DELINEATING THE TRAJECTORIES OF VENETOCLAX-AZACITIDINE COMBINATION THERAPY RESISTANCE IN ACUTE MYELOID LEUKEMIA

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AML-672

1. INTRODUCTION

The treatment regimen for acute myeloid leukemia (AML) patients has largely diversified beyond the conventional 3+7 chemotherapy treatment.

The combination of venetoclax with hypomethylating agents such as azacitidine and decitabine was approved by the US FDA in November 2018 for the treatment of AML patients.

Reports from the VIALE-A trial suggest better rates of complete remission (CR) along with improved overall survival. The composite response rates (CRR) were 61% with a median overall survival (OS) of 14.2 months.

However, another study by Stahl et al. 2021 suggests that in relapsed refractory AMLs, the CRR is as low as 24%. Also, about 60% of the treated patients present with a relapsed disease, which necessitates **the study of genetic and clonal trajectories of resistance to venetoclax-based combination regimens and the discovery of additional molecular therapeutic targets for the treatment of venetoclax-resistant patients**.

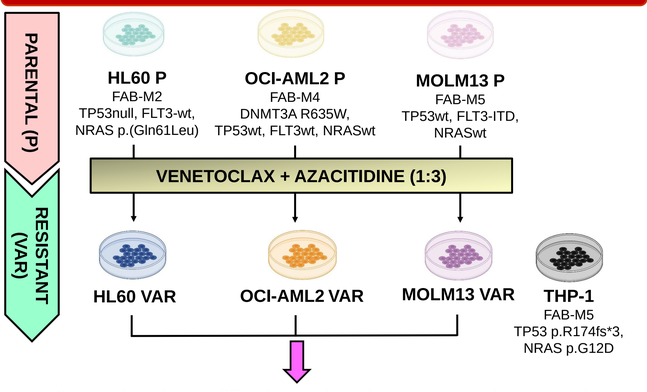
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2. HYPOTHESIS AND AIM

We hypothesize that the **Ven-Aza-resistant leukemic cells shift their apoptotic dependencies from BCL2**, which helps them survive venetoclax treatment. They also have **altered metabolic preferences** through which they bypass the Ven-Aza-mediated metabolic inhibition. These resistant cells may also have **a differential mitochondriome** compared to the sensitive cells.

To decipher the underlying mechanisms of Ven-Aza resistance and evaluate the efficacy of new therapeutics in Ven-Aza-resistant AMLs

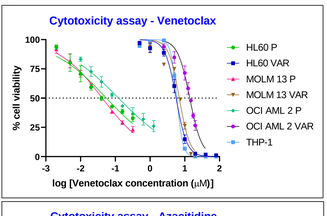
3. METHODOLOGY

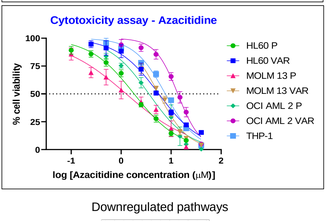


Comprehensive profiling for markers for Ven-Aza resistance and introduction of new small molecule inhibitors in this disease setting

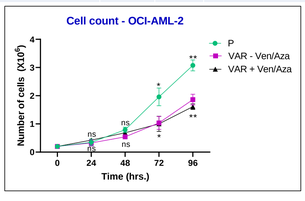
3. RESULTS

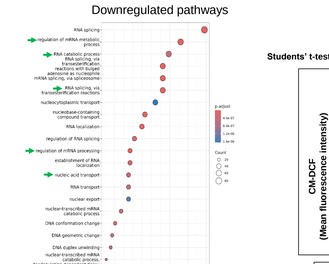
3.1. Establishment and characterization of Ven-Aza-resistant AML cell lines



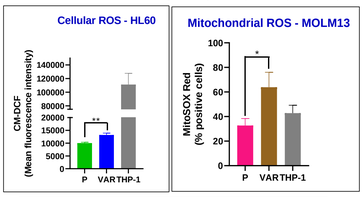


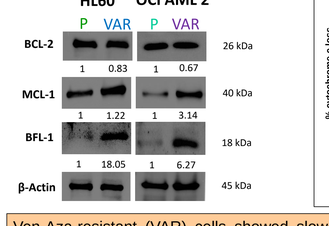
Cell line	IC50 (in μM)		RI	
	VEN	AZA	VEN	AZA
HL60 P	0.055	1.819	**102.5**	**3.12**
HL60 VAR	5.637	5.674		
OCI-AML2 P	0.123	3.6	**112.9**	**3.78**
OCI-AML2 VAR	13.89	13.6		
MOLM13 P	0.042	1.261	**165.8**	**4.47**
MOLM13 VAR	6.962	5.633		
THP-1	5.886	7.666		

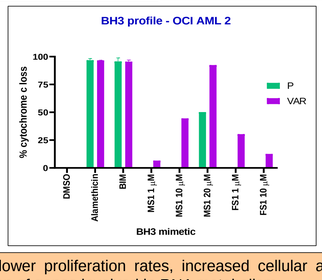




Students' t-test, two-tailed. *Mean ± SD.* ns not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

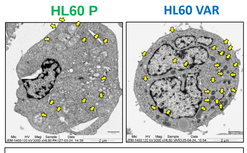




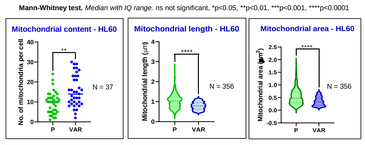


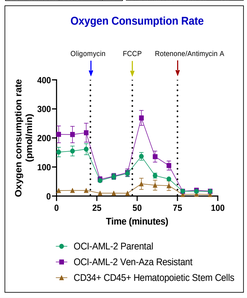
Ven-Aza-resistant (VAR) cells showed slower proliferation rates, increased cellular and mitochondrial ROS, and had low expression of genes involved in RNA metabolism.

