Ex vivo venetoclax sensitivity testing predicts treatment response in acute myeloid leukemia

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Received: June 30, 2022.
Accepted: November 28, 2022.
Early view: December 15, 2022.

https://doi.org/10.3324/haematol.2022.281692

Supplementary Appendix:

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Supplemental methods

Samples and shipping

Local bone marrow evaluation included BM morphology, FAB classification, immunophenotyping, chromosomes (Giemsa staining), and a gene panel for myeloid mutations. For drug sensitivity testing twenty ml of bone marrow (BM) was aspirated into four 9 ml ethylenediaminetetraacetic acid (EDTA) tubes (N=37). To ensure sufficient sample material for ex vivo drug sensitivity testing, the position of the aspiration needle was changed after aspirating 10 ml of bone marrow and samples for clinical laboratories were drawn subsequently. In case the bone marrow aspiration was unsuccessful, and the blast count was >10 % in the peripheral blood (PB), 30 ml PB was taken into six EDTA tubes (N=2). The tubes were opened to remove a vacuum.

The tubes were stored and shipped from five trial sites to the central laboratory at room temperature. Samples from Helsinki University Hospital (HUS) were delivered by a courier from a short distance; samples from Kuopio University Hospital, Oulu University Hospital, Tampere University Hospital and Turku University Hospital University Hospital were shipped by airplane or train.

Compound plates for drug sensitivity profiling

The compound plates were obtained from the High Throughput Biomedicine Unit (FIMM, HiLIFE, University of Helsinki). Compounds were dissolved in 100% dimethyl sulfoxide (DMSO) and dispensed on plates using an acoustic liquid handling device, Echo 550 (Labcyte Inc). The plates were stored under nitrogen gas at low oxygen and humidity levels in storage pods. The plate for CTG measurement was a 384-well polypropylene plate (Greiner) and compounds were plated in seven concentrations across a 10,000-fold concentration range (**Supplementary Table S1**). The plate used for FC measurement was a 96-well conical bottom polystyrene plate (Nunc) and compounds were plated in seven concentrations across a 10,000-fold concentration range (**Supplementary Table S1**). The FC drug plate consisted of venetoclax as quadruplicate and three BCL-2 family inhibitors navitoclax, S-63845 and A-1331852. Venetoclax efficacy was measured for cells cultured in three different cell culture medium: 1) RPMI 2) conditioned medium (CM, duplicate) 3) StemSpan SFEM II + 20 ng/ml of FLT3L + SCF + TPO (SPM). Azacitidine was added as 300 nM or 1000 nM on top of the venetoclax concentration series for two additional columns for cells cultured in CM. The plates contained DMSO-control wells as negative controls and benzethonium chloride wells as positive controls. Fresh plates were prepared every second month and their quality was confirmed using MOLM13 AML control cell line.

Sample preparation for drug sensitivity profiling

Samples from HUS were processed immediately (within 6 hours); whereas samples from the other trial sites were processed the following morning but not later than 26 h from the sampling. Mononuclear

cells (MNCs) were isolated with Ficoll gradient centrifugation, using Ficoll-Paque Plus solution (Cytiva, #17144003) according to manufacturer's instructions. In case there was a red blood cell contamination left after the MNC isolation, red blood cells were lysed using ACK lysing buffer for 3 minutes. The MNCs were then divided for two different drug sensitivity profiling assays: CellTiter-Glo (CTG) and flow cytometry (FC).

For the CTG-based cell viability assay, cells were suspended into conditioned medium (CM) in $0.4x10^6$ live cells/ml and plated with a multichannel pipette into 384-well plates with 10,000 cells/well in 25 µl, followed by 48-hour incubation at 37°C, under 5% CO_2.

For FC-based cell viability assay, three different culture conditions were used to measure venetoclax efficacy 1. RPMI, 2. Conditioned medium (CM), and 3. StemSpan SFEM II + 20 ng/ml of FLT3L + SCF + TPO (SPM) (**Supplementary Table S2**). Cells were suspended into media in $1x10^6$ live cells/ml and plated with a multichannel pipette into 96-well plates with 100,000 cells/well in 100 µl, followed by 48-hour incubation at 37°C, under 5% CO_2.

Drug sensitivity profiling

To immunophenotype the cells and to assess cell viability, FC analysis was done right after sample processing on an empty 96-well plate. Following the 48-hour incubation, cells on the drug plates were centrifuged at 500 × g and supernatant was discarded by flipping the plate. Cells were stained with 25 µl of a monoclonal antibody mixture containing anti-CD45, anti-CD34, anti-CD117, anti-CD14, anti-CD11b, anti-CD64 and anti-CD38 antibodies in RPMI + 10% FBS using a multichannel pipette (**Supplementary Table S7**). After 20 min incubation in the dark at RT cells were centrifuged at 500 × g and supernatant was removed. For apoptotic and dead cell exclusion, 25 µl of Annexin-V and nucleic acid dye 7-AAD mixture was added on each well (both diluted 1:50 in Annexin V binding buffer). Followed by 10 min incubation in the dark, data acquisition was performed on iQue Screener PLUS instrument (Sartorius). ForeCyt software (Sartorius) was used to analyze the cells. Gating strategy is presented in Appendix Figure S1 and S2. The total number of viable blasts in each well was counted and normalized to DMSO containing control wells. Drug sensitivity score (DSS) derived from the area under the dose response curve calculations indicated efficacy (**Supplementary Figure S3**).

For CTG-based cell viability assay, 25 µl CTG was added to each well after the 48-hour incubation, and the luminescence intensity was measured using the PHERAstar plate reader (BMG Labtech).

