Advancing Precision Therapy in Pediatric Acute Myeloid Leukemia Through PDX Models and Mitochondrial Targeting

SUPPLEMENTAY DATA

SUPPLEMENTAY METHODS

Study approval

AML primary cells derived from patients affected by *de novo* AML in accordance with AIEOP AML 2002/01 and 2013/01 protocol and the LnLA working party. All in vivo experimental procedures have been performed at Venetian Oncologic Institute (IOV)-animal facility. Procedures involving animals and their care were in accordance with institutional guidelines that comply with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 12 December 1987) and with "ARRIVE" guidelines (Animals in Research Reporting In Vivo Experiments). All animal procedures were authorized by Italian Ministry of Health: 622/2017-PR and 512/2019-PR.

Patient samples

Bone marrow (BM) or peripheral blood (PB) samples of pediatric patients affected by AML were provided by the BBOP biobank at the pediatric Onco-Hematology Lab of the Padova University-Hospital, reference center for the pediatric AML diagnosis, in accordance with AIEOP AML 2002/01 [1] and 2013/01 trials and the LnLA working party. Samples were obtained at diagnosis (n=56) or relapse (n=8) of *de novo* AML and patients' characteristics reported in Table 1 and Supplementary Table 1.

Primary cells culture and *in vitro* treatment

Primary cells were cultured at 37°C in RPMI Medium 1640 with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 2mM glutamine, 100U/mL streptomycin/penicillin (Gibco, Thermo Fisher Scientific), and supplemented with 50 ng/mL thrombopoietin (TPO), 50 ng/mL stem cell factor (SCF), 50 ng/mL FMS-like tyrosine kinase 3 ligand (Flt3L), 20 ng/mL interleukin-3 (IL-3) and 20 ng/mL interleukin-6 (IL-6), all of them purchased from Miltenyi Biotec (Miltenyi Biotec, Bergisch Gladbach, DE). Primary cells were seeded at 0.75 x 10^6/ml and treated with Venetoclax, Azacitidine, (Sigma, Merck), IACS-010759, SNDX-5613 and Atovaquone (Selleckchem, Houston, TX, USA). Their viability was evaluated after 72 hours or 6 days, using PrestoBlue Cell Viability (Invitrogen, Thermo Fisher Scientific).

Isolation and culture of Mesenchymal Stromal Cells (MSCs)

Isolation, culture, expansion and characterization of MSCs from BM were conducted as previously published [2]. Briefly, cells from BM of pediatric AML patients and healthy donors were plated at 100.000 cells/cm^2 in StemMACS^{TM} MSC Expansion Media (Miltenyi Biotec), supplemented with 100 U/mL penicillin/streptomycin (Gibco, Life Technologies) and incubated at 37°C. After one day of culture, the non-adherent cells were removed and fresh medium was added to the adherent ones, which were expanded until 90% confluence, then split after detaching by trypsin (Trypsin/EDTA Solution, Biochrom, Merck, Darmstadt, DE) and plated at 5.000 cells/cm2 in fresh medium. These

cells (AML-MSCs) were expanded and characterized as per ISCT guidelines as we previously described[2]. Briefly, we confirmed positivity for CD73, CD90, and CD105, and negativity for CD34, CD45, and CD11b, and their multipotency to differentiate into adipogenic, osteogenic, and chondrogenic lineages. For co-culture experiments AML-MSCs were used between passages 2 to 5, indiscriminately with related or unrelated AML blasts.

Flow cytometry

For the detection of the surface markers, the following anti-human antibodies conjugated with proper fluorophores were used: CD45 (Beckman Coulter, Brea, CA, USA), CD11b (Beckman Coulter), CD34 (BD Biosciences, Franklin Lakes, NJ, USA), CD38 (BD Biosciences), CD33 (BD Biosciences), HLADR (BD Biosciences), NG2 (Beckman Coulter), CD56 (Beckman Coulter), CD7 (Beckman Coulter), CD11a (BD Biosciences), CD38 (BD Biosciences). Briefly, cells obtained after hemolysis of PB or BM samples, or from spleen after mechanical dissociation and subsequent hemolysis, were stained with the conjugated primary antibody for 30', rinsed in PBS 1X and then fluorescence was detected with Cytometer FC500 Instrument (Beckman Coulter) or FACSCanto (BD Biosciences).

Mitochondrial dependence assessment, by SCENITH methodology

Mitochondrial dependence was assessed on *KMT2A*-r and non *KMT2A*-r ex vivo blasts by SCENITH, as described in Argüello R. et al., 2020[3]. Briefly, cells were treated at a concentration of 1x106 cells/mL for 15 minutes with Control (DMSO), 2-Deoxy-Glucose (DG, 100 mM), Oligomycin (O, 1 µM) and a combination of DG and Oligomycin. Successively, Puromycin 10 µg/mL was added to cells for 40 minutes at 37°C. Then, cells were washed in cold FACS buffer (PBS+2% FBS+2 µM EDTA) and stained with the LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (Invitrogen) for 15 minutes at 4°C, then cells were stained with human CD45 antibody (Beckman Coulter). After washing, cells were fixed and permeabilized using the Fix&Perm™ Cell Permeabilization Kit (Invitrogen) following the manufacturer's instruction. Then, coupled Alexa-Fluor 647 anti-puromycin antibody (BioLegend, San Diego, CA, USA) was added for one hour at 4°C. After a final wash, cells were resuspended for the analysis using Cytoflex cytometer (Beckman Coulter). Inhibitors and Puromycin were obtained from www.scenith.com/try-it. Mitochondrial dependence, is calculated as detailed in Argüello R. et al.[3]: briefly, mitochondrial dependence = 100*(Co−O)/(Co−DGO), where Co is the geometric mean fluorescence intensity (GeoMean) of anti-puromycin-fluorochrome in cells analyzed after control treatment, O represent the GeoMean of anti-puromycin-fluorochrome on cells treated with Oligomycin and DGO is the GeoMean of anti-puromycin-fluorochrome in cells upon DG + O treatment.

