SUPPLEMENTARY APPENDIX

Pevonedistat and azacitidine upregulate NOXA (*PMAIP1*) to increase sensitivity to venetoclax in preclinical models of acute myeloid leukemia

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Supplementary Tables

Supplementary Table 1: Sequences of CRISPR crRNAs and oligonucleotides for PCR and sequencing.

crRNA/DNA	Sequence
***BAK1* crRNA**	GCTCACCTGCTAGGTTGCAG
***BAK1* gDNA-PCR (F)**	ATGGTTGTGACATGACAGAG
***BAK1*-gDNA-PCR (R)**	ATTTCTTTCCATGGGTGAGG
***BAK1* Sequencing (F)**	AGCAACCATGAGAGAAGGGC
***BAK1* Sequencing (R)**	CTCCCTGACTCCCAGCTTTG
***BAX* crRNA**	TCGGAAAAAGACCTCTCGGG
***BAX* gDNA-PCR (F)**	GCTCCTTCAGGACACAGGAC
***BAX*-gDNA-PCR (R)**	TGAGAGCAGGGATGTAGCCT
***BAX* Sequencing (F)**	CTTGTCCCCTTCCCTTGTCC
***BAX* Sequencing (R)**	TGATACCACGATCCCCAGGT
***ATF4* crRNA 1**	AGGTCTCTTAGATGATTACC
***ATF4* crRNA 2**	AGATGACCTTCTGACCACGT

Supplementary Table 2: Primary antibodies used in western blotting and flow cytometry

Antibody	Vendor	Catalog Number	RRID
anti-BCL2	Abcam	ab32124	AB_725644
anti-BCL-X_L	Abcam	ab32370	AB_725655
anti-MCL1	Cell Signaling Technology	#94296	AB_2722740
anti-PUMA	Abcam	ab9645	AB_296538
anti-NOXA	Abcam	ab13654	AB_300536
anti-PARP	BD Biosciences	556494	AB_396433
anti-ATF4	Cell Signaling Technology	#11815	AB_2616025
anti-CHOP	Cell Signaling Technology	#2895	AB_2089254
anti-β-actin	Sigma-Aldrich	A2228	AB_476697
anti-BAX (D2E11)	Cell Signaling Technology	#5023	AB_10557411
anti-BAK	Abcam	ab32371	AB_725624
anti-DNMT1	Cell Signaling Technology	#5032	AB_10548197
anti-BIM	Abcam	ab230531	
human CD45-APC	Biolegend	Clone 2D1	AB_2566371
murine CD45-PE	Biolegend	Clone 30-F11	
human CD33-PE-Cy7	Biolegend	Clone P67.6	

Supplementary Table 3: AML patient samples mutational background, karyotype, and treatment.

PATIENT	MUTATIONS AT BIOPSY	KARYOTYPE	TREATMENTS FAILED
AML001	*DNMT3A, FLT3-ITD, IDH1, NPM1*	46 XY	Conventional chemotherapy
AML002	*DNMT3A, FLT3-ITD, IDH2, SRSF2*	46 XX, del(12)(p12)[15]	Conventional chemotherapy
AML003	*FLT3-ITD, IDH1*	46 XY	VEN/AZA
AML004	*DNMT3A, FLT3-ITD, IDH2, NPM1*	46 XX	Conventional chemotherapy + midostaurin
AML005	*TP53, TET2*	Complex karyotype	VEN/AZA
AML006	*TP53, FLT3, ASXL1, TET2*	47,XY,+8[6]/46,idem,add(8)(p11.2),-21[cp14]	Decitabine X 1 week
AML007	*TP53, FLT3, TET2*	45,XX,-16,add(17)(p11.2)[18]/46,XX[2]	Conventional chemotherapy
AML008	*TP53, DNMT3A*	Complex karyotype	Conventional chemotherapy
AML 009	*FLT3-ITD, WT1*	Complex karyotype	Conventional chemotherapy, decitabine + sorafenib

Supplementary Materials and Methods

Reagents

PEV, VEN and AZA were prepared in DMSO by AbbVie; or PEV (Takeda), AZA (Sigma-Aldrich), VEN (Selleckchem). *In vivo* formulations: PEV: 20% (2-hydroxypropyl)-β-cyclodextrin; VEN: 60% phosal 50 propylene glycol, 30% polyethylene glycol-400 and 10% ethanol; AZA: 0.9% sodium chloride.

