Voruciclib, an Oral, Selective CDK9 Inhibitor, Enhances Cell Death Induced by the Bcl-2 Selective Inhibitor Venetoclax in Acute Myeloid Leukemia

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Introduction

5 year survival rates for patients with acute myeloid leukemia (AML) remain frustratingly low (65% for children and 27% for adults). Resistance to frontline chemotherapy (cytarabine and an anthracycline-based) often develops; therefore, a new treatment modality is urgently needed.

Bcl-2 family proteins play an important role in balancing cell survival and apoptosis. The anti-apoptotic protein Bcl-2 is overexpressed in both bulk AML cells and leukemic stem cells. ABT-199 (Venetoclax), a BH3 mimetic, was developed to selectively target Bcl-2. Even though ABT-199 has demonstrated promising anti-AML activity, another anti-apoptotic Bcl-2 family protein, Mcl-1, impairs its activity. Previous studies, including our own, have shown that direct targeting of both Bcl-2 and Mcl-1 with small molecule inhibitors in AML is effective. Alternatively, indirect targeting of Mcl-1 may preserve or enhance ABT-199 activity in AML cells, as well.

One approach to indirectly target Mcl-1 is to transcriptionally downregulate Mcl-1 through CDK9 inhibition. The CDK9 inhibitor flavopiridol (alvocidib) has progressed to phase II clinical trials in AML. However, off-target effects and toxicity remains a concern. A more selective CDK9 inhibitor, voruciclib, represses Mcl-1 and sensitizes high risk diffuse large B-cell lymphoma to Bcl-2 inhibition. Based on these data, we hypothesize that voruciclib will also downregulate Mcl-1 and therefore synergize with ABT-199 in AML cells.

Analogous to flavopiridol, voruciclib induced apoptosis in AML cell lines and primary patient samples at clinically achievable concentrations. Both voruciclib and flavopiridol were found to synergistically induce apoptosis in AML cells when combined with ABT-199. Voruciclib and flavopiridol were found to downregulate Mcl-1 transiently. The combination treatment was greatly enhanced when using a concentration of voruciclib or falvopiridol that downregulated Mcl-1.

Additional studies are underway to further elucidate the molecular mechanisms and to determine the *in vivo* anti-leukemia efficacy in NSGS mouse AML models.