Proteomic analysis by Reverse-Phase Protein Arrays

Reverse-phase protein array (RPPA) analysis was performed as previously described[4].

3D *in vitro* model set up and treatment

The 3D model consists of a scaffold composed by 70% hydroxyapatite/30% collagen type I (Typeone Biomaterias S.R.I., Calimera, LE, Italy) with 2% 1,4-butanediol diglycidyl ether (BDDGE), of 2 mm in height and diameter, synthesized by Dr. M. Sandri's laboratory at "Istituto di Scienza, Tecnologia e Sostenibilità per lo Sviluppo dei Materiali Ceramici" (ISSMC-Consiglio Nazionale delle Ricerche

CNR, Faenza, Italy). Previously, we demonstrated that AML-MSCs seeded on the scaffold adhered to the inner trabecular pores and formed a stable fibrous 3D network by day 7. Scanning electron microscopy showed tight attachment of MSCs to the trabecular surface, with cytoplasmic extensions ensuring strong connections between cells and the scaffold. Seven days after MSC seeding, primary AML blasts were added, allowing the establishment of blast–stroma interactions. We documented that within the first 3 hours, AML cells established contact with MSCs, showing morphological changes and forming membrane nanotubes. We also observed gap junctions and cytoplasmic calcein transfer, confirming functional communication[2]. Briefly, prior to cell seeding, scaffolds were soaked in DMEM medium (Gibco, Thermo Fisher Scientific) overnight. After medium removal, scaffolds were equally divided into 4 parts, then $1x10^5$ AML-MSCs in 10 µL of StemMACS were slowly seeded on the upper surface of the scaffold. Scaffolds seeded with AML-MSCs were incubated for 4 hours at 37°C, to facilitate cell spreading, then 0.5 mL of StemMACS was gently added to each well of a 48 well plate. After 7 days of culture, the medium was removed and $2x10^5$ AML primary cells in 10 µL of proper medium were slowly seeded on the upper surface of the AML-MSCs coated-scaffold. Seeded scaffolds were incubated for 15' at 37°C, prior to gently add 0.5 mL of primary cells medium, with or without selected drugs, then incubated at 37°C for 72 hours prior to cell viability assay.
Drugs used are Venetoclax, Azacitidine, Trametinib, γ-Secretase inhibitor X (Sigma, Merck), IACS-010759, ICG-001, and Quizartinib (Selleckchem).

3D Cell viability assay

Scaffolds were individually transferred into wells of flat-bottom white-opaque 96-well plates (Corning, Merck) with 100 µL of RPMI, then 100 µL of CellTiter-Glo® 3D reagent (Promega, Fitchburg, WI) was added into each well. Plates were shaken for 5' to induce scaffold and cells lysis. Samples were then incubated for additional 20' in the dark at room temperature, to stabilize the bioluminescent signal. Absorbance was recorded using the Spark® multimode microplate reader (TECAN_Männedorf, CH) and cell viability was compared to the control sample treated with dimethyl sulfoxide (DMSO). The combination index was calculated as reported by Slinker BK et al.[5].

***In vivo* PDX models**

Female NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice (NSG) 4-8 weeks old were used. First mice recipients (P0) were conditioned (1.5 Gy) 24 hours prior to leukemic cell transplantations. Primary AML cells were pre-emptively CD3-depleted by immune-magnetic cell separation using CD3 MicroBead Kit (Miltenyi Biotec) and $1x10^6$ of viable blasts were injected. To ensure a cell viability greater than 70%, gradient density centrifugation (Lymphoprep, Stemcell Technologies, Vancouver, Canada) was performed when necessary to remove dead cell and increase cell viability before downstream experiments. Cells were resuspended in RPMI, in details into 100 µl for intravenous injection (i.v.), 50 µl for intrafemoral (i.f.) injection or 30 µl for intrahepatic (i.h.) injection in newborn mice (3 days). For scaffold implantation, scaffolds were seeded with $0,4x10^6$ AML-MSCs as previously described, and $1x10^6$ of AML primary cells in 10 µL of proper medium were slowly seeded after 24h on AML-MSCs coated-scaffold. Seeded scaffolds were incubated for 15' at 37°C, prior to gently add 1 mL of primary cells medium and the day after were implanted in the back of mice. Mice engraftment has been evaluated once *per* month, for at least one year, by flow cytometric measurement of human CD45 (hCD45) positive cells in PB. Mice were sacrificed when $hCD45^+$ cells exceeded 20% in peripheral blood, or earlier if they reached a humane endpoint, and organs (femur and spleen) were

recovered; femurs were flushed and spleens were mechanically dissociated to harvest cells and analyze organ AML infiltration by flow cytometry. Then, $1x10^6$ hCD45 cells harvested from P0 mice were intravenously injected into P1 and subsequently P2-PDX, as well as cryopreserved in our biobank. P3-PDX instead were generated and used to evaluate the model stability at transcriptomic level.

