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# Tambiciclib (SLS009), a CDK9 inhibitor, promotes apoptosis and suppresses MCL-1 levels in AML cell lines

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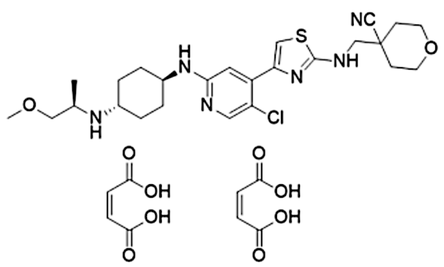
## Background

- Tambiciclib (SLS009) is a potent, selective CDK9 inhibitor that has shown promising results in Phase I clinical trials in hematological malignancies (NCT04588922).
- **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, and induces caspase-3 cleavage, an indicator of apoptosis.
- The **inhibition of CDK9** causes **stalling** of **RNAPII** and transcriptional arrest leading to DNA damage and **apoptosis**.
- Inhibiting CDK9 is a strategy to trigger **apoptosis** downstream of p53, making **mutated p53 less relevant**.

| | P53 mut. | MLL-AF9 rearr. | ASXL1 mut. | NRAS mut. | FLT3 mut. | P53 KO |
|---|---|---|---|---|---|---|
| THP1 | X | X | | X | | |
| NOMO-1 | X | X | X | | | |
| MOLM-13 (WT) | | X | | X | X | |
| MOLM-13 (p53 KO) | | X | | X | X | X |

**Table 1.** Cell lines tested and their phenotypes.




**Tambiciclib (SLS009)**

## Aims

- Establish **optimal conditions**, **doses**, and **exposure times** of tambiciclib and other chemotherapy agents that lead to apoptosis of TP53 mutated AML cell lines, like **THP1**.
- Examine the **synergistic** effects of CDK9 inhibition with standard chemotherapy agents.
- Examine the mechanistic effects of tambiciclib exposure correlated with apoptosis and cell death in AML cell lines.

## Apoptotic proteins

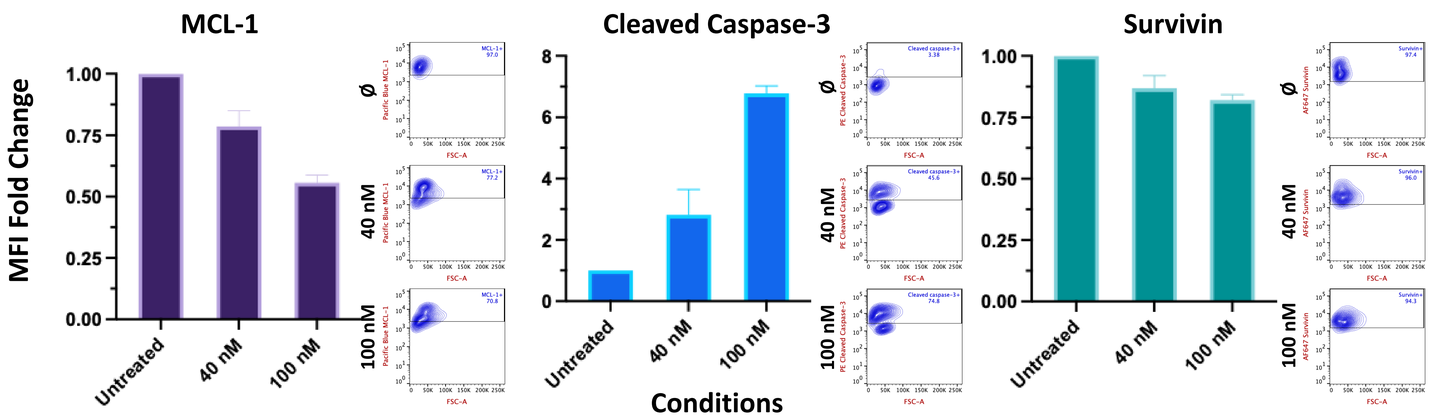



**Figure 1. (A)** THP1 cells were treated with 40 nM or 100 nM **SLS009** for **6 hours**. Cells were fixed 24 hours after the beginning of treatment, stained, then analyzed by **flow cytometry**. The median fluorescence intensity (MFI) was measured and normalized to the untreated condition.

## Repeated 8-hour treatments cause cell death

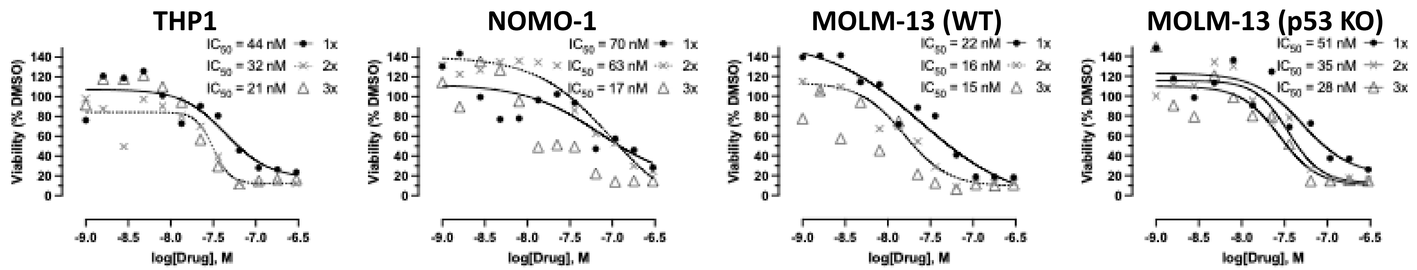



**Figure 2. THP1, NOMO-1, MOLM-13 (WT), and MOLM-13 (p53 KO)** cells were treated with **SLS009** for **8 hours** and treatments were repeated for one, two, and three days. Cell viability was measured using Cell Titer-Glo.