Preparation of conditioned medium

The HS-5 human bone marrow stromal cell line was obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, penicillin (100 U/mL), and streptomycin (100 µg/mL). When the cells reached 170 million, they were seeded to Corning Hyperflask, 1720 cm^2 at a density of 100,000

cells /cm^2 with 500 ml of medium in the Hyperflask. After 72-hour of incubation, conditioned medium was collected from 70-80% confluent plates and centrifuged at 6,000 × g for five minutes. Supernatant was divided in 120 x 4 ml aliquots and stored at -80 ºC. Upon arrival of a trial sample, fresh 12,5% CM medium was produced by adding 4 ml of CM to 28 ml of complete RPMI. Cytokine content of HS-5 derived conditioned medium was analyzed with Olink Target 48 cytokine panel (**Supplementary Table S2**).

Quality control and reproducibility

Technical quality was controlled with various processes. First, standard operating procedures were created and followed. Personnel both in hospital and laboratories were trained and they followed GCP and GLP practices. Compound plate provider evaluated the selected compound libraries frequently with cell lines (MOLM-13, IGR-OV1, DU-445 and HDQ-P1) to confirm compound stability. In addition, we evaluated the compound plates with MOLM-13 cell line for every new plate batch in 2–3-month intervals (**Supplemental Figure S4**). Commercial antibodies, media and supplements were purchased from established vendors providing validated products. Flow cytometer operation was tested prior to each sample run with quality control beads. Compound plates contained several controls both for dose response curve normalization (negative and positive controls) and cell population gating purposes (staining controls). Reproducibility and repeatability were confirmed with replicates and repeating tests by different operators. For seven samples, two different persons processed, stained, and analyzed the same sample in parallel without communicating to each other. In addition, we performed drug sensitivity testing at two different time-points (BM sampling one week apart) for two patient samples (**Supplemental Figure S4**).

Single-cell RNA sequencing

Mononuclear cells were enriched using Ficoll-Paque gradient centrifugation after they were frozen in 5 % DMSO + 95% fetal bovine serum (FBS) and stored in liquid nitrogen. Upon thawing, cells were treated with DNAse for 15 minutes after dead cells were removed with dead cell removal kit (Miltenyi Biotec) and cells were subjected to scRNA-seq.

The gel beads in emulsion (GEM) generation, cDNA amplification, and library preparation were performed according to the manufacturer's instructions using Chromium Single Cell 3' v3.1 Dual Index Reagent Kit (10x Genomics) with target loading of 10,000 cells per Chromium chip lane. Libraries were sequenced using Illumina NovaSeq 6000 system with paired-end reads 28 bp and 90 bp.

The Cell Ranger (6.0.2) mkfastq and count analysis pipelines (10x Genomics) were used to demultiplex and convert Chromium single-cell sequencing barcode and read data to FASTQ files and to align reads and generate gene-cell matrices. The raw data were aligned to the GRCh38 reference genome. UMI counts were quantified using the 10x Genomics Cell Ranger pipeline (6.0.2) with default parameters.

R package HGNChelper was used for mapping the gene symbols to the most recent symbols for both platforms, resulting in a total of 22,763 genes.

After quality control (mitochondrial transcripts < 12.5%, ribosomal transcripts > 5 % and < 50%, number of genes between 1,000 and 10,000 per cell, number of UMI reads between 2,500 and 100,000 counts), the data was log-normalized (scaling factor of 10 000) and scaled using the top 2 000 genes with the highest variance with Seurat (4.0.4) in R (4.0.0). The effect of the cell cycle was regressed out with scores assigned to each cell using the CellCycleScoring function with cell cycle phase markers provided in Seurat. The non-malignant populations (including B cells, T cells, NK cells, and red blood cells) were removed from the analysis to focus on the malignant cells.

To overcome batch effect, scVI tools (0.8.0) in Python (3.6.8) was used to calculate latent embeddings with default parameters. The latent embeddings were used for graph-based clustering and UMAP dimensionality reduction in Seurat with default parameters. Cell clusters were annotated with an ensemble method including analysis of canonical marker genes, calculating the most differentially expressed genes (Wilcoxon test), and analysis with reference-based method SingleR (1.2.4) performed with default parameters.

Statistical analyses

Efficacy analyses were performed on all participants who received at least one dose of venetoclax. Both recruiting cohorts and trial phases are analyzed separately. If the sample was non-evaluable for ex vivo drug sensitivity, the participant was eligible to receive the venetoclax–azacitidine combination, but was not evaluable for the study's endpoints.

To compare the difference in DSSs between the two groups during analyses of the cell culture method and clinical response, Mann-Whitney *U* test or Wilcoxon matched-pairs signed-rank test were used. To define the best cutoff values for drug sensitivity testing, a receiver operating characteristic curve (ROC) analyses were performed using the Wilson/Brown method and Prism 8 (GraphPad). Specificity, sensitivity, and likelihood ratio were calculated for each cutoff value and method.

Overall survival (OS) was defined as the number of days from the date of the first dose to the date of death from any cause. Progression-free survival (PFS) was defined as the number of days from the date of the first dose to the date when deemed refractory or the earliest evidence of relapse or death. All disease progression was included regardless whether the event occurred while the participant was taking the study drug or had previously discontinued the study therapy. If the participant achieved response and did not experience relapse or death, the data was censored at the cutoff date. For the participants starting other therapy after the study the data was censored at the start date of the other therapy. Four

of the censored participants were continuing the study therapy and three continued with azacytidine monotherapy after the end of the study.

For this interim analysis, the clinical data cutoff date was January 20, 2022. OS and PFS were assessed using the Kaplan-Meier method, and the difference between two groups was compared using a log-rank test. The survival rate was estimated along with the corresponding 95% confidence interval (CI). Statistical analyses and graphic illustrations were conducted using IBM SPSS Statistics version 25 and R version 4.1.2.

