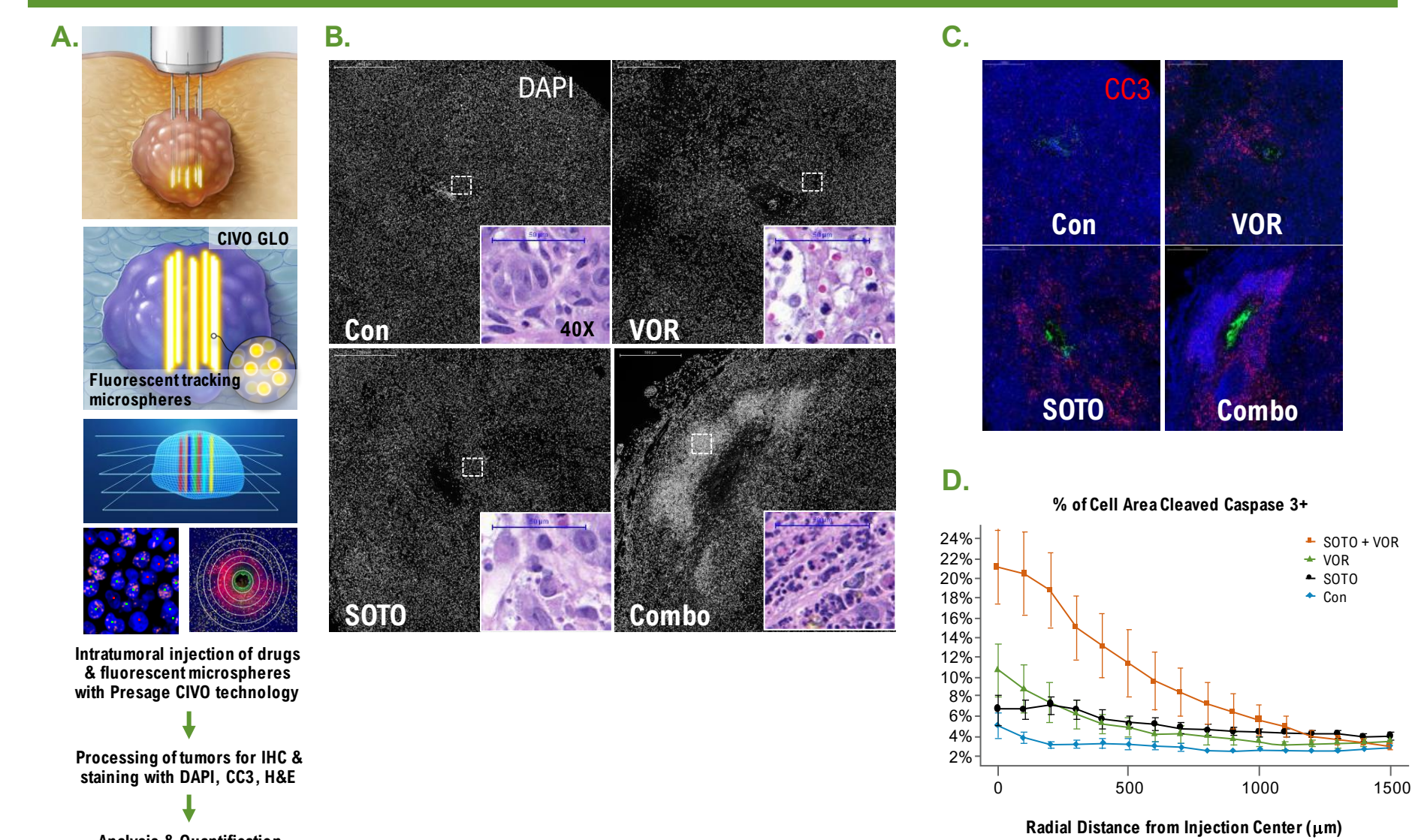


Figure 6. Voruciclib synergizes with sotorasib *in vivo*. (A) Presage CIVO technology^{4,6} was used to inject MIA PaCa-2 cell tumors *in vivo* in a murine xenograft model. Tumors were injected with either vehicle (Con), voruciclib (VOR), sotorasib (SOTO), or voruciclib + sotorasib (Combo). Tumors were 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 \pm SEM) (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 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.

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