PDX treatments

NSG mice were transplanted with $1x10^6$ *ex vivo* PDX-derived blasts and monitored for AML engraftment by PB sampling. At leukemia onset, when hCD45 positive cells in PB ranged between 5 and 10% (indicated as day 0), mice were randomized into groups (n=6 mice/group) for treatment. Mice were treated at day 1-3-5 for 4 weeks with the following drugs: Venetoclax (Merck Millipore) 25 mg/kg, dissolved in 60% Phosal-50PG (Lipoid), 30% polyethylene glycol-400 (Sigma, Merck), and 10% ethanol, administered by oral gavage; IACS-010759 (Selleckchem) 7.5 mg/kg, DMSO stock solution dissolved in 96% water, 3% ethanol, 1% TWEEN80 (Sigma /Merck), administered by oral gavage; Lercanidipine (Sigma, Merck) 1,5 mg/kg, DMSO stock solution dissolved in sterile saline solution, by i.p. injection. Cytarabine (AraC, Selleckchem) was subministered daily, by i.p.injection at 12.5 mg/kg. To generate an AML-PDX modeling a relapse event, mice with high tumor burden (40% of hCD45 positive cells in PB) were treated with chemotherapy induction cycle which consisted in three days of intravenous injection of Cytarabine (AraC, Selleckchem) 50mg/kg + Doxocyclin (Sigma, Merck) 1.5mg/kg, followed by two days of i.p. injection of Cytarabine 50mg/kg. Doxorubicin, Cytarabine powder were dissolved in DMSO for stock solutions, then freshly diluted in sterile saline solution at appropriate concentration for daily injection.
Mice weight was monitored weekly to adjust drug dosage and to eventually evaluate drug toxicity. Appropriate vehicle was used for control group. Mice were monitored by flow cytometric measurement of hCD45 percentage in PB, remission was defined when blasts in PB were <5% and survival analysis (humane endpoint) were performed.

RNA extraction

Total RNA was isolated using Trizol (Invitrogen, Thermo Fisher Scientific) according to manufacturer's protocol and nucleic acids were quantified by using Qbit RNA BR Assay Kit on a Qbit fluorometer (Thermo Fisher Scientific). Quality of RNA samples has been checked using the 2100 Bioanalyzer instrument (Agilent, Santa Clara, CA, USA).

DNA extraction

DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Hilden, DE) according to manufacturer's protocol and nucleic acids were quantified by using Qbit dsDNA BR Assay Kit on a Qbit fluorometer (Thermo Fisher Scientific). Quality of DNA samples has been analyzed by gel electrophoresis.

RNA sequencing

Libraries were prepared using NEB Next Ultra II with RiboZero Plus kit following the manufacturer's protocol, then sequenced 150x2 bp (paired-end) on the NovaSeq 6000 System -S4 (Illumina, San Diego, CA, USA). Reads were trimmed to clean the sequencing adapters, and to filter or trim the reads for sequence quality (Phred quality > 10 and minimum length of trimmed sequence of 30nt). These two steps were performed with AdapterRemoval v.2.1.7 tool. Cleaned reads were aligned on

human reference genome hg38 with the STAR v2.6.1 [3] pipeline. An additional processing step was adopted in the case of PDX samples, to remove the host genome by the tool disambiguate using the mm39 as murine reference genome. Samtools v1.9 was used to remove the sequencing duplicates (optical and PCR related) and to index the alignment files. The number of reads for known genes, included in the Ensembl release 95 (http://www.ensembl.org) were counted using htseq-count (Python package HTseq) function. Raw counts were imported in DESeq2 (v.1.34.0) for subsequent analysis, and only genes summing at least 10 reads for all samples were considered. Log transformation and normalization by DESeq2 integrated functions were applied. Principal Component Analysis (PCA) was computed in DESeq2 and represented by ggplot2 (v 3.3.6) package. Pearson correlations and their *p*-values were calculated using the *cor.test* function of the *stats* package in R 4.2.2. Barplot of Pearson correlations were plotted using *graphics* (v.3.5.0.9000) packages. Gene Set Enrichment Analysis (GSEA) was performed using GSEA (v 4.2.3) software and c5 Gene Ontology-Biological Processes from MSigDB, using 1000 permutations on gene set and Signal2Noise metric in *KMT2A*-r genotype versus all the others genetic subtypes, both for P2-PDX and patients. In order to stabilize GSEA statistics, a pseudocount of 1 was added to count matrix. Gene sets with an FDR <0.05 and belonging to mitochondria and oxidative phosphorylation biological processes were selected and represented by a circular barplot using tidyverse (v.2.0.0) R packages.

Whole exome sequencing (WES)

Libraries were prepared using SureSelect V6+UTR kit (Agilent) following the manufacturer protocol, then sequenced 150x2 bp (paired-end mode) on the NovaSeq 6000 System-S4 (Illumina).

Paired-end short reads were processed for trimming as previously described for RNA sequencing data. Cleaned reads were then aligned on human reference genome hg38 with Burrows–Wheeler Aligner mem (BWA v0.7.17). For PDX samples host genome was removed as specified for RNA sequencing data. Single Nucleotide Variants (SNVs) and small indels were called by Mutect2 with tumor-only mode in Genome Analysis Toolkit (GATK, v4.2.1.0) pipeline. Variant prioritization was set up considering total depth > 10, localization within coding exons and non-silent effect on protein sequence (non-synonymous and nonsense SNVs, frameshift and non-frameshift InDels). Variants were annotated with Annovar tool and filtered using databases of human variability (1000 Genomes; ExAC) to discard polymorphism (allele frequency in population <0.01). In order to additionally refine the set of interesting variants we applied the following selection criteria: 1) patients' variants reported on COSMIC in the exact or near position (±10 nt); 2) patients' variants showing an increased allelic frequency (>25%) in P2-PDX (coverage >50X); 3) variants detected in all PDXs passages and not detected in the corresponding patient. Finally, only variants with potential functional consequences were selected according to the prediction of deleteriousness provided by at least one of the following tools: PolyPhen-2, SIFT, FatHMM, MutationTaster-2, MutationAssessor, CADD, LRT. Variants were represented in an oncoplot, generated using a customized R script, based on ComplexHeatmap (v.2.14.0) R package.