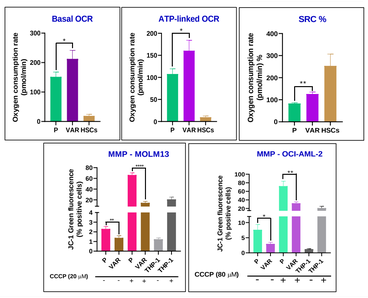
3.2. Investigating mitochondrial plasticity in Ven-Aza resistant AMLs



Mann-Whitney test. *Median with IQ range.* ns not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

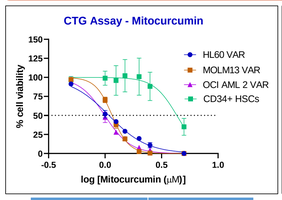


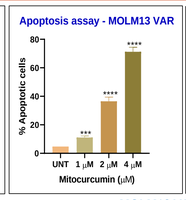


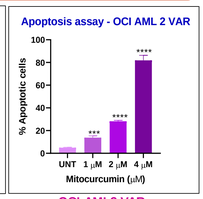


Ven-Aza-resistant (VAR) cells had a higher number of smaller and rounded mitochondria and also had high basal and ATP-linked OCRs and high spare reserve capacity. They also possessed hyperpolarized mitochondria and showed a preference for OXPHOS.

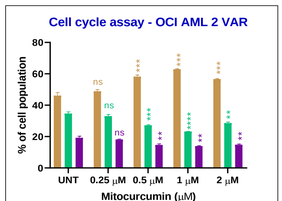
3.3. Exploring the efficacy of mitocurcumin in Ven-Aza resistant AMLs

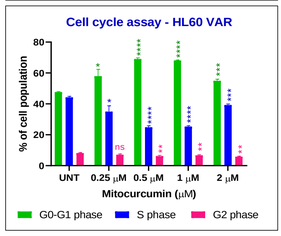


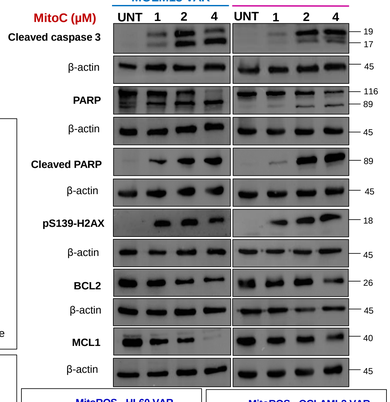


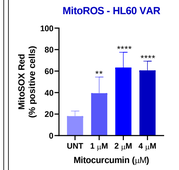


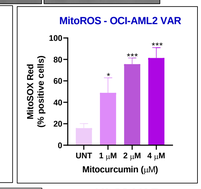
CELL LINE	IC50 (in μM)
HL60 VAR	1.082
MOLM13 VAR	1.156
OCI-AML-2 VAR	0.9943
CD34+ HSCs	4.715

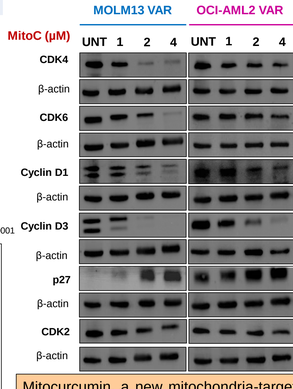


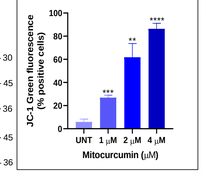


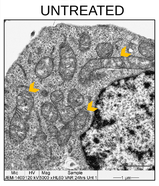


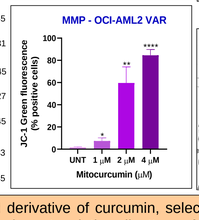


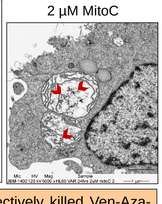












Mitocurcumin, a new mitochondria-targeted derivative of curcumin, selectively killed Ven-Aza-resistant leukemic cells while sparing healthy hematopoietic cells. It was found to localize in the mitochondrial matrix, causing mitochondrial blebbing, elevated mitochondrial ROS, triggering depolarization of the mitochondrial membrane potential, and arresting the cell cycle in the G0-G1 phase, ultimately activating the intrinsic apoptotic pathway in these cells.

4. CONCLUSIONS

VAR leukemic cells shift their apoptotic dependencies, undergoing distinct metabolic reprogramming for survival. Mitocurcumin may be considered one of the leading molecules for the treatment of VAR AMLs.

5. ACKNOWLEDGEMENTS

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