Enhancing Sensitivity of Leukemia Cells to Venetoclax Through PINK1 Alternative Splicing

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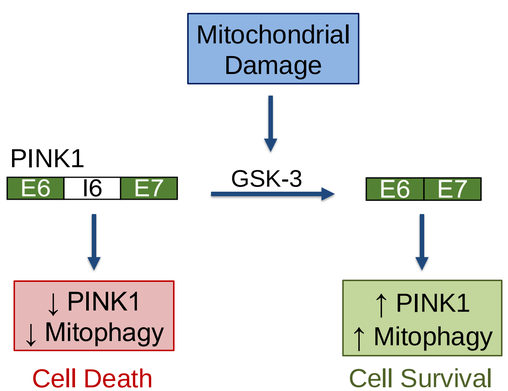
Background

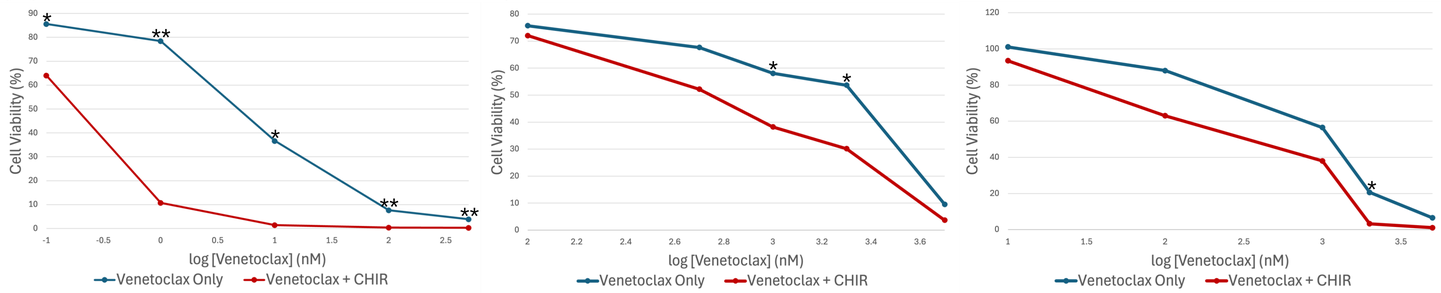
- Acute Myeloid Leukemia (AML) is a cancer of the blood in which there's an over-proliferation of immature white blood cells.
- The BCL2 inhibitor Venetoclax is a commonly used drug to treat AML through permeabilizing mitochondria and promoting apoptosis of leukemia cells.
- However, ~30% of patients fail to respond and most eventually become resistant. Cells can develop resistance to Venetoclax through increased mitophagy, the digestion and recycling of damaged mitochondria.
- Mitophagy is regulated by alternative splicing of intron 6 in the PINK1 gene by GSK-3.
- **We hypothesized that the inhibition of GSK-3 with the drug CHIR99021 (CHIR) would enhance sensitivity of AML cells to Venetoclax by limiting mitophagy through PINK1 splicing.**



