A Novel Isoflavone, ME-344, Enhances Venetoclax Antileukemic Activity Against AML via Suppression of Oxidative Phosphorylation and Purine Biosynthesis

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Introduction

The 5-year survival rate for adult patients with acute myeloid leukemia (AML) treated with cytarabine (AraC)-based chemotherapy remains less than 30%, due to drug resistance and relapse.[1] Recently, a selective inhibitor of anti-apoptotic Bcl-2, venetoclax (VEN), was approved by the FDA in combination with low-dose AraC or hypomethylating agents for treating newly diagnosed AML patients 75 years of age or older or are unfit for standard chemotherapy. However, with the response rate to these new combination therapies reported to be 70%, the median overall survival is only 10-18 months.[2] Therefore, novel therapeutic agents are in demand to enhance venetoclax activity against AML and combat AraC resistance.

AraC-resistant AML cells induce relapse and rely on oxidative phosphorylation (OXPHOS) for survival.[3] Additionally, it is reported that targeting OXPHOS enhances venetoclax activity against preclinical models of AML,[4] providing a strategy for targeting AraC-resistant AML.

ME-344 is an investigational isoflavone that has been shown to suppress OXPHOS in solid tumor cells.[5] However, it has not been tested extensively in hematologic malignancies. This is the first study that analyzes the ability of ME-344 to enhance the antileukemic activity of venetoclax against AML.

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2. Knight, T., et al. (2019). "Evaluating venetoclax and its potential in treatment-naive acute myeloid leukemia." Cancer Manag Res 11: 3197-3213.
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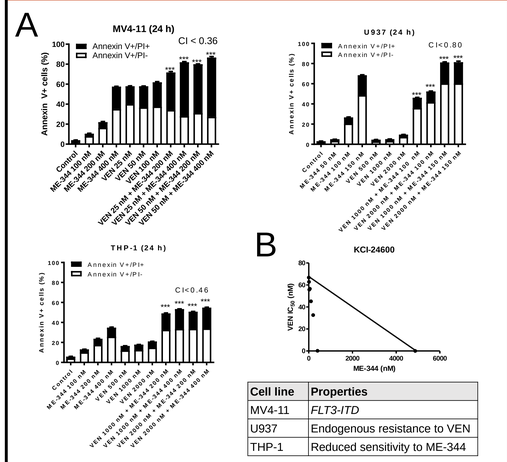
Hypothesis

ME-344 enhances the antileukemic activity of venetoclax against AML cells, including those that are resistant to AraC, via targeting oxidative phosphorylation and/or cellular metabolism.

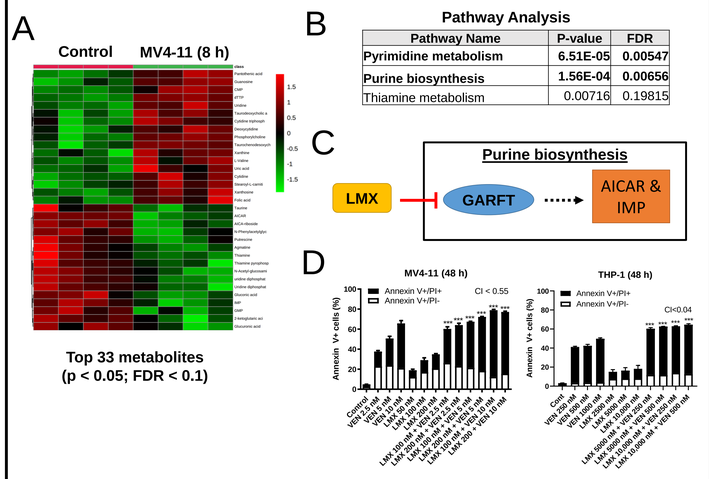
ME-344 synergistically enhances venetoclax activity against AML



Cell line	Properties
MV4-11	*FLT3-ITD*
U937	Endogenous resistance to VEN
THP-1	Reduced sensitivity to ME-344

Figure 1. ME-344 synergistically enhances the antileukemic activity of venetoclax against AML cells. AML cell lines were treated with multiple concentrations of ME-344 and venetoclax (VEN), alone or in combination, or vehicle control for 24 hours, then subjected to Annexin V-FITC/PI staining and flow cytometry analysis. Combination Index (CI) values were calculated using CompuSyn software to determine synergy. CI < 1.0, CI = 1.0, and CI > 1.0 indicate synergistic, additive, and antagonistic effects, respectively. *** indicates p<0.001 compared to vehicle control. **(B)** Primary patient sample, KCI-24600, was treated with variable concentrations of ME-344 and VEN, alone or combined, for 72 hours. Viable cells were determined using MTT assay. IC_{50}s of ME-344 and venetoclax were calculated and plotted as a standard isobologram graph. All data points falling under the line indicate synergistic antileukemic activity between VEN and ME-344 against the primary patient sample.