Figure S1

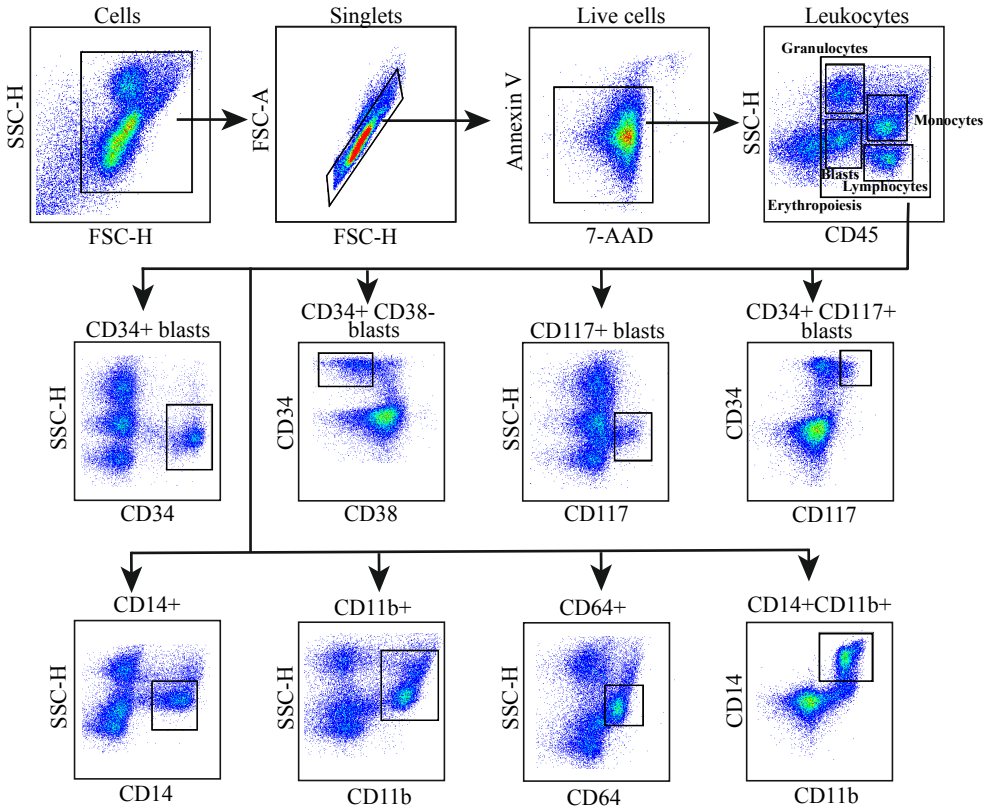


Figure S1. Gating strategy for flow cytometry analysis. Cells were gated and debris removed based on SSC-H/FSC-H after singlets were identified with FSC-A/FSC-H. Viable cells were gated by excluding the apoptotic and dead cells using Annexin V and 7-AAD, respectively. SSC-H/CD45 was used to gain an overview from the cell composition of the sample: blasts – SSClow/CD45dim, lymphocytes – SSClow/CD45bright, granulocytic cells (present after Ficoll gradient centrifugation) – SSChigh/CD45dim and monocytic cells – SSCmid/CD45bright. Blasts were identified with CD34 and CD117 antibodies and leukemic stem cells were gated as CD34+/CD38-. Additional markers CD11, CD64, and CD14 were used to gate cells differentiated towards monocytic and granulocytic lineages.

Figure S2

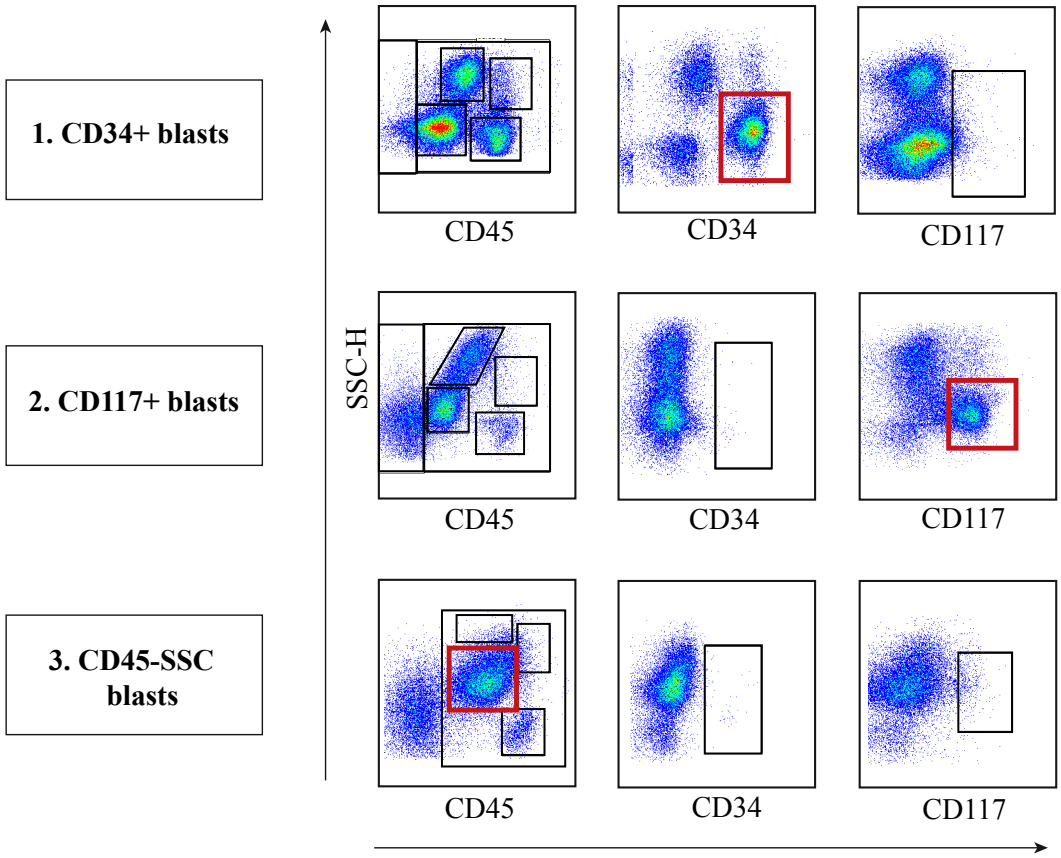


Figure S2. Gating strategy for blasts. CD34 positivity was used as the main marker to calculate drug sensitivity of blasts, and it was used for 34/39 samples. If blasts did not express CD34, CD117 was used to identify blasts (2/39 samples). If blasts were negative for both CD34 and CD117, blasts were identified as SSClow/CD45dim (3/39) samples.

