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ME-344, a novel isoflavone mitochondrial inhibitor, in combination with venetoclax constitutes a new metabolism-targeted approach to overcome resistance to Bcl-2 inhibition and standard of care treatment in AML

Katie H. Hurrish[1], Yongwei Su[2,3], Shraddha Patel[4], Johnny Busquets[5], Alessia Lodi[5], Sandra E. Wiley[6], Zhanjun Hou[2,3], Jenna Carter[1,7], Hasini Kalpage[8], Maik Hüttemann[8], Connie Weng[4], Holly Edwards[2,3], Lisa Polin[2,3], Jing Li[2,3], Jay Yang[2,3], Larry H. Matherly[1, 2, 3], Sergey Konoplev[9], Jeffrey W. Taub[1,3,10,11], Stefano Tiziani[5], Marina Konopleva[4], Yubin Ge[1,2,3], Natalia Baran[4]

[1] Cancer Biology Graduate Program, Wayne State University School of Medicine, Detroit, MI 48201, USA; [2] Department of Oncology, Wayne State University School of Medicine, Detroit, MI 48201, USA; [3] Molecular Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA; [4] Department of Leukemia, the University of Texas, MD Anderson Cancer Center, Houston, TX 77030, USA; [5] Department of Nutritional Sciences, Dell Pediatric Research Institute, University of Texas at Austin, Austin, TX 78723, USA; [6] MEI Pharma, Inc., San Diego, CA 92130, USA; [7] MD/PhD Program, Wayne State University School of Medicine, Detroit, MI 48201, USA; [8] Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, USA; [9] Department of Hematopathology, MD Anderson Cancer Center, The University of Texas, Houston, TX 77030, USA [10] Division of Pediatric Hematology/Oncology, Children's Hospital of Michigan, Detroit, MI 48201, USA; [11] Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI 48201, USA

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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 who 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 VEN activity against AML and combat AraC resistance.

AraC-resistant AML cells induce relapse and rely more on oxidative phosphorylation (OXPHOS) for survival than the parental cells.[3] Additionally, it is reported that targeting OXPHOS enhances VEN 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 VEN 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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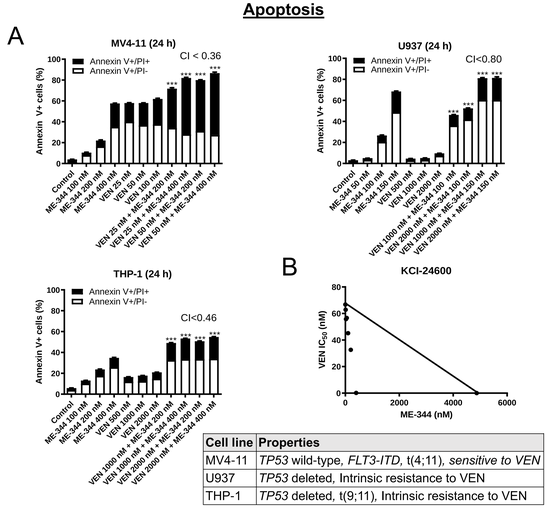
Hypothesis

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.

Apoptosis



Cell line	Properties
MV4-11	*TP53* wild-type, *FLT3-ITD*, t(4;11), *sensitive to VEN*
U937	*TP53* deleted, Intrinsic resistance to VEN
THP-1	*TP53* deleted, t(9;11), Intrinsic resistance to VEN

Figure 1. ME-344 synergistically enhances the antileukemic activity of venetoclax against AML cells. (A) 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 and single drug treatments. **(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.

