# CDK9 inhibition enhances apoptosis of TP53 mutated AML when combined with standard chemotherapy

#1626

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## Background

- Outcomes for patients with TP53 mutated acute myeloid leukemia remain extremely poor with both chemotherapy alone and with the addition of stem cell transplantation.
- Inhibiting CDK9 is a strategy to trigger apoptosis downstream of p53, making mutated p53 less relevant.
- CDK9 is required for mRNA processing and elongation.
- CDK9 inhibition results in loss of short half-life proteins such as MCL-1, and survivin—molecules AML cells depend on.
- These proteins are downstream from p53, hence, CDK9 inhibition will target these proteins regardless of p53 status.
- The inhibition of CDK9 causes stalling of RNAPII, transcriptional arrest, and loss of short half-life proteins leading to DNA damage and apoptosis.
- Dinaciclib and SLS009 are potent CDK9 inhibitors.

## Aims

- Establish optimal conditions, doses, and exposure times of CDK9 inhibitors and other chemotherapy agents, which lead to apoptosis of TP53 mutated AML cell lines.
- Determine an optimal chemotherapy regimen for CDK9 inhibitors with additional agents for an in vivo study.
- Examine the synergistic effects of CDK9 inhibition with standard chemotherapy agents.

## Azacitidine, venetoclax, dinaciclib, and SLS009

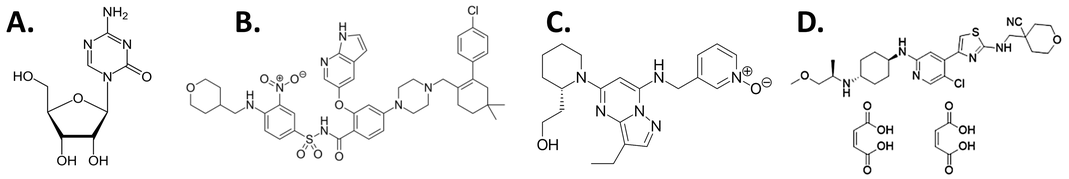


**Figure 1.**
**(A) Azacitidine** acts as a hypomethylating agent, reactivating tumor suppressor genes.
**(B) Venetoclax** inhibits anti-apoptotic protein BCL-2, promoting apoptosis.
**(C) Dinaciclib** is a potent inhibitor of CDK1, CDK2, CDK5 and CDK9.
**(D) SLS009** is a highly selective CDK9 inhibitor.

## Single-agent treatments cause cell death

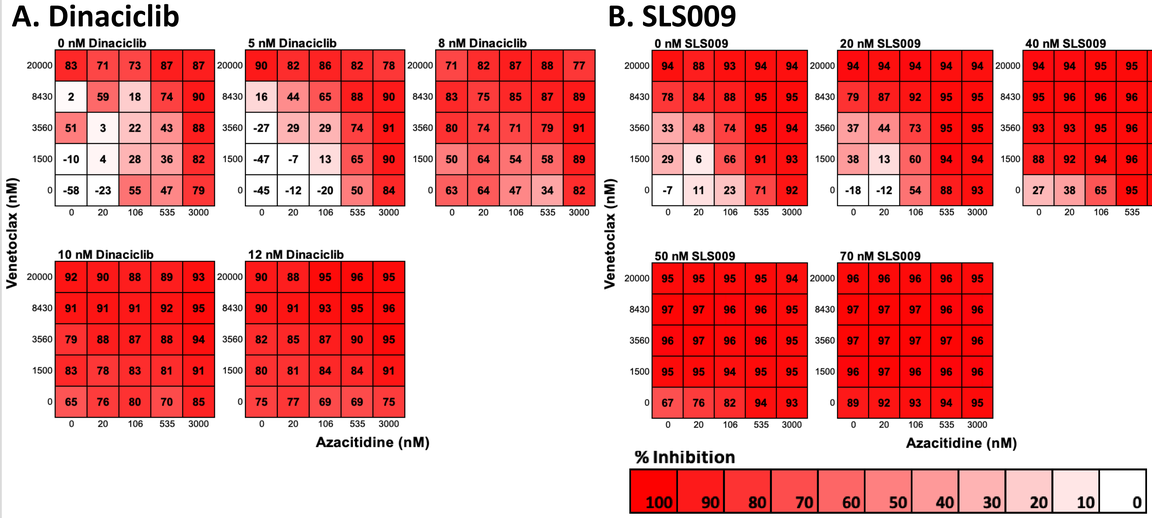


**Figure 2. THP1 cells were treated for 72-hours to establish IC50's. Cell viability was measured using Cell Titer-Glo.** Treatment of THP1 cells with azacitidine, venetoclax, dinaciclib and SLS009 demonstrates a dose-dependent decrease in cell viability, with $IC_{50}$'s of 237 nM, 7158 nM, 6.3 nM, and 43.3 nM, respectively.

## Triple-drug treatments decrease cell viability




**Figure 3. THP1 cells were treated with azacitidine, venetoclax, and dinaciclib or SLS009 for 8-hours. Cell Titer-Glo was used to measure cell viability after 72-hours.**
**(A)** Triple-drug therapy with **azacitidine**, **venetoclax**, and **dinaciclib** results in high cytotoxicity in the p53 mutated THP1 cell line.
**(B)** Triple-drug therapy with **azacitidine**, **venetoclax**, and **SLS009** results in high cytotoxicity in the p53 mutated THP1 cell line.
**(C)** Triple-drug therapy with **azacitidine**, **venetoclax**, and **dinaciclib** results in low cytotoxicity in the normal peripheral blood mononuclear cells (PBMC's).

## Apoptotic proteins

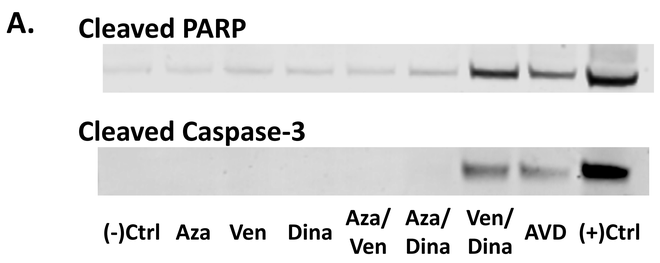


**Figure 4**. **PARP cleavage** and **cleaved caspase-3** expression according to single-agent and combination treatments.
**(A)** Immunoblot of THP1 cells were exposed to 200 nM azacitidine, 6000 nM venetoclax, and 10 nM dinaciclib for 8-hours.
**(B)** Quantification of protein levels using fluorescence. Protein levels were normalized so the (-)ctrl is 0% and (+)ctrl is 100%.

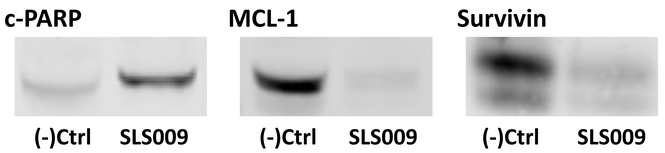


**Figure 5.** THP1 cells exposed to 50 nM **SLS009** for 8-hours undergo **PARP cleavage** in association with **loss** of both **MCL-1** and **survivin** expression.

## Conclusions

- CDK9 inhibition results in cytotoxicity of TP53 mutated AML cells at low concentrations of dinaciclib and SLS009.
- CDK9 inhibition results in apoptosis associated with low expression of short half-life molecules.
- Cytotoxicity of conventional chemotherapy agents is enhanced by CDK9 inhibition.
- Testing triple therapy in a clinical trial is warranted.

## Contact

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## References

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