Figure S3

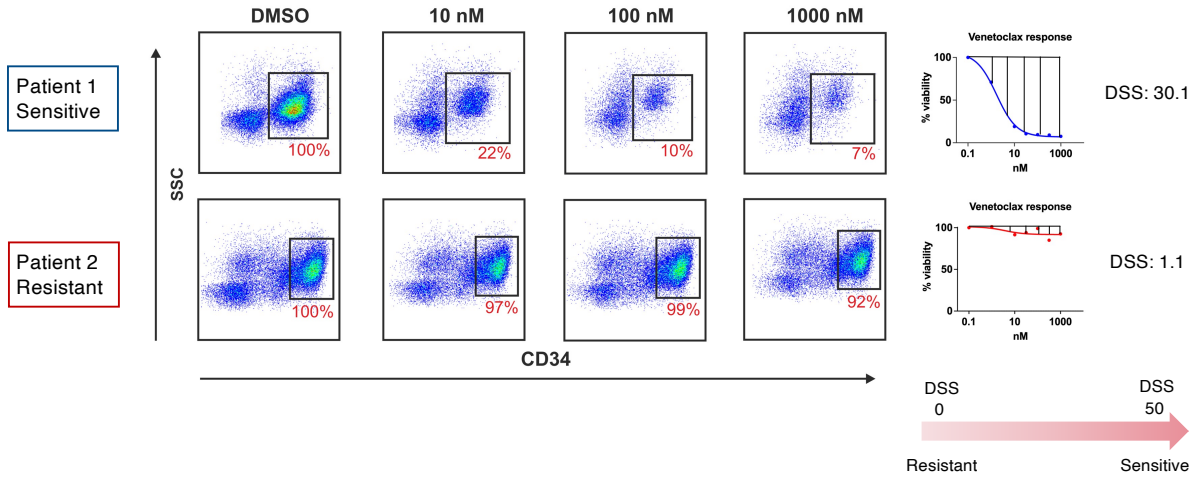


Figure S3. Conversion of venetoclax sensitivity to drug sensitivity scores (DSS). Illustration of an ex vivo venetoclax sensitive and a resistant sample. The number of the viable blasts (CD34) after venetoclax treatment relative to DMSO control were converted to dose response curves (seven concentrations) and then to DSS. DSS is calculated based on optimized area under the dose response curve; higher number reflecting higher sensitivity (range 0-50, Yadav et al., Scientific reports, 2014).