## Synergy

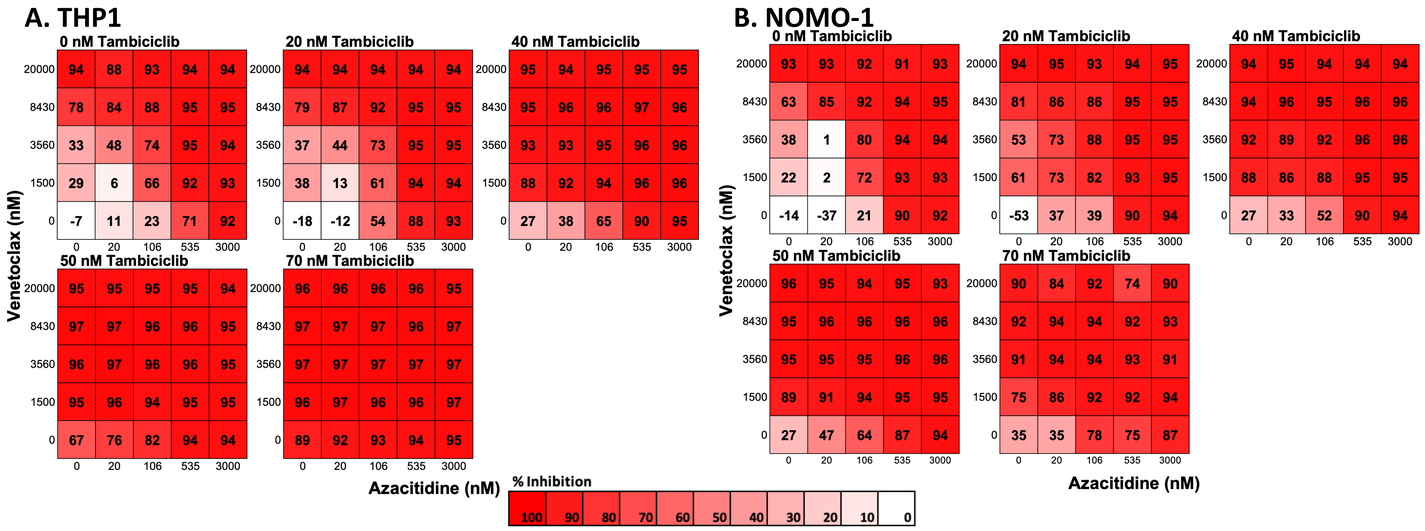
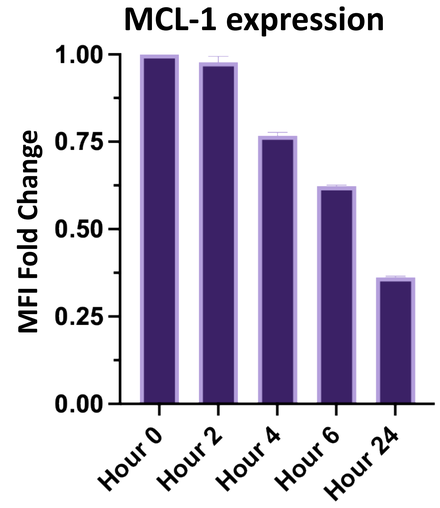



**Figure 3. THP1 (A) and NOMO-1 (B) cells** were treated with **azacitidine, venetoclax,** and **SLS009** for **8 hours. Cell Titer-Glo** was used to measure **cell viability** and examine **synergy** after 72-hours.

## MCL-1



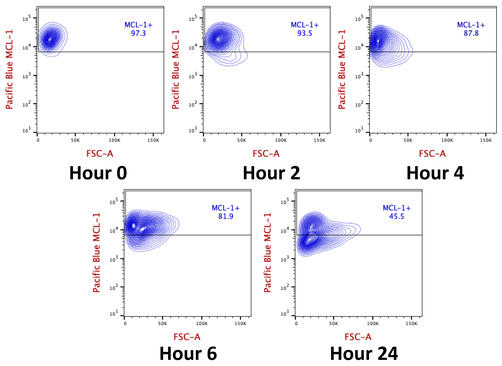




**Figure 4.** THP1 cells were exposed to 100 nM **SLS009** for 6 hours.
Samples were fixed at the beginning (Hour 0), throughout (Hour 2, Hour 4), end (Hour 6), and 18-hours after drug-washout (Hour 24). Intracellular staining of MCL-1 was performed and analyzed by flow cytometry.

## Conclusions

- **CDK9 inhibition** results in **cytotoxicity** of AML cell lines with high-risk mutations at low nanomolar concentrations of SLS009.
- **CDK9 inhibition** results in **apoptosis** associated with changes in the expression of short half-life molecules.
- Preliminary analysis of short-half life proteins and apoptotic molecules suggest tambiciclib may be used to decrease MCL-1.
- **Cytotoxicity** of conventional chemotherapy agents is **enhanced by CDK9 inhibition**.

## Contact

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## References

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