Copy number alterations, cancer cell fraction and clonal dynamics

Copy number alterations were computed using a dockerized version of cnvkit package (https://registry.hub.docker.com/r/etal/cnvkit/, v.0.9.10), with default parameters, target capture *.bed* panel and a normal reference generated using in house available sequencing data obtained with the

same kit for library preparation and from healthy bone marrow donors. Cancer cell fraction (CCF) was calculated for each variant, using the definitions and formulas as in Tarabichi M et al., Nat Met 2021 considering WES variant allele frequency, local copy number and purity defined as percentage of blasts. For PDX samples purity has been set up to 1 considering the demousing step. Clusters of variants were generated according to CCF and graphical representations of clonal evolution were generated using fishplot (v.0.5.2) R package.

SUPPLEMENTAY REFERENCES

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2. Borella G, Da Ros A, Borile G, et al. Targeting the plasticity of mesenchymal stromal cells to reroute the course of acute myeloid leukemia. *Blood*. 2021;138(7):557-570. doi:10.1182/blood.2020009845

3. Argüello RJ, Combes AJ, Char R, et al. SCENITH: A Flow Cytometry-Based Method to Functionally Profile Energy Metabolism with Single-Cell Resolution. *Cell Metab*. 2020;32(6):1063-1075.e7. doi:10.1016/j.cmet.2020.11.007

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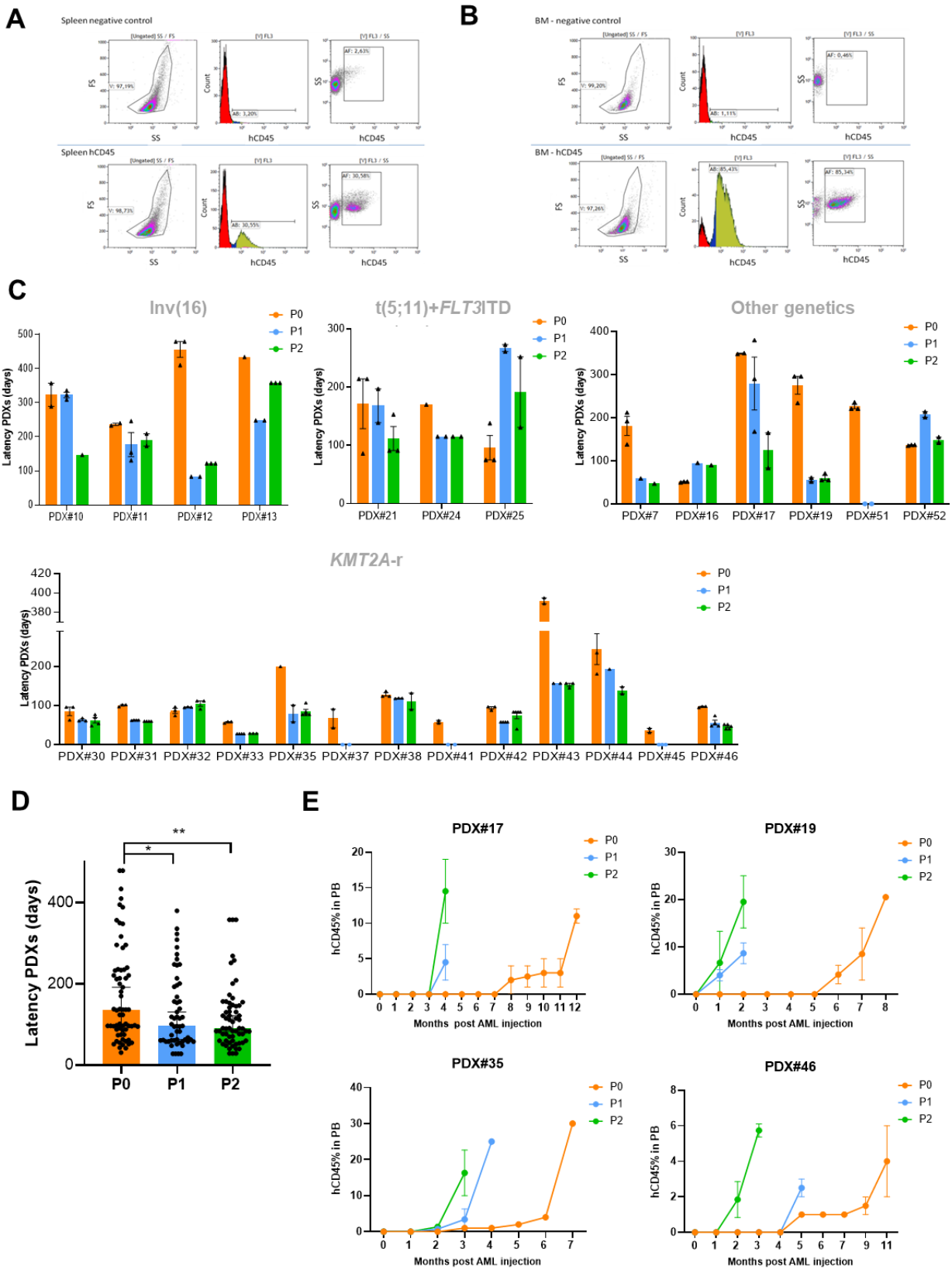
5. Slinker BK. The Statistics of Synergism. *J Mol Cell Cardiol*. 1998;30(4):723-731. doi:10.1006/jmcc.1998.0655