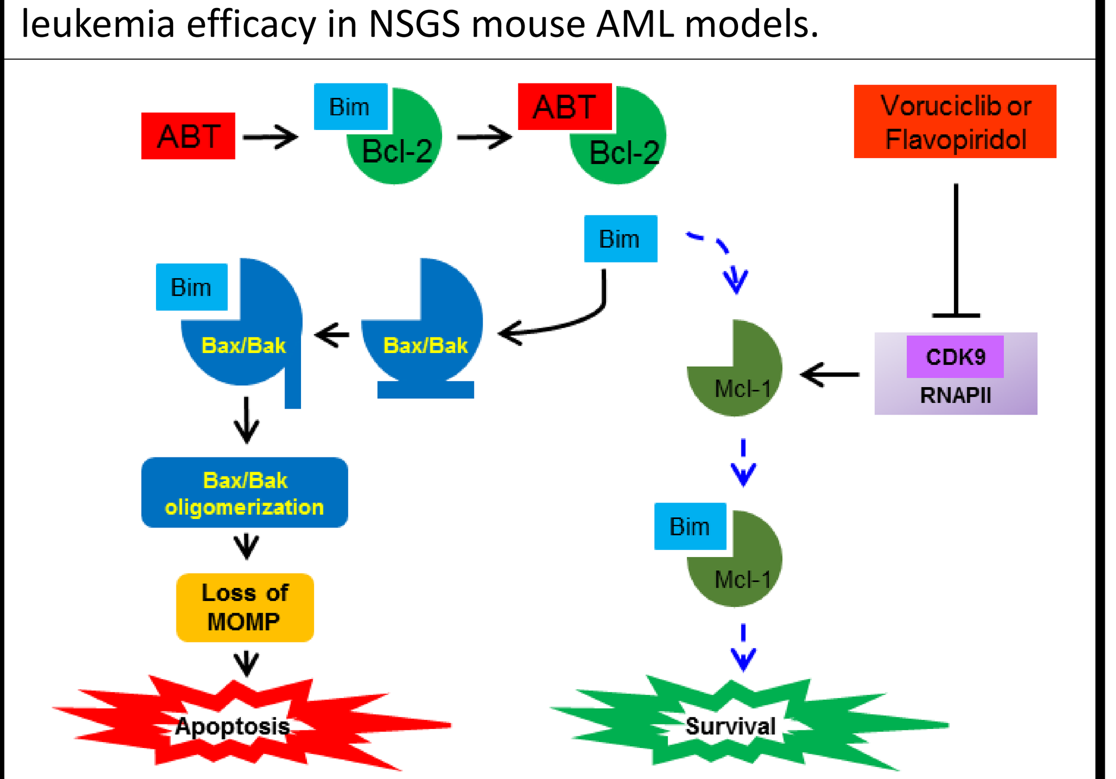

Figure 1. Proposed mechanism. ABT-199 (ABT) treatment releases Bim from Bcl-2. In sensitive cells, there is an inadequate amount of Mcl-1 to sequester all of the released Bim, resulting in free Bim, which can then activate the canonical apoptosis pathway. In ABT-199-resistant cells, the Bim released from Bcl-2 is sequestered by Mcl-1, stabilizing Mcl-1, and ultimately resulting in survival. CDK9 inhibition by voruciclib or flavopiridol reduces Mcl-1 protein levels by decreasing transcription, leading to reduced sequestration of Bim by Mcl-1. This, in combination with ABT-199, can free Bim to bind to Bak and/or Bax and induce apoptosis.