In vivo Murine Modeling for Patient-derived Xenografts

Animal experiments were conducted in accordance with guidelines approved by the IACUC at Vanderbilt University Medical Center. Female NSGS [NOD-scid IL2Rgnull3Tg(hSCF/hGM-CSF/hIL3)] mice, purchased from Jackson laboratories at 7-9 weeks old, were 18-20 grams on arrival. Food and water were provided ad libitum. They were irradiated with 1Gy cesium irradiation 1 week after arrival to facility. Twenty-four hours later, mice were intravenously xenografted with 2×10^6 AML mononuclear cells. Mice were randomized post-cell injection into cages of 5 or 6. Mice without peripheral chimerism of human AML were removed from the study. Chimeric mice were treated with either vehicle, PEV (Takeda) by three times weekly i.p. injection for 4 weeks, AZA (Sigma-Aldrich) daily by i.p. injection for 7 days, VEN (Selleckchem) by gavage for 5d/week for 4 weeks, combination dosing of VEN/AZA, or triple therapy with the same dosing schedules as above. PEV was formulated in 20% (2-hydroxypropyl)-β-cyclodextrin [HPbCD], VEN was formulated in 60 % phosal 50 propylene glycol (PG), 30 % polyethylene glycol (PEG) 400 and 10 % ethanol, and 5-azacytidine was formulated with 0.9 % sodium chloride. Peripheral blood was assessed weekly for human chimerism. Chimerism was assessed in the bone marrow and spleen on the final day of the experiment at time of tissue harvest.

Cell line-derived Xenografts

Female Fox Chase SCID Beige (MV4-11 experiment) and Female NOD SCID gamma (NSG) (OCI-AML2-Red-Fluc experiment) mice were purchased from Charles River Laboratories (Wilmington, MA). Body weights upon arrival were 18-20 g. Food and water were provided ad libitum. Animals were on the light phase of a 12 h light: 12 h dark cycle. All animal studies were conducted in accordance with the guidelines established by the AbbVie Institutional Animal Care and Use Committee.

In the flank xenograft model, female Fox Chase SCID Beige (Charles River Laboratories; Wilmington, MA) mice were inoculated with 5×10^6 MV4-11 cells subcutaneously into the right flank. Mice were injected with a 0.1 mL inoculum of 1:1 cell mixture in culture media and Matrigel (BD Biosciences, Bedford, MA). When tumors reached approximately 225 mm^3, the mice were size-matched into treatment and control groups. Mice were treated QD×14 PO 50 mg/kg with venetoclax, QD×14 IP 60mg/kg with pevonedistat or combination of both. Tumor volume was measured twice per week with electronic calipers and calculated according to the formula, $(L \times W^2)/2$. All treatment groups consisted of 8 mice per group.

In the systemic engraftment model, the OCI-AML2-Red-Fluc stable *F.luciferase*-expressing cell line was generated by infecting the parental OCI-AML2 with the RediFect Red-FLuc-Puromycin lentiviral particles (PerkinElmer, Waltham, MA). Female NOD SCID gamma mice (Charles River Laboratories; Wilmington, MA) were inoculated with 1×10^6 OCI-AML2-Red-Fluc cells intravenously into the tail vein with 0.2 ml inoculum of culture media. Bioluminescence (BLI) signals were obtained 10 min after IP injection of luciferin (150 mg/kg) using an IVIS imaging system (PerkinElmer, Waltham, MA). Signal intensity was determined by drawing regions of interest around the whole mouse. Data was quantified using Living Image® software (PerkinElmer, Waltham, MA) and mice were size-matched at 3×10^6 signal into treatment and control groups. Mice were treated QD×14 IP 60mg/kg with PEV, QD×14 PO 50 mg/kg with VEN, Q7D×3 IV 8 mg/kg with AZA or various combinations. BLI signal was monitored 1-2 times weekly and mice were euthanized when a BLI signal of 1×10^{10} was observed. Whole body ROI to quantify tumor burden with a signal of photons×sec^{-1}. All treatment groups consisted of 6-8 mice. In all studies all groups received appropriate vehicles. PEV was formulated in 20% (2-hydroxypropyl)-β-cyclodextrin [HPbCD], VEN was formulated in 60 % phosal 50 propylene glycol (PG), 30 % polyethylene glycol (PEG) 400 and 10 % ethanol, and 5-azacytidine was formulated with 0.9 % sodium chloride.

Colony-forming Unit (CFU) Assays

Cyropreserved CD34+ bone marrow cells (Lonza Bioscience) or primary patient normal bone marrow mononuclear cells were treated with DMSO, VEN(0.01uM)/AZA(0.3uM), or VEN(0.01uM)/AZA(0.3uM)/PEV(0.3uM). Cells were plated in Methocult H4034 Optimum (Stem Cell Technologies, Vancouver, Canada) at a density of 500 cells per mL for purchased $CD34^+$ cells or 1×10^4 cells per mL for normal bone marrow mononuclear cells. After 12-14 days, burst forming

unit-erythroid (BFU-E), colony forming unit-granulocyte, macrophage (CFU-GM) and colony forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) colonies were counted and analyzed.