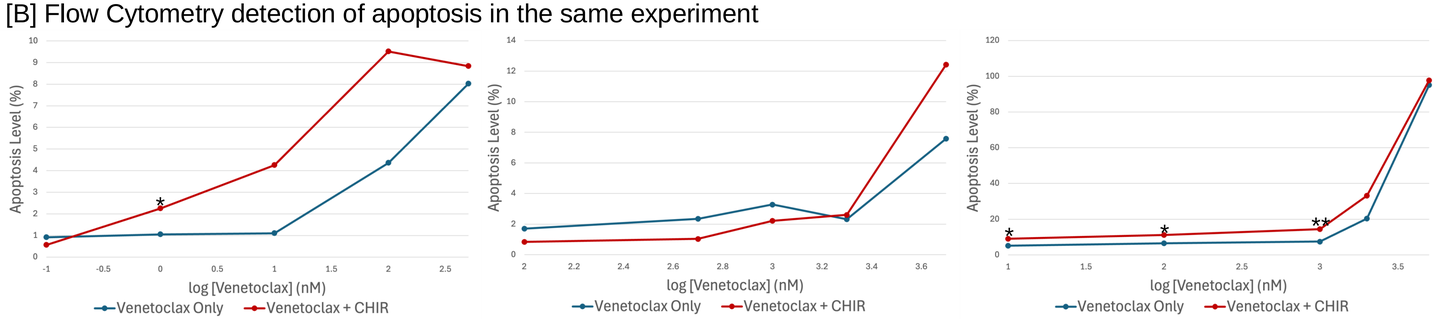
Results

MV4-11 | OCI-AML3 | MOLM-13

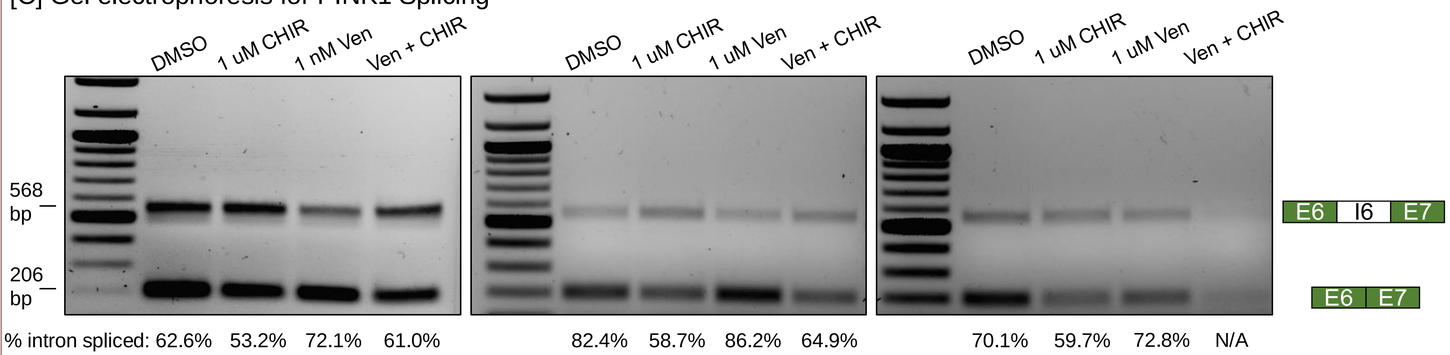
[A] Cell Proliferation of Venetoclax dose-response with and without CHIR



[B] Flow Cytometry detection of apoptosis in the same experiment



[C] Gel electrophoresis for PINK1 Splicing



% intron spliced: 62.6% 53.2% 72.1% 61.0% | 82.4% 58.7% 86.2% 64.9% | 70.1% 59.7% 72.8% N/A

[A] GSK-3 inhibition enhanced sensitivity to Venetoclax in all cell lines, but most significantly in MV4-11. Cell lines were treated every other day with varying concentrations of Venetoclax with and without 1 uM CHIR. Cell numbers were counted on Day 6 using a hemocytometer and compared to a DMSO-only control group to calculate cell viability percentage. **[B] Treatment with both drugs induced higher levels of apoptosis for MV4-11 and MOLM-13 cell lines but not for OCI-AML3.** Cells were stained on Day 6 with 7-AAD and Annexin V to quantify apoptosis through flow cytometry. It's possible that these results were not as significant as cell proliferation because most cells were already dead by Day 6. **[C] PINK1 splicing increased with Venetoclax treatment and decreased with CHIR treatment for all cell lines.** Agarose gels were run with DNA acquired from purifying RNA on Day 4, treating with DNAse, reverse transcribing, and performing PCR with primers from Exon 6 and 7 of PINK1. Percent intron retention was calculated through ImageJ analysis of grayscale levels.
* $p < 0.05$. ** $p < 0.005$.

Conclusion

- Treatment of Venetoclax and CHIR has a synergistic effect for all three cell lines.
- PINK1 Splicing decreased with GSK3 inhibition and increased with Venetoclax treatment.
- **These data support our hypothesis that inhibition of mitophagy with CHIR enhances cell sensitivity to Venetoclax.**

Next Steps

- Reconduct flow cytometry for all cell lines at an earlier date in the experiment.
- Create a Venetoclax-resistant MV4-11 cell line and test if GSK-3 inhibition will re-sensitize the cells.
- Test if overexpression of PINK1 among cell lines will confer resistance to Venetoclax.

Acknowledgments

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