Figure S4

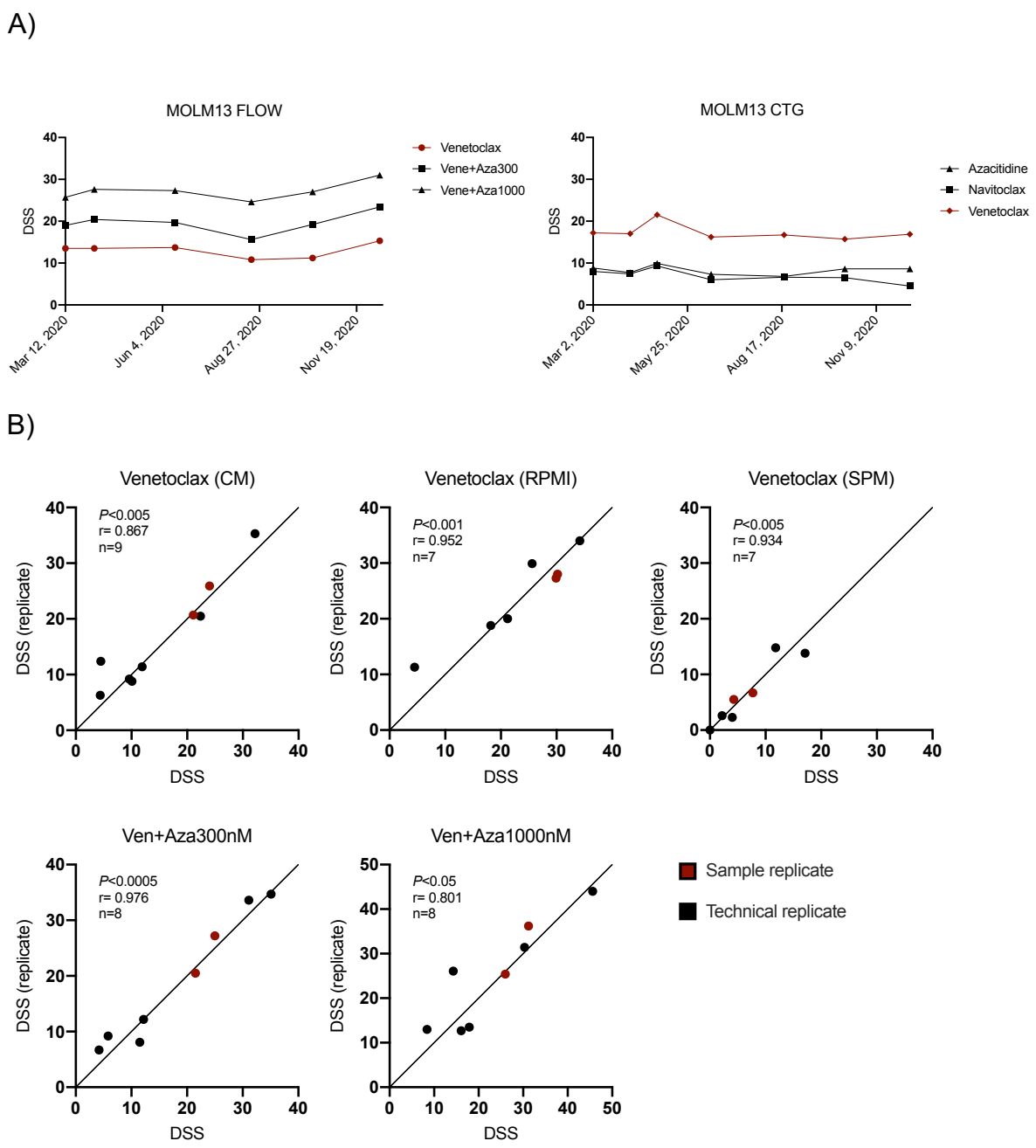


Figure S4. Quality control assessment and reproducibility of the ex vivo drug sensitivity testing. (A) To verify the efficacy of the compounds, MOLM13 AML cell line was screened in 96-well (flow) and 384-well (CTG) plates every 2-3 months as the new plate batch was prepared. Cells were incubated for 48-hours, and drug sensitivity was assessed as described in the methods. **(B)** To assess the reproducibility of the FC blast-specific drug sensitivity testing seven samples were processed, stained, and analysed in parallel by two different persons (colored as black). For two samples, drug sensitivity testing was performed at two different time-points (BM sampling one week a part, colored as red). Correlation coefficient and p-value was calculated using two-tailed Spearman correlation.

Figure S5

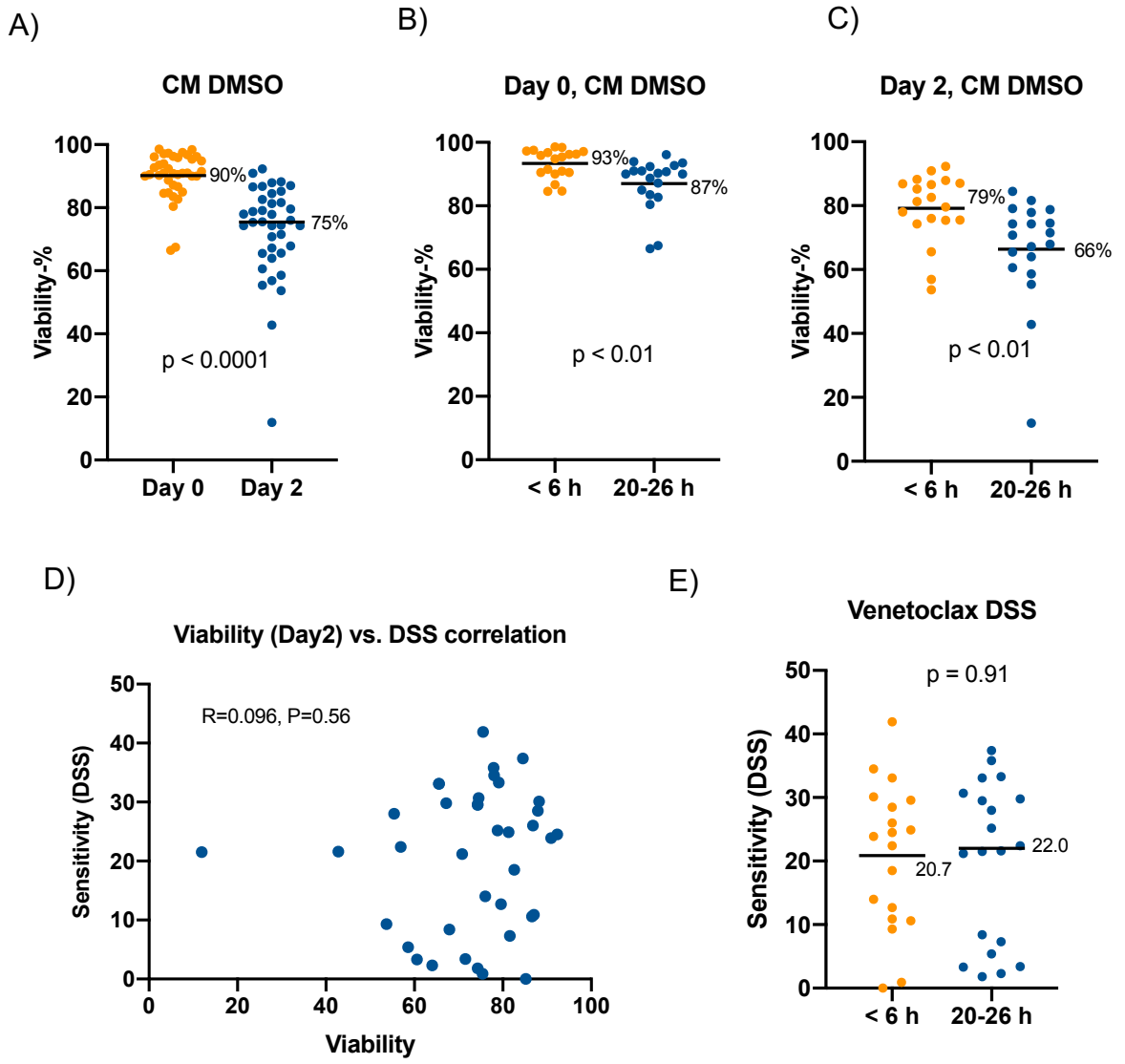


Figure S5. Sample viability. Viability was measured using Annexin V and 7-AAD. **(A)** Sample viability was assessed immediately after sample processing (Day 0) and after 48h incubation (Day 2) in CM medium from DMSO wells. Lines represent the median mononuclear cell viability and p-value was calculated using two-tailed Wilcoxon matched-pairs signed-rank test. **(B)** Samples from the neighboring hospital were processed right away (< 6 h), whereas the samples from the other hospitals were processed the following morning (20-26 h). Median viability is presented in the figure and p-value was calculated with Mann–Whitney *U* test. **(C)** Median viability of the samples processed within 6 h and 20-26 h after two-day incubation in DMSO wells in CM medium. p-value was calculated using Mann–Whitney *U* test. **(D)** Correlation plot of blast-specific venetoclax sensitivity (DSS) vs. cell viability at Day 2 in DMSO wells in CM medium (n=38). **(E)** Blast-specific venetoclax sensitivity (DSS) in samples processed within 6 h and between 20-26 h. Lines represent median value and p-value was calculated using Mann–Whitney *U* test.