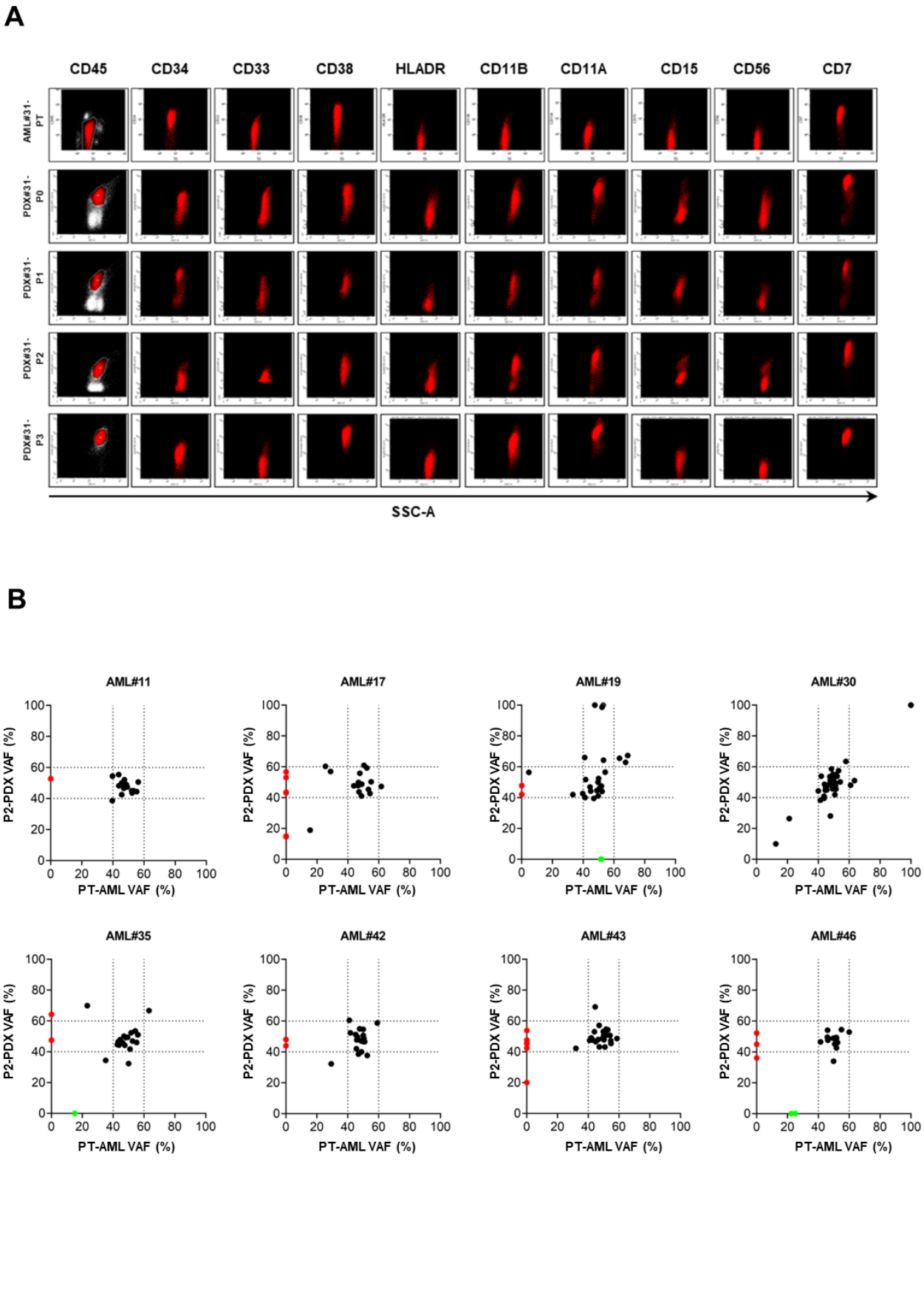
SUPPLEMENTAY FIGURES

Figure S1



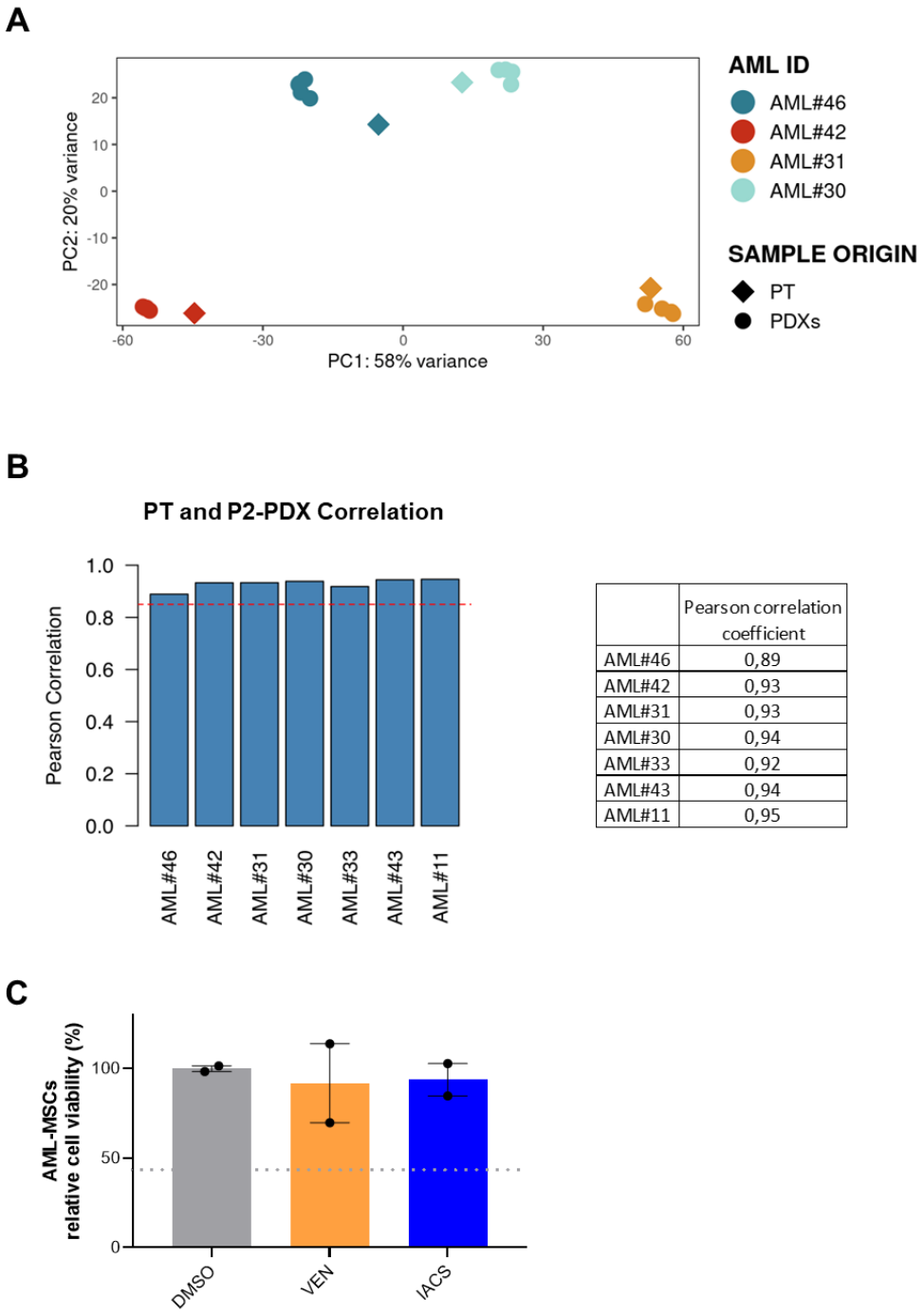
Supplementary Figure 1. AML-PDX engraftment. **(A-B)** AML infiltration in PDX organs: representative image of organs invasion by flow cytometry monitoring of hCD45 in cells harvested from mouse spleen (A) and bone marrow (B). **(C)** Latency (days from AML cell inoculation to mice sacrifice) relative to each PDX model from PT-AML to P0, from P0 to P1, from P1 to P2-PDXs. Light blue dots at 0 days indicate failure to passage. **(D)** Median of disease latency in P0- P1- and P2-PDXs (mice n=65, n=54, n=63, respectively). **E)** Representative monitoring of hCD45+ AML cells percentage in mice PB during PDX establishment process, exhibiting progressively higher rising levels at the second and third passages compared with the first (P0-P1-P2). All data are presented as mean±standard error of the mean (SEM). *p*-value <0.05(*), *p*-value <0.01(**).

Figure S2