Results

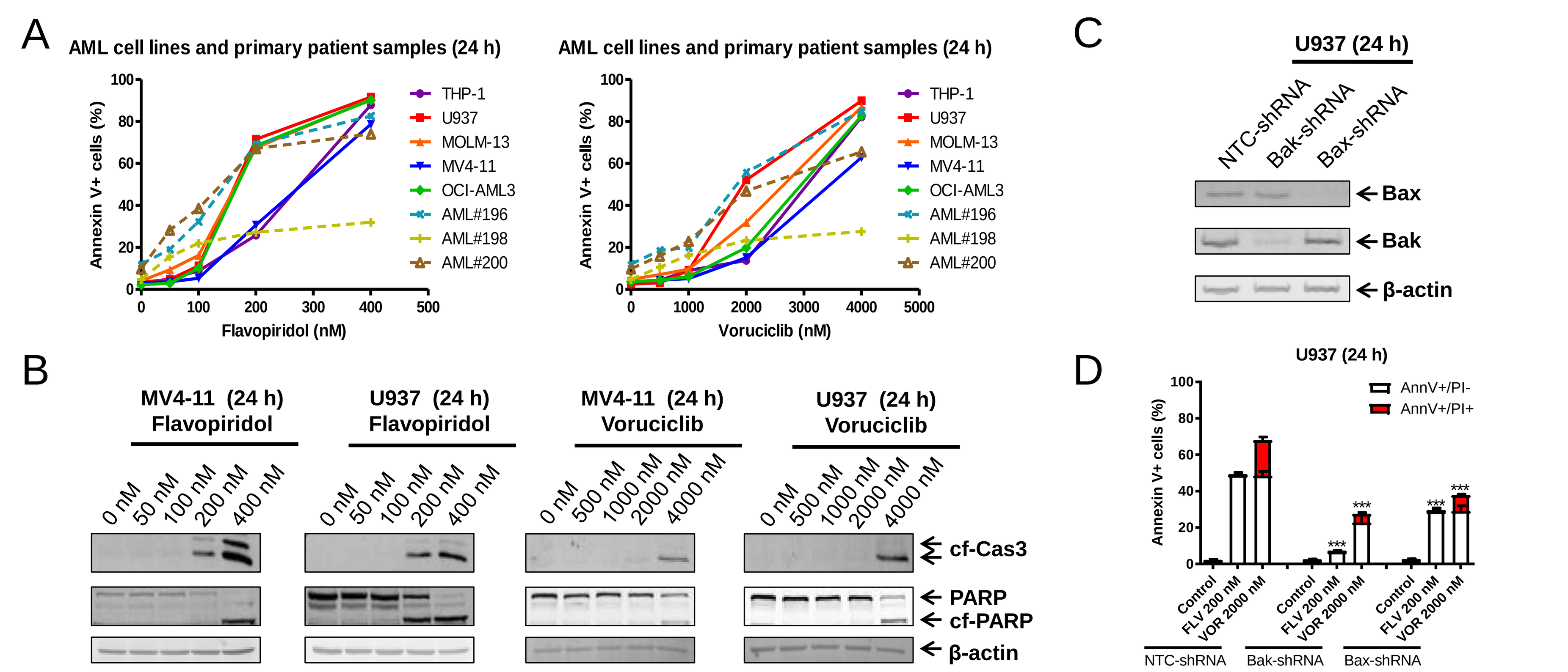

Figure 2. Voruciclib and flavopidiol induce apoptosis in AML cell lines and primary patient samples. (A) THP-1, U937, MOLM-13, MV4-11, OCI-AML3 AML cell lines and three primary patient samples were treated with voruciclib or flavopiridol for 24 h and then subjected to annexin V/PI staining and flow cytometry analyses. (B) MV4-11 and U937 cells were treated with voruciclib or flavopiridol for 24 h. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. (C) U937 cells were infected with non-template control (NTC)-, Bak-, or Bax-shRNA lentivirus. Whole cell lysates were subjected to Western blotting and probed with the indicated antibody to confirm the knockdown. (D) U937 NTC, Bak, and Bax knockdown cells were treated with voruciclib (VOR) or flavopiridol (FLV) for 24 h and then subjected to annexin V/PI staining and flow cytometry analyses. *** indicates p<0.001.