Figure S6

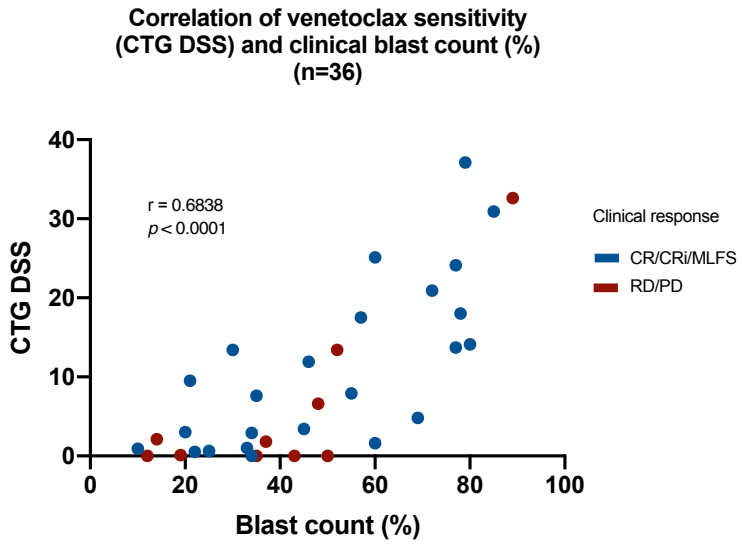


Figure S6. Correlation of ex vivo venetoclax sensitivity and clinical blast count. CTG viability assay was used to measure ex vivo venetoclax sensitivity for all trial participants. Two-tailed Mann-Whitney *U* test and Spearman's correlation were used to calculate p-value and correlation coefficient, respectively.

Figure S7

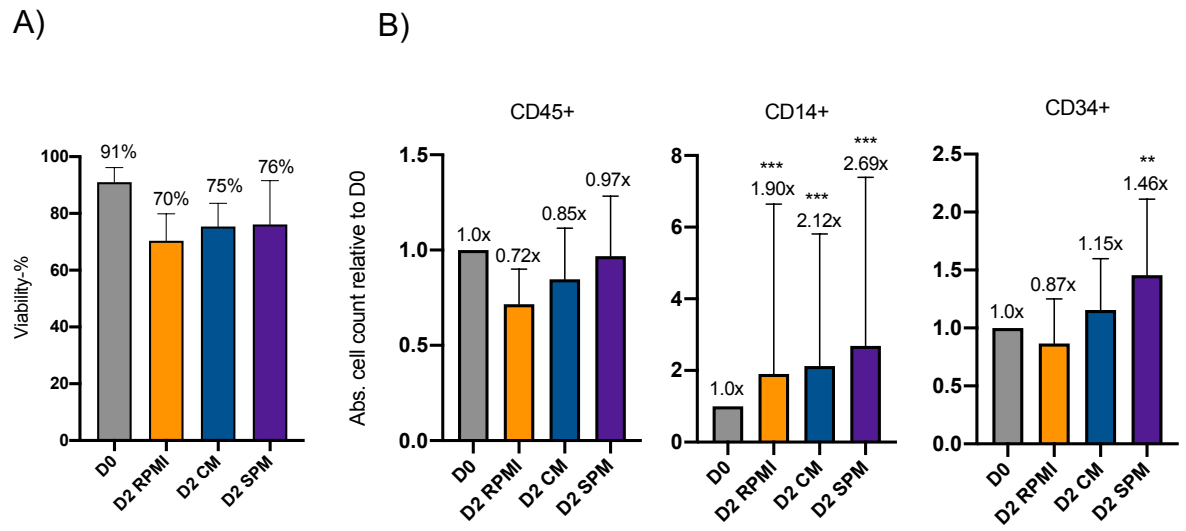


Figure S7. Comparison of the effect of culture media on viability and cell count. (A) Median cell viability with interquartile range of MNCs (n = 41) at Day 0 and after 2-day culture in RPMI, CM and SPM media. Viability was assessed by Annexin V and 7-AAD staining in control wells. **(B)** Median fold change with interquartile range of absolute numbers of CD45, CD14 and CD34 positive cells after 2-day culture relative to Day 0 sample. Each sample was normalized to cell count calculated at Day 0. Statistically significant differences compared to Day 0 control are indicated with asterix, **p<0.005, ***p<0.0001. p-value calculated with Wilcoxon matched-pairs signed-rank test.