A
CD45
CD34
CD33
CD38
HLADR
CD11B
CD11A
CD15
CD56
CD7
AML#31-PT
PDX#31-P0
PDX#31-P1
PDX#31-P2
PDX#31-P3
SSC-A
B
AML#11
AML#17
AML#19
AML#30
AML#35
AML#42
AML#43
AML#46
P2-PDX VAF (%)
PT-AML VAF (%)

Supplementary Figure 2. Immunophenotype and genomic analysis of AML blasts derived from matched patients (PT) and PDXs. A) Representative flow cytometry plots relative to immunophenotypic profile of patients' AML#31 cells (PT) and matched PDX#31 derived blasts (at P0-P1-P2-P3 passages). **B)** Correlation plots of Variant Allele Frequency (VAF) of PT-AML (x-axis) and matched P2-PDX (y-axis) in each AML patient. Red dots are relative to variants only detected in P2-PDX, whereas green dots represent variants exclusively identified in PT-AML. PCC: Pearson Correlation Coefficient. AML#11: PCC=0.31, ns; AML#17: PCC=0.42, ns; AML#19: PCC=0.14, ns; AML#30: PCC=0.94, *p*-value<0.0001; AML#35: PCC=0.14, ns; AML#42: PCC=0.17, ns; AML#43: PCC=0.40, *p*-value<0.05; AML#46: PCC=0.35, ns.