Mitochondrial Respiration

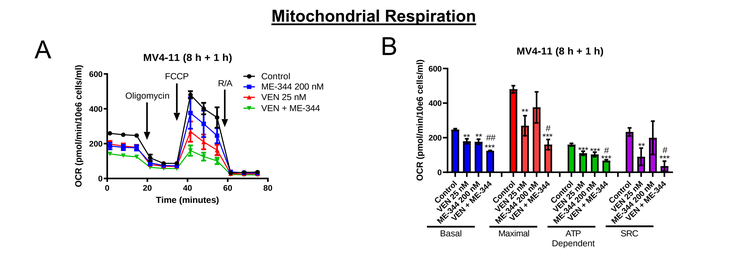


Figure 2. Variable suppression of OXPHOS by ME-344 and venetoclax in AML. AML cell lines, MV4-11 and U937, were pre-treated with 200 nM ME-344 for 8 h, followed by 25 nM VEN for 1 h. **(A)** Oxygen consumption rate (OCR) in treated and control cells was measured over time using a XFe96 Seahorse analyzer. Baseline OCR measurements were collected at the beginning of the Mito stress test assay. All data was normalized to viable cell number at time of assay initiation. **(B)** OCR linked to ATP production, Maximal OCR, and spare respiratory capacity (SRC) were collected by the Seahorse XFe96 under following treatment conditions (1.5 µM oligomycin, 1.0 mM FCCP, and 0.5 µM antimycin/rotenone). Statistical significance of the changes was determined with GraphPad Prism 9.0 software. ** indicates p<0.01 and *** indicates p<0.001 compared to untreated control, while # indicates p<0.05 and ## indicates p<0.01 compared to single drug treatments. **(C)** MV4-11 cells were pre-treated with 200 nM ME-344 for 8 h, followed by 25 nM VEN treatment for 1 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. **(D)** MV4-11 cells were treated with 200 nM ME-344 for 8 h followed by 25 nM VEN for 1 h. Treated and control cells were collected and underwent targeted LC-MS/MS for ATP. * indicates p<0.05. **(E)** U937 cells were pre-treated with 200 nM ME-344 for 8 h, followed by 25 nM VEN for 1 h. OCR was measured in treated and control cells using XFe96 Seahorse respirometery as in 2A. All data was normalized to viable cell number at time of assay initiation.

Metabolomics

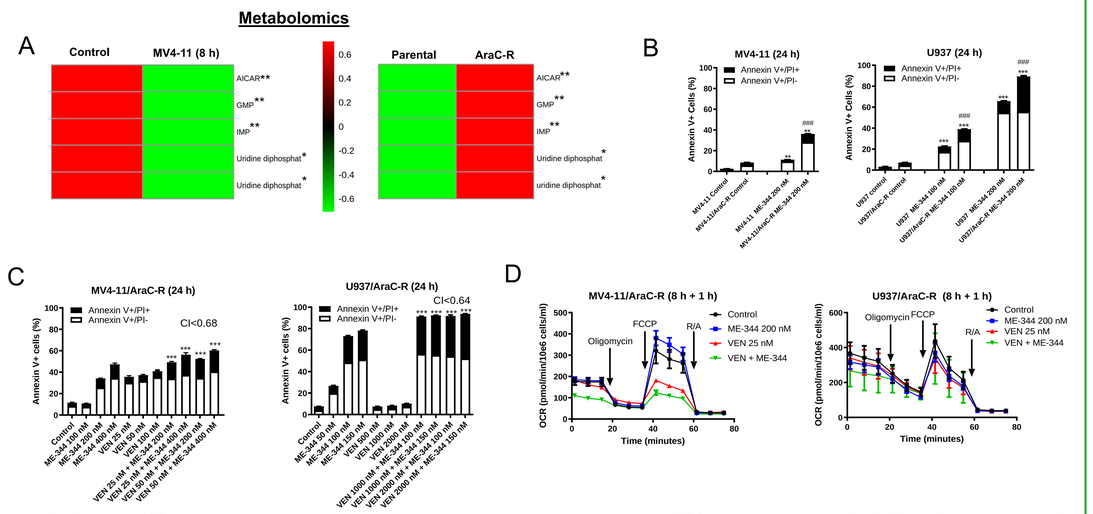
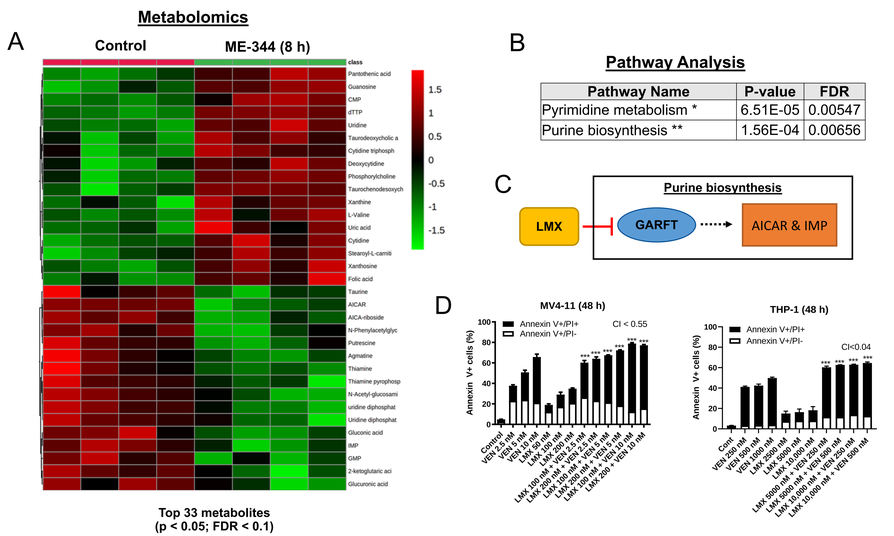