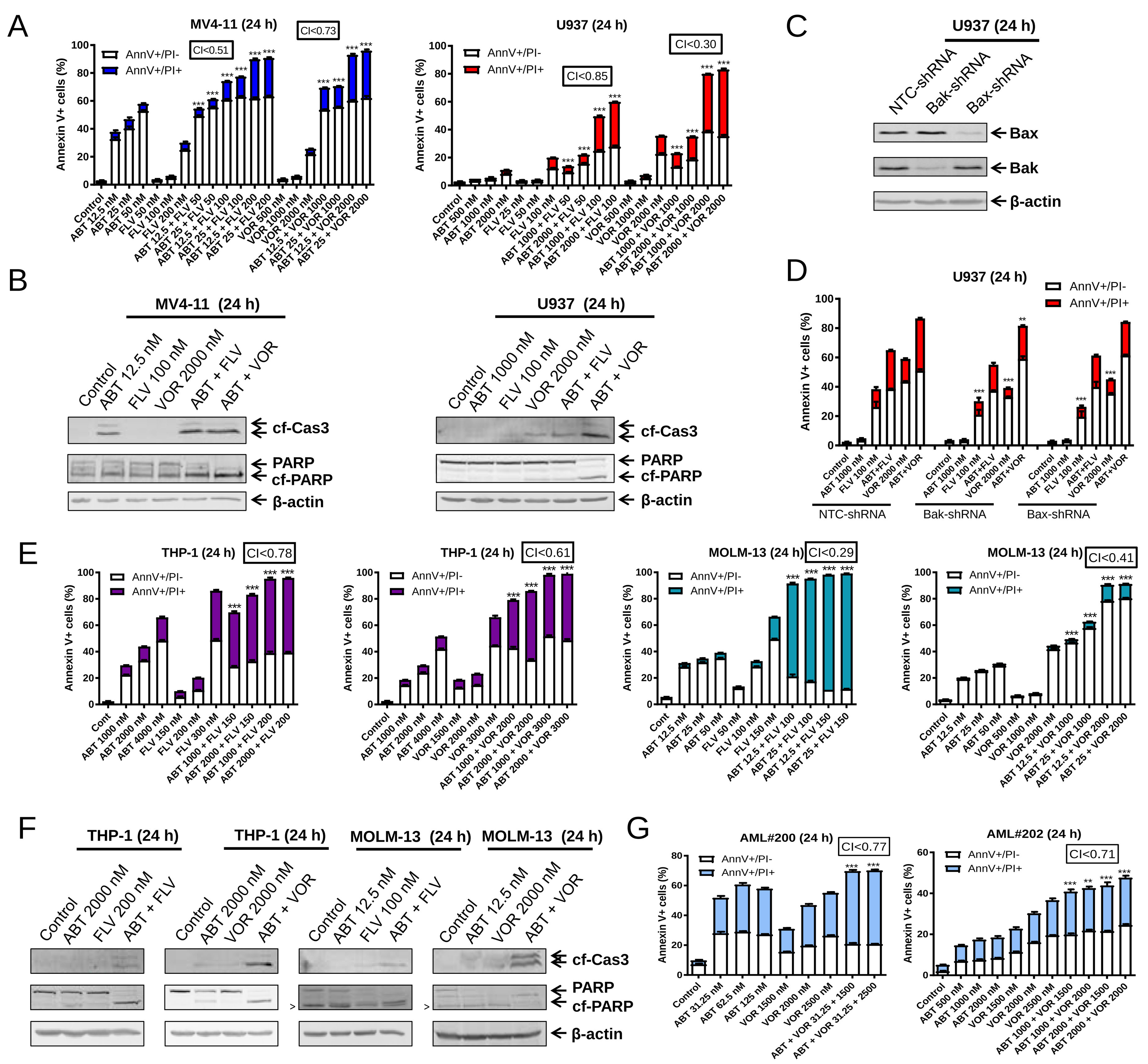

Figure 3. CDK9 inhibition synergizes with ABT-199 in AML cells. (A, E, and G) MV4-11, U937, THP-1, MOLM-13, and primary AML patient samples were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination, for 24 h and then subjected to annexin V/PI staining and flow cytometry analyses. CI values were calculated using CompuSyn software. ** indicates p<0.01, *** indicates p<0.001. (B, F) MV4-11, U937, THP-1, and MOLM-13 cells were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination, for 24 h. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. > indicates a non-specific band in MOLM-13 cells probes with PARP antibody. (C) U937 cells were infected with non-template control (NTC)-, Bak-, or Bax-shRNA lentivirus. Whole cell lysates were subjected to Western blotting and probed with the indicated antibody to confirm the knockdown. (D) U937 NTC, Bak, and Bax knockdown cells were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination, for 24 h and then subjected to annexin V/PI staining and flow cytometry analyses. ** indicates p<0.01, *** indicates p<0.001.