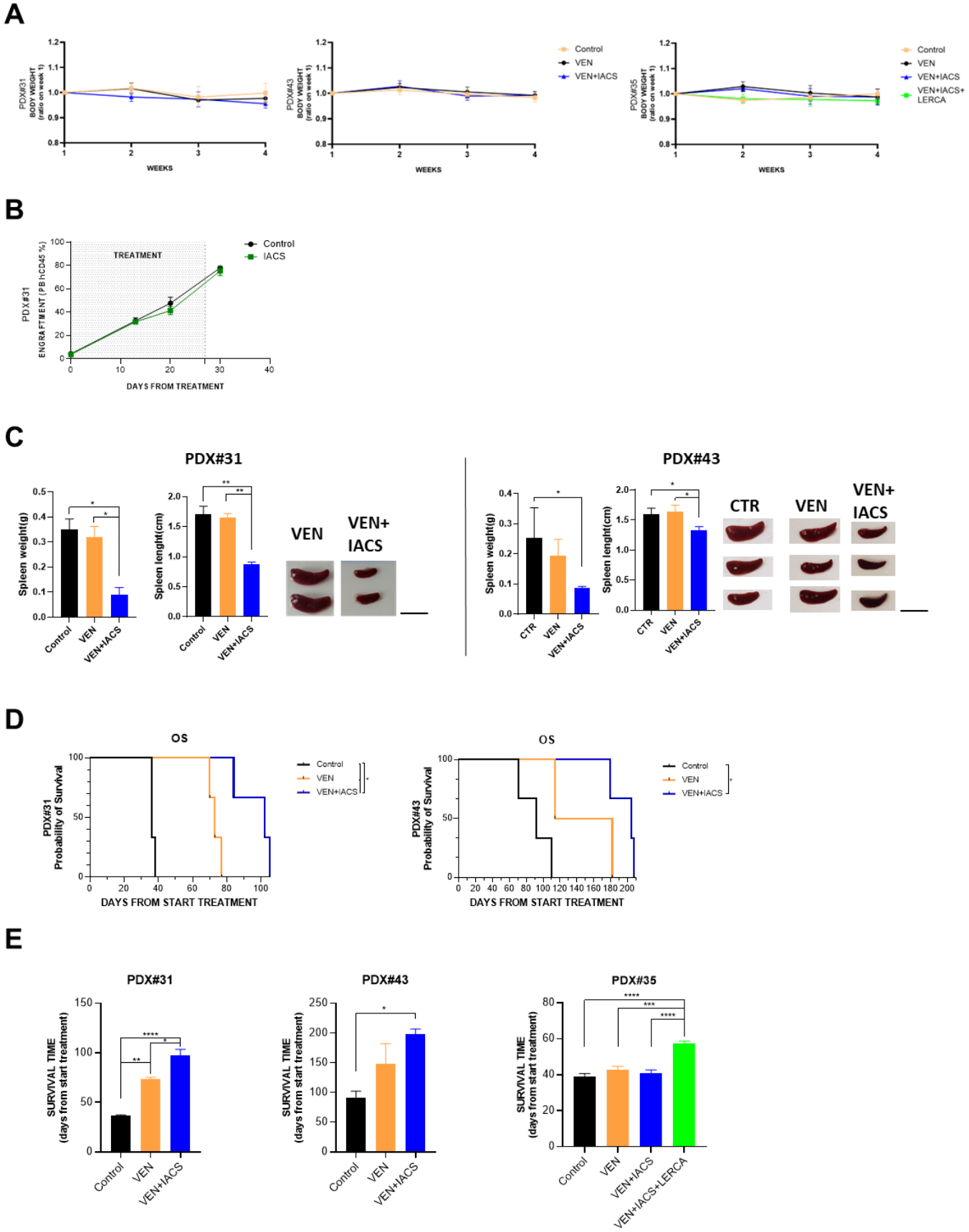
Figure S3



	Pearson correlation coefficient
AML#46	0,89
AML#42	0,93
AML#31	0,93
AML#30	0,94
AML#33	0,92
AML#43	0,94
AML#11	0,95

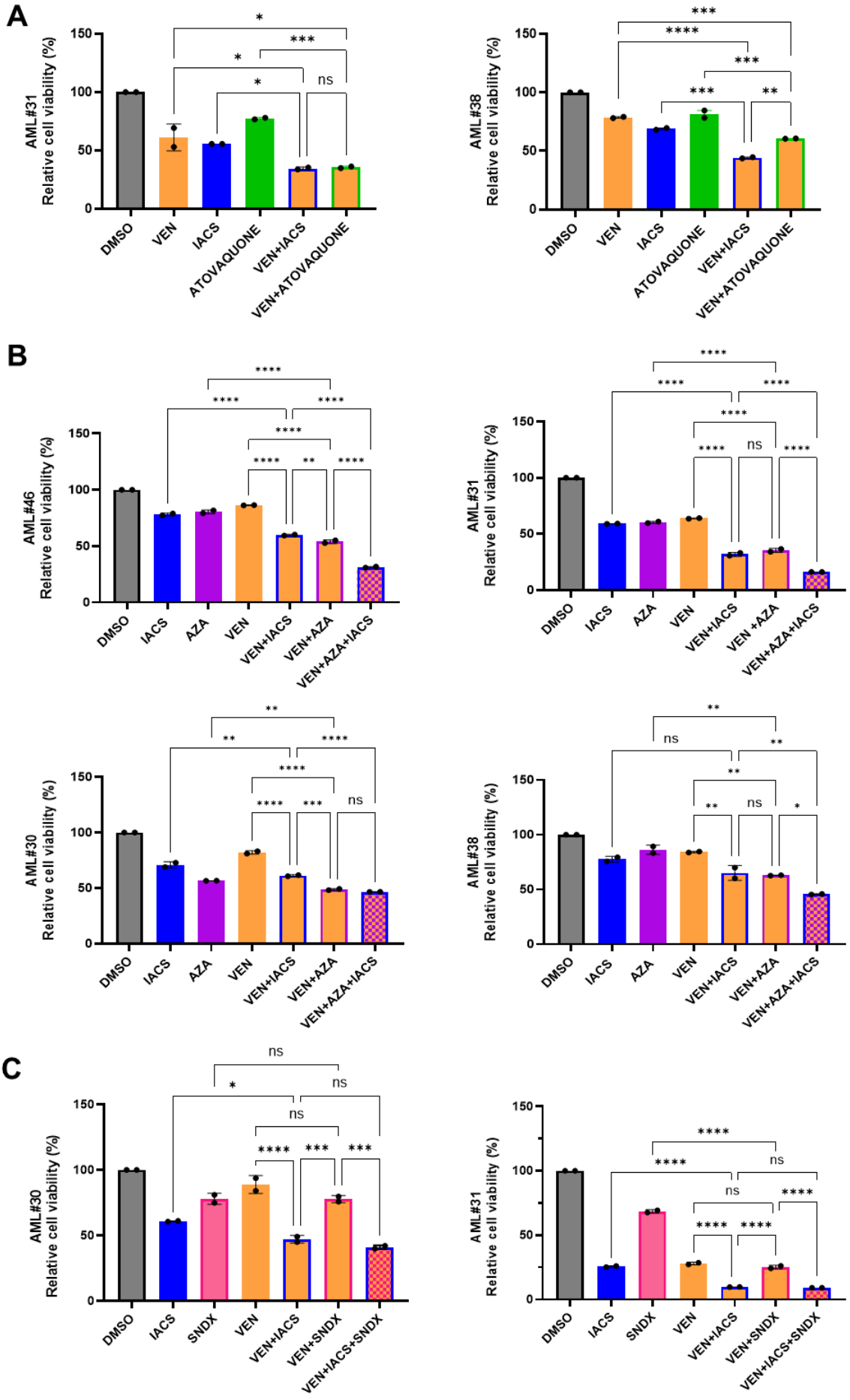
Supplementary Figure 3. **Transcriptomic analysis of AML blasts derived from patients (PT) and matched PDXs. (A)** Unsupervised Principal Component Analysis (PCA) of PT-AML and AML at P0-P1-P2-P3 mice generation (n=4 models). **(B)** Pearson correlation between PT and P2-PDX log2-transformed gene expression profiles for each AML. Bars represent the correlation coefficient calculated across all genes and reported in the adjacent table. The red dashed line indicates a correlation of 0.85; *p*-value < 0,0000000001**. (C)** Cell viability of AML-MSCs in 3D system, treated with VEN (7µM) and IACS (2µM), normalized to respective controls. All data are presented as mean±standard error of the mean (SEM).