Figure S8

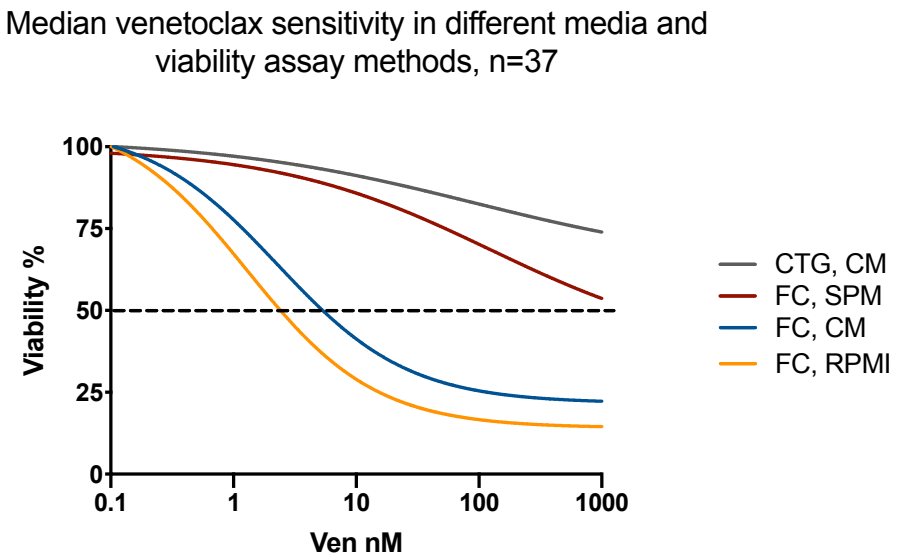


Figure S8. Median venetoclax sensitivity assessed in different media and viability assay methods. Median venetoclax dose response curves were calculated separately for RPMI, CM and SPM media using flow cytometry (FC) viability assay (n=37). For CTG viability assay, median dose response curve was derived only in CM medium (n=36).

Figure S9

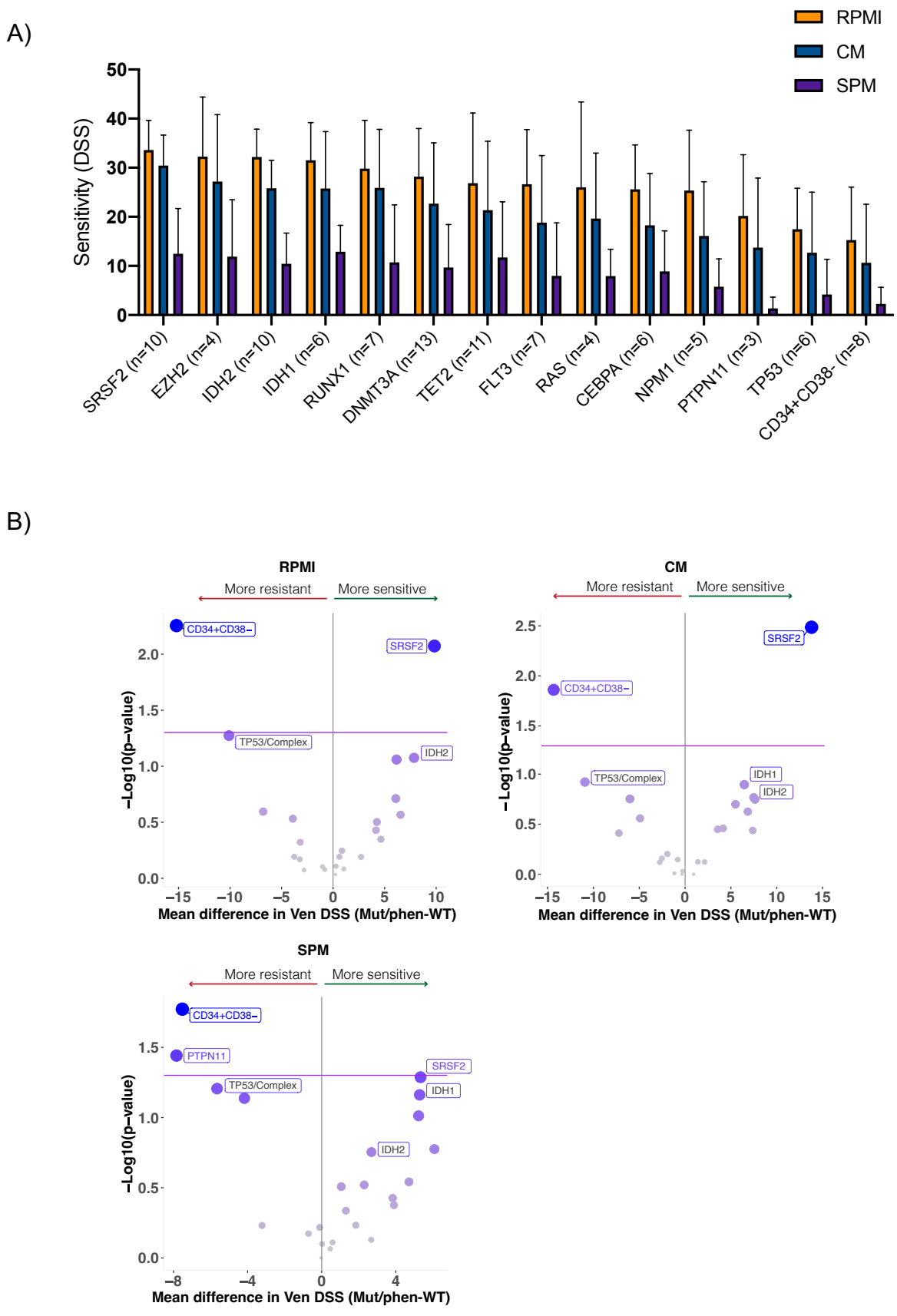
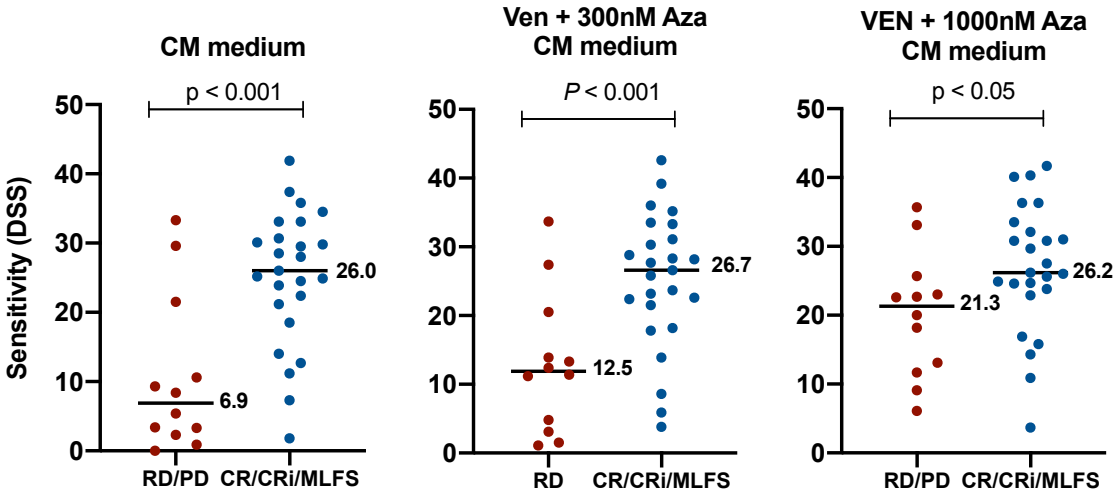

