# #1863 Development and Characterization of the Venetoclax-Resistant AML Model to Enable Preclinical Drug Discovery

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## Abstract

Acute myeloid leukemia (AML) is an aggressive type of hematological cancer, causing over 100,000 death per year globally. Venetoclax, a BCL-2 antagonist, was approved in 2018 for the treatment of AML in combination with Azacytidine[1]. Through neutralizing BCL-2 function, Venetoclax (Ven) restores the apoptotic cascade in tumor cells. However, long-term efficacy of Venetoclax is often limited by the development of drug resistance, underscoring the need for preclinical Venetoclax-resistant (Ven-R) tumor models[2].

In this study, we successfully established two Venetoclax-resistant cell lines, Ven-R-MV4-11 and Ven-R-MOLM-13, through chronic exposure to Venetoclax. Compared to the parental cell lines, both resistant cell lines exhibited increased expression of MCL-1, an anti-apoptotic protein implicated crucial in Venetoclax resistance. Cell viability assays in these resistant cell lines demonstrated strong synergy between Venetoclax and two MCL-1 inhibitors, AMG-176 and MIK665. *In vivo*, both Ven-R-MV4-11 and Ven-R-MOLM-13 models showed increased growth rate and substantial resistance to Venetoclax alone and in combination with Azacytidine (standard therapy). Interestingly, the MCL-1 inhibitor, MIK665 effectively overcame the resistance in the Ven-R-MV4-11 tumor model when used in combination with Venetoclax and Azacytidine (Aza).

In conclusion, we have developed two Venetoclax-resistant AML models, which serve as a promising tool for mechanistic research and drug discovery aimed at overcoming the BCL2 inhibitor resistance.

## Methods

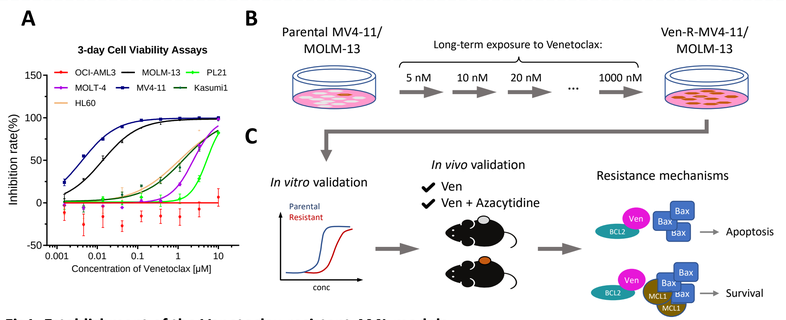
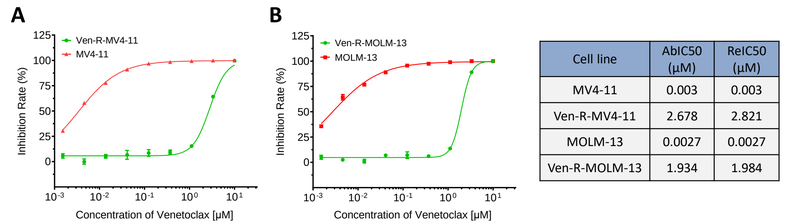



**Fig1. Establishment of the Venetoclax-resistant AML models**

(a) Among AML cell lines with high BCL-2 expression, both MV4-11 and MOLM-13 showed pronounced *in vitro* sensitivity to Venetoclax. (b) Ven-R-MV4-11 and Ven-R-MOLM-13 cell lines were established through long-term exposure to escalated dose of Venetoclax *in vitro* for over 6 months. (c) Acquired Venetoclax resistance was validated *in vitro* by CTG assays and in xenograft models. Western blot was performed to explore the potential resistance mechanism, which was further validated using combinatorial treatment.

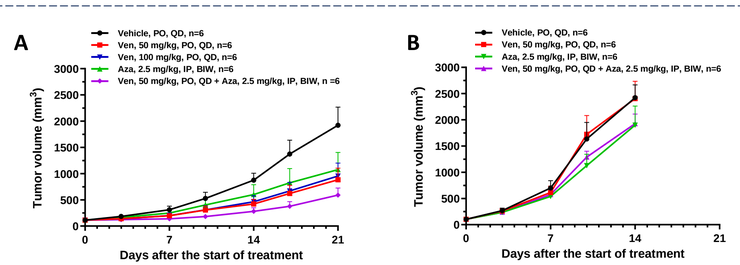
## Results

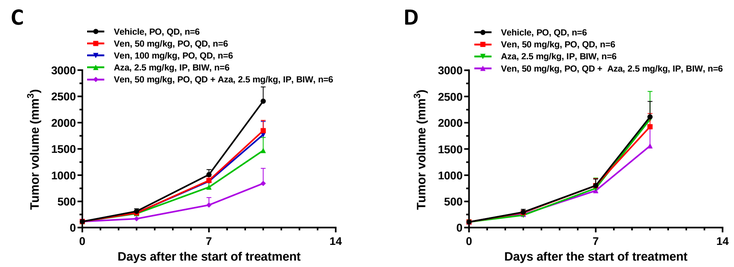



| Cell line | AbIC50 (μM) | ReIC50 (μM) |
|---|---|---|
| MV4-11 | 0.003 | 0.003 |
| Ven-R-MV4-11 | 2.678 | 2.821 |
| MOLM-13 | 0.0027 | 0.0027 |
| Ven-R-MOLM-13 | 1.934 | 1.984 |