Figure 4. AraC-resistant AML cells show elevated metabolites in the purine biosynthesis pathway and respond to ME-344 and venetoclax. (A) LC-MS/MS detection of cellular metabolites was performed in both parental and AraC-resistant MV4-11 cells (MV4-11/AraC-R). Levels of metabolites were measured and 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)** To measure apoptosis, MV4-11, MV4-11/AraC-R, U937 and U937/AraC-R cells were treated with ME-344 at the indicated concentrations for 24 hours, stained with Annexin V-FITC/PI and subjected to flow cytometry analyses. ** indicates p<0.01 and *** indicates p<0.001 compared to untreated control, while ### indicates p<0.001 compared to parental with the same treatment. **(C)** To measure apoptosis, 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 and single drug treatments. **(D)** MV4-11/AraC-R and U937/AraC-R cells were pre-treated with 200 nM ME-344 for 8 h, followed by 25 nM VEN for 1 h. Treated and control cells were then adhered to XFe96 Seahorse culture plates with Seahorse media. Baseline OCR measurements were collected during the programed Mito stress test assay (1.5 µM oligomycin, 1.0 mM FCCP, and 0.5 µM antimycin/rotenone). All data was normalized to viable cell number at time of assay initiation.

Metabolomics



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

Figure 3. ME-344 treatment results in drastic changes to the metabolome of AML cells. (A) 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 or ME-344 treated cells. 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)** Analysis of the top altered metabolites using the Pathway Analysis tool of MetaboAnalyst identified purine biosynthesis and pyrimidine metabolism as the most significantly altered pathways. **(C)** Lometrexol (LMX) inhibits a key enzyme of purine biosynthesis, glycinamide ribonucleotide formyltransferase (GARFT), that is upstream of AICAR and IMP production. **(D)** Apoptosis was measured in MV4-11 and THP-1 cells after treatment with variable concentrations of the purine biosynthesis inhibitor, lometrexol (LMX), VEN, or LMX+VEN for 48 hours. Cells were stained with Annexin V-FITC/PI and subjected to flow cytometry analyses. CI value calculation was performed as described in Figure 1. *** indicates p<0.001 compared to vehicle control and single drug treatments.

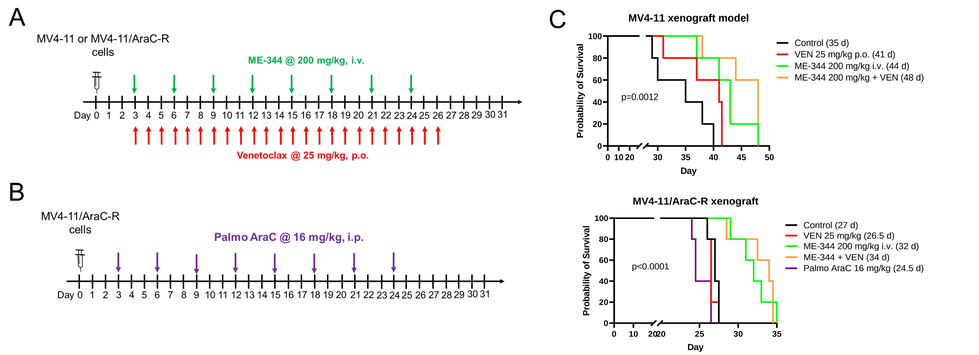


Figure 5. ME-344 alone and in combination with venetoclax prolong survival of parental and MV4-11/AraC-R-derived NSGS xenograft models. (A & B) NSGS mice were inoculated with $1x10^6$ cells/mouse of parental MV4-11 (n=20) or MV4-11/AraC-R (n=25) cells through the tail vein, then randomized into each treatment arm (n=5), including vehicle control, ME-344 alone, VEN alone, ME-344 + VEN. **(B)** 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.

Conclusions

- ME-344 enhances the antileukemic activity of venetoclax against AML
- The combination of ME-344 and venetoclax compromises mitochondrial function in some 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
- **Overall: ME-344 enhances venetoclax activity against AML cells including those resistant to AraC by suppressing purine biosynthesis**

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