Suppression of purine biosynthesis enhances venetoclax activity



Pathway Name	P-value	FDR
Pyrimidine metabolism	6.51E-05	0.00547
Purine biosynthesis	1.56E-04	0.00656
Thiamine metabolism	0.00716	0.19815

Figure 3. ME-344 treatment results in drastic changes to the metabolome of AML cells. MV4-11 cells were treated with 200 nM ME-344 for 8 hours. Cell pellets were processed and LC-MS/MS detection of approximately 300 cellular metabolites was performed. Levels of metabolites were measured in 4 replicate samples for vehicle control or ME-344 treated cells. **(A)** Data from the targeted LC-MS/MS was analyzed using the MetaboAnalyst software. 33 metabolites were determined as significantly altered by ME-344 treatment with p<0.05 and FDR (false positive rate)<0.1. These 33 metabolites readily distinguish ME-344 treated cells from vehicle control treated cells when clustered into a heatmap. **(B)** Names of the top 33 altered metabolites were logged into the Pathway Analysis tool of MetaboAnalyst. Pathways were ranked based on the impact each one had on the involved pathway. **(C)** Lometrexol (LMX) inhibits a key enzyme of purine biosynthesis, glycinamide ribonucleotide formyltransferase (GARFT), that is upstream of AICAR and IMP production. **(D)** MV4-11 and THP-1 cells were treated with variable concentrations of LMX alone, VEN alone or in combination, for 48 hours, and stained with Annexin V-FITC/PI, and subjected to flow cytometry analyses. CI value calculation was performed as described in previous figures. *** indicates p<0.001 compared to vehicle control.

ME-344 and venetoclax prolong survival of NSGS mice bearing MV4-11-derived AML

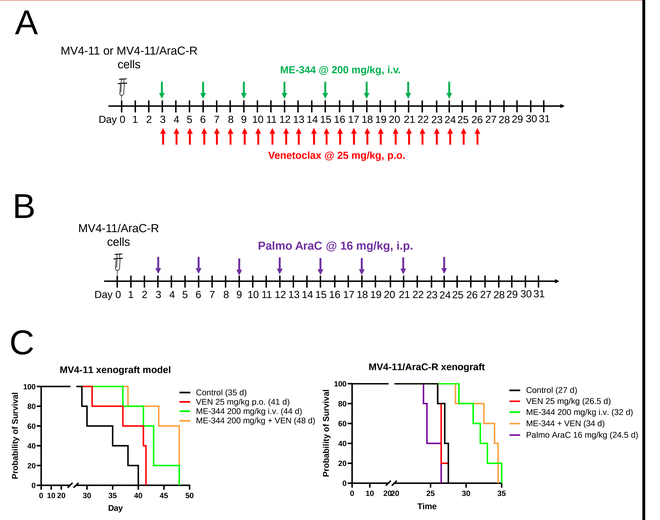
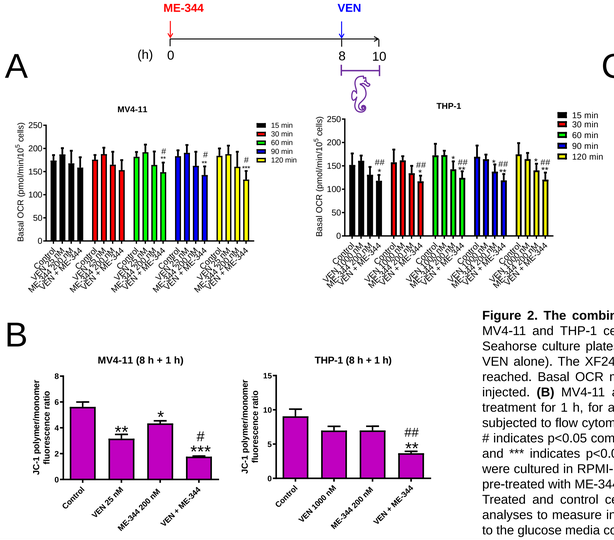


Figure 5. ME-344 alone and in combination with venetoclax prolong survival of parental and AraC-resistant MV4-11-derived NSGS xenograft models. (A & B) NSGS mice were inoculated with $1x10^6$ cells/mouse of parental MV4-11 (n=20) or MV4-11/AraC-R (n=25) cells through the tail vein, then randomized into each treatment arm (n=5), including vehicle control, ME-344 alone, VEN alone, ME-344 + VEN. One randomized arm of MV4-11/AraC-R inoculated mice received Palmo-AraC (panel B). Day 3 post-inoculation, mice were treated with ME-344 (intravenously, i.v.), VEN (oral gavage, p.o.), or palmo-AraC (intraperitoneal, i.p.). Treatments were terminated once signs of leukemia were presented. **(C)** Survival for each treatment arm was estimated using the Kaplan-Meier method and log-rank test for statistical significance.