Figure S4


A
PDX#31 BODY WEIGHT (ratio on week 1)
PDX#43 BODY WEIGHT (ratio on week 1)
PDX#35 BODY WEIGHT (ratio on week 1)
WEEKS
Control
VEN
VEN+IACS
VEN+IACS+ LERCA
B
PDX#31 ENGRAFTMENT (PB hCD45 %)
TREATMENT
Control
IACS
DAYS FROM TREATMENT
C
PDX#31
PDX#43
Spleen weight(g)
Spleen lenght(cm)
Control
VEN
VEN+IACS
CTR
VEN
VEN+ IACS
D
OS
PDX#31 Probability of Survival
PDX#43 Probability of Survival
DAYS FROM START TREATMENT
E
PDX#31
PDX#43
PDX#35
SURVIVAL TIME (days from start treatment)
Control
VEN
VEN+IACS
VEN+IACS+LERCA

Supplementary Figure 4. Testing of novel combination VEN+IACS *in vivo* on AML-PDXs. (A) Changes in mice body weight, during treatment (*p*=ns). **(B)** Monitoring of hCD45+ AML cells percentage in PB of PDX#31 during treatment with IACS-010759 (7.5 mg/kg). Mice/group n=3. **(C)** Weight, length and representative images of spleen harvested from mice 15 days after treatment discontinuation. Results are relative to untreated mice (Control), animals treated with VEN and mice receiving the combinations VEN+IACS-010759. Mice n=2/3 *per* group; scale bar=1cm. **(D)** Overall survival Kaplan-Meier analysis relative to control mice, mice treated with VEN or VEN+IACS. Mice n=3 *per* group; Log rank Mantel-Cox test. **(E)** Average survival (days) from the start of treatment with VEN or the combinations VEN+IACS-010759 and VEN+IACS-010759+LERCA; mice/group n=3. All data are presented as mean±standard error of the mean (SEM). *p*-value <0.05(*), *p*-value <0.01(**), *p*-value <0.001(***), *p*-value <0.0001(****).

Figure S5


A
AML#31
Relative cell viability (%)
DMSO
VEN
IACS
ATOVAQUONE
VEN+IACS
VEN+ATOVAQUONE
AML#38
Relative cell viability (%)
DMSO
VEN
IACS
ATOVAQUONE
VEN+IACS
VEN+ATOVAQUONE
B
AML#46
Relative cell viability (%)
DMSO
IACS
AZA
VEN
VEN+IACS
VEN+AZA
VEN+AZA+IACS
AML#31
Relative cell viability (%)
DMSO
IACS
AZA
VEN
VEN+IACS
VEN +AZA
VEN+AZA+IACS
AML#30
Relative cell viability (%)
DMSO
IACS
AZA
VEN
VEN+IACS
VEN+AZA
VEN+AZA+IACS
AML#38
Relative cell viability (%)
DMSO
IACS
AZA
VEN
VEN+IACS
VEN+AZA
VEN+AZA+IACS
C
AML#30
Relative cell viability (%)
DMSO
IACS
SNDX
VEN
VEN+IACS
VEN+SNDX
VEN+IACS+SNDX
AML#31
Relative cell viability (%)
DMSO
IACS
SNDX
VEN
VEN+IACS
VEN+SNDX
VEN+IACS+SNDX

Supplementary Figure 5. Testing of Atovaquone and novel triplet regiments *in vitro*: VEN+IACS plus Azacitidine or Menin inhibitors. (**A**) Cell viability of AML#31 and AML#38 *ex vivo KMT2A*-r blasts treated with VEN at patient-specific dosage (0.01µM and 5 µM, respectively), IACS (2 µM) or Atovaquone (15µm) and combinations for 96 hours. **(B)** Cell viability of 4 different *ex vivo KMT2A*-r primary AML cells treated with patient-specific VEN dosage (AML#30 and #31 at 0.01µM, AML#38 and #46 at 5µM), IACS (2 µM), Azacitidine (AZA, 3 µM) and combinations for 72 hours. (**C**) Cell viability of 2 different *ex vivo KMT2A*-r primary AML cells treated with patient-specific VEN dosage (AML#30 and #31 at 0.01µM), IACS (2 µM) or SNDX-5613 (SNDX, 500 nM) and combinations for 6 days. All data are normalized to respective controls and presented as mean±standard error of the mean (SEM). *p*-value <0.05(*), *p*-value <0.01(**), *p*-value <0.001(***), *p*-value <0.0001(****).

SUPPLEMENTARY TABLES 1-9

(see Excel file)