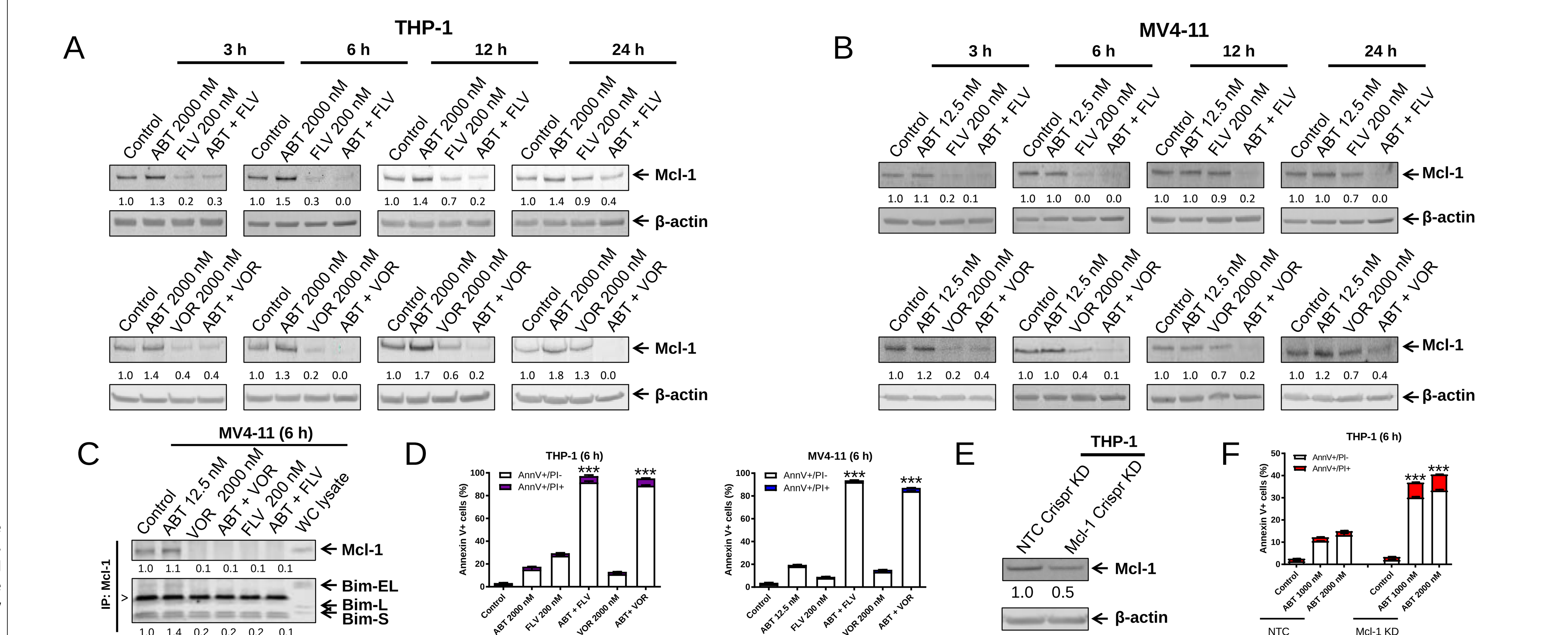

Figure 4. Mcl-1 dependent effects of voruciclib and flavopiridol on ABT-199 activity. (A and B) THP-1 and MV4-11 cells were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination, for the indicated times. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. Relative densitometry measurements were determined using Odyssey Software V3.0. (C) MV4-11 cells were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination for 6 h. Cells were immunoprecipitated from whole cell lysates and then subjected to Western blotting and probed with the indicated antibodies. Relative densitometry measurements of Mcl-1, Bim, and Bcl-2 were measured using Odyssey Software V3.0. > indicates the light chain of IgG. (D) THP-1 and MV4-11 were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination, for 6 h and then subjected to annexin V/PI staining and flow cytometry analyses. *** indicates p<0.001. (E) Crispr knockdown of Mcl-1 was generated with the indicated control (NTC). Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies to confirm the knockdown. (F) THP-1 Mcl-1 and NTC knockdown cells were treated with ABT-199 for 6 h and then subjected to annexin V/PI staining and flow cytometry analyses. *** indicates p<0.001.