ME-344 and venetoclax target mitochondria in AML



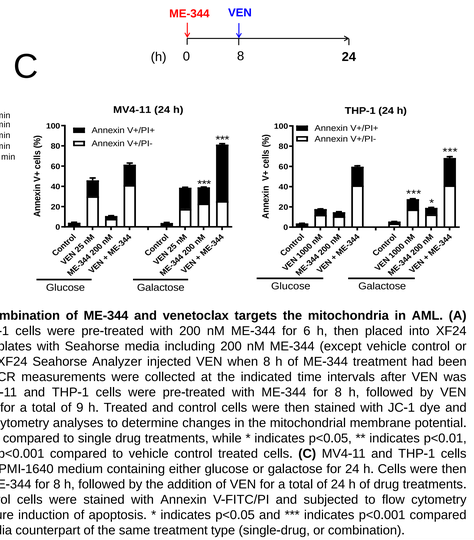


Figure 2. The combination of ME-344 and venetoclax targets the mitochondria in AML. (A) MV4-11 and THP-1 cells were pre-treated with 200 nM ME-344 for 6 h, then placed into XF24 Seahorse culture plates with Seahorse media including 200 nM ME-344 (except vehicle control or VEN alone). The XF24 Seahorse Analyzer injected VEN when 8 h of ME-344 treatment had been reached. Basal OCR measurements were collected at the indicated time intervals after VEN was injected. **(B)** MV4-11 and THP-1 cells were pre-treated with ME-344 for 8 h, followed by VEN treatment for 1 h, for a total of 9 h. Treated and control cells were then stained with JC-1 dye and subjected to flow cytometry analyses to determine changes in the mitochondrial membrane potential. # indicates p<0.05 compared to single drug treatments, while * indicates p<0.05, ** indicates p<0.01, and *** indicates p<0.001 compared to vehicle control treated cells. **(C)** MV4-11 and THP-1 cells were cultured in RPMI-1640 medium containing either glucose or galactose for 24 h. Cells were then pre-treated with ME-344 for 8 h, followed by the addition of VEN for a total of 24 h of drug treatments. Treated and control cells were stained with Annexin V-FITC/PI and subjected to flow cytometry analyses to measure induction of apoptosis. * indicates p<0.05 and *** indicates p<0.001 compared to the glucose media counterpart of the same treatment type (single-drug, or combination).

ME-344 enhances venetoclax activity against AraC-resistant AML cells

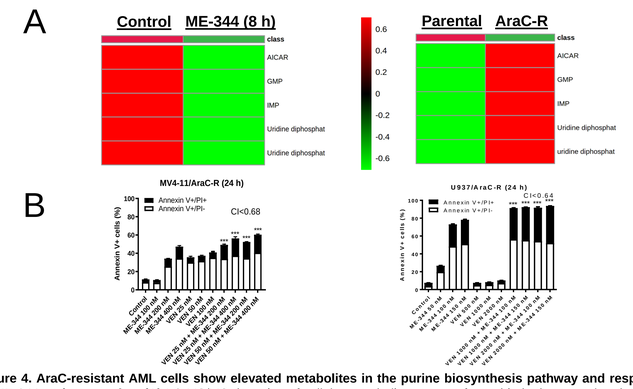


Figure 4. AraC-resistant AML cells show elevated metabolites in the purine biosynthesis pathway and respond to ME-344 and venetoclax. (A) LC-MS/MS detection of cellular metabolites was performed in both parental and AraC-resistant MV4-11 cells (MV4-11/AraC-R). Levels of metabolites were measured and analyzed under the same conditions as in Figure 3. 100 metabolites were determined to be significantly altered between parental and MV4-11/AraC-R cells with p<0.05 and FDR <0.1. Heatmaps represent the levels of all overlapping metabolites that changed in opposite directions between this data set and the ME-344 treated vs vehicle control treated data set. **(B)** MV4-11/AraC-R and U937/AraC-R cells were treated with variable concentrations of ME-344 or VEN, alone or combined, or vehicle control for 24 hours. The cells were then stained with Annexin V-FITC/PI and subjected to flow cytometry analyses. CI value calculation was performed as described in previous figures. *** indicates p<0.001 compared to vehicle control.

Conclusion

- ME-344 enhances the antileukemic activity of venetoclax against AML
- The combination of ME-344 and venetoclax compromises mitochondrial function in AML cells
- ME-344 reduces essential metabolites in the purine biosynthesis pathway that are elevated in the MV4-11/AraC-R cells compared to the parental cells
- Inhibition of purine biosynthesis enhances venetoclax activity against AML
- ME-344 and venetoclax prolong survival in MV4-11- and MV4-11/AraC-R-derived xenograft AML models

Acknowledgements

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