**Fig2. *In vitro* validation of the Venetoclax-resistant cell lines**

In the 3-day cell viability assays, both Ven-R-MV4-11 (a) and Ven-R-MOLM-13 (b) cell lines showed robust resistance to Venetoclax, with an IC50 > 1 μM.




| Treatment | TGI | | | |
|---|---|---|---|---|
| | MV4-11 | Ven-R-MV4-11 | MOLM-13 | Ven-R-MOLM-13 |
| Ven, 50 mg/kg | 57.5% | 0.8% | 24.5% | 9.3% |
| Ven, 100 mg/kg | 54.6% | NA | 27.8% | NA |
| Aza, 2.5 mg/kg | 47.8% | 22.6% | 40.9% | 2.4% |
| Aza, 2.5 mg/kg + Ven, 50 mg/kg | 73.7% | 21.4% | 68.3% | 27.6% |

**Fig3. *In vivo* validation of the Venetoclax-resistant models**

(a, b) Compared to parental MV4-11 tumors, Ven-R-MV4-11 tumors showed strong resistance to Venetoclax, Azacytidine, and combination treatment with Venetoclax and Azacytidine (standard therapy). (c, d) Ven-R-MOLM-13 tumors showed decreased sensitivity to Venetoclax, Azacytidine, and resistance to Ven + Aza combination treatment, comparing with the parental MOLM-13 tumors.

## Results

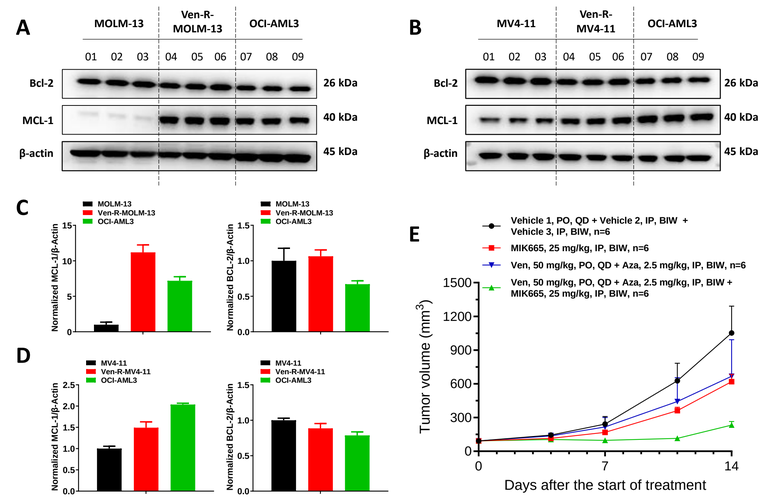



**Fig4. MCL-1 overexpression as the mechanism of Venetoclax resistance.**

(a-d) Ven-R-MOLM-13 and Ven-R-MV4-11 cell lines showed increased MCL-1 expression compared to the parental cell lines, with unchanged BCL-2 levels. As a positive control, the OCI-AML3 cell line with intrinsic Venetoclax resistance exhibited consistently high levels of both BCL-2 and MCL-1. (e) Overcoming the resistance to Ven + Aza therapy using an MCL-1 inhibitor, MIK665 in the Ven-R-MV4-11 model.

## Conclusion

In this study, we have established two Venetoclax-resistant tumor models: Ven-R-MOLM-13 and Ven-R-MV4-11. *In vitro* and *in vivo* experiments demonstrated robust resistance to Venetoclax and the standard therapy (Venetoclax + Azacytidine). Further mechanistic exploration suggests MCL-1 overexpression as an important resistance mechanism. Via transcriptomic and genomic profiling, future studies will provide further insights into the mechanisms of Venetoclax resistance. These Venetoclax drug resistance models represent a valuable platform to accelerate drug discovery in overcoming the BCL-2 inhibitor resistance.

## References

1. DiNardo, Courtney D et al. "Azacytidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia." *The New England journal of medicine* vol. 383,7 (2020): 617-629. doi:10.1056/NEJMoa2012971

2. Nwosu, Gus O et al. "Venetoclax therapy and emerging resistance mechanisms in acute myeloid leukemia." *Cell death & disease* vol. 15,6 413. 12 Jun. 2024, doi:10.1038/s41419-024-06810-7

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