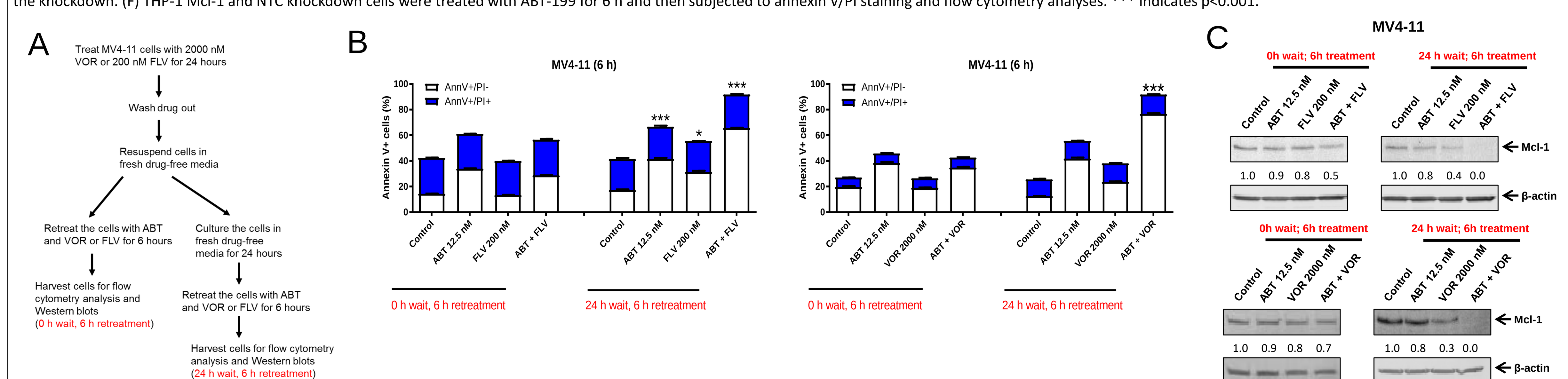

Figure 5. Effect of voruciclib or flavopiridol pretreatment on ABT-199 and voruciclib or flavopiridol activity . (A) Schematic for the rechallenge treatments. (B&C) MV4-11 cells were treated with flavopiridol or voruciclib alone for 24 h. The cells were spun and washed with PBS. Cell were cultured in drug-free medium for 0 or 24 h, then treated as indicated for 6 h. Retreated cells were subjected to annexin V/PI staining and flow cytometry analyses. * indicates p<0.05, *** indicates p<0.001 (B). Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. Relative densitometry measurements were determined using Odyssey Software V3.0 (C).

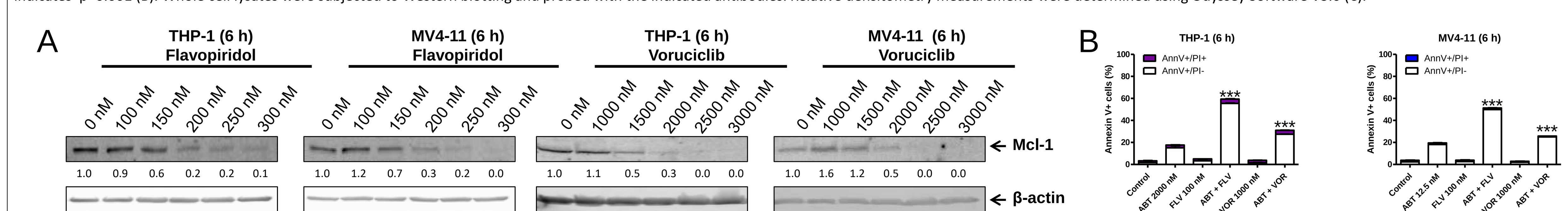

Figure 6. Mcl-1 independent effects of voruciclib and flavopiridol on ABT-199 activity. (A) THP-1 and MV4-11 cells were treated with with various concentrations of flavopiridol or voruciclib for 6h. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. Relative densitometry measurements were determined using Odyssey Software V3.0. (B) THP-1 and MV4-11 were treated with flavopiridol or voruciclib, and ABT-199, alone or in combination, for 6 h and then subjected to annexin V/PI staining and flow cytometry analyses. *** indicates p<0.001.

Conclusions and Future Work

Conclusions:

- Voruciclib and flavopiridol both synergize with ABT-199 to induce apoptosis in both venetoclax sensitive and resistant AML cells
- Voruciclib and flavopiridol both transiently downregulate Mcl-1
- Mcl-1 downregulation is likely responsible for the bulk of the synergy between voruciclib and ABT-199 as well as flavopiridol and ABT-199

Future studies:

- Further determination of the molecular mechanism underlying the synergy between ABT-199 and voruciclib or flavopiridol
- *In vivo* efficacy in MV4-11- and patient-derived xenograft models in NSGS mice
- Alternative molecular mechanisms

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