A)
RPMI
CM
SPM
Sensitivity (DSS)
50
40
30
20
10
0
SRSF2 (n=10)
EZH2 (n=4)
IDH2 (n=10)
IDH1 (n=6)
RUNX1 (n=7)
DNMT3A (n=13)
TET2 (n=11)
FLT3 (n=7)
RAS (n=4)
CEBPA (n=6)
NPM1 (n=5)
PTPN11 (n=3)
TP53 (n=6)
CD34+CD38- (n=8)
B)
RPMI
More resistant
More sensitive
CD34+CD38−
SRSF2
TP53/Complex
IDH2
−Log10(p−value)
Mean difference in Ven DSS (Mut/phen-WT)
CM
More resistant
More sensitive
SRSF2
CD34+CD38−
TP53/Complex
IDH1
IDH2
−Log10(p−value)
Mean difference in Ven DSS (Mut/phen-WT)
SPM
More resistant
More sensitive
CD34+CD38−
PTPN11
TP53/Complex
SRSF2
IDH1
IDH2
−Log10(p−value)
Mean difference in Ven DSS (Mut/phen-WT)

Figure S9. Ex vivo venetoclax sensitivity of the blasts by genotypic/phenotypic subgroups. (A) Mean drug sensitivity scores (DSS) of blasts with specific genotypic/phenotypic characteristics evaluated by FC in RPMI, CM and SPM media. Number of patient samples is indicated in the brackets while standard deviation in each column. **(B)** Volcano plot of the most significant genotypic/phenotypic characteristics of the blasts associated with altered ex vivo venetoclax sensitivity in RPMI, CM and SPM media. Immunophenotypes of the blasts were defined in clinical laboratories and included CD34, CD64, CD123, CD7, CD71 and CD38. Purple line represents a p-value < 0.05. p-values were calculated using Mann-Whitney *U* test and detailed values for each group are presented in Supplementary Table S4.

Figure S10

A)



B)

C)

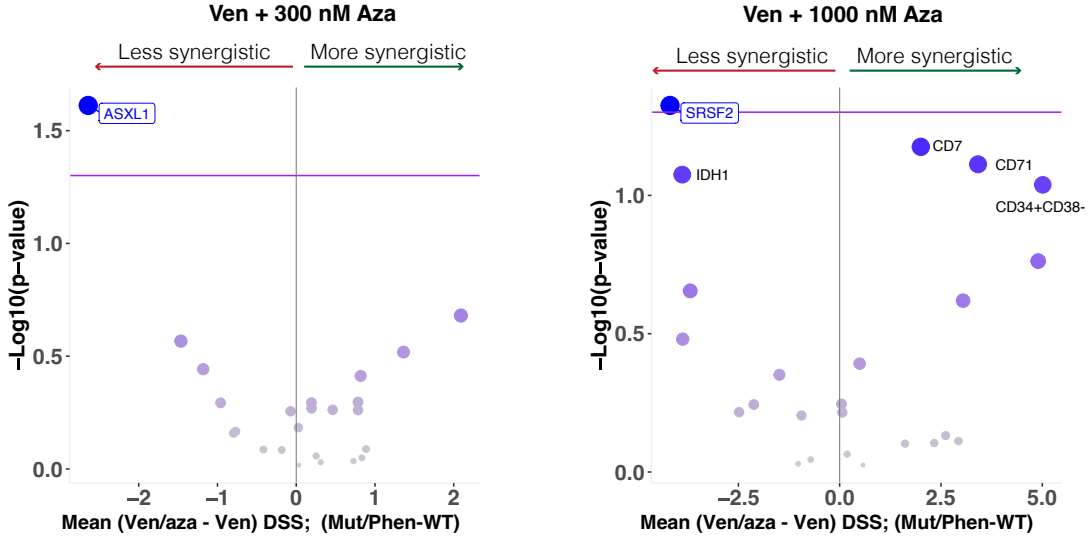


Figure S10. Venetoclax+azacitidine drug sensitivity/synergy. **(A)** Blast-specific venetoclax and venetoclax+azacitidine DSS using FC in conditioned medium (CM). Combination efficacy was measured by adding 300 nM or 1000 nM of azacitidine to the venetoclax-containing wells across the entire concentration range. Participants were divided between responders (CR/CRi/MLFS, n = 25) and non-responders (PD/RD, n = 12). Line represents the median, and p-value was calculated using one-tailed Mann-Whitney *U* test**. (B)** Volcano plot of the most significant genotypic/phenotypic characteristics of the blasts associated with altered ex vivo venetoclax+azacitidine synergy. Venetoclax DSS was subtracted from venetoclax+azacitidine DSS and the mean delta value of each subgroup (mut/phen) was compared to WT blasts. p-value < 0.05 is indicated with a purple line. p-values were calculated using Mann-Whitney *U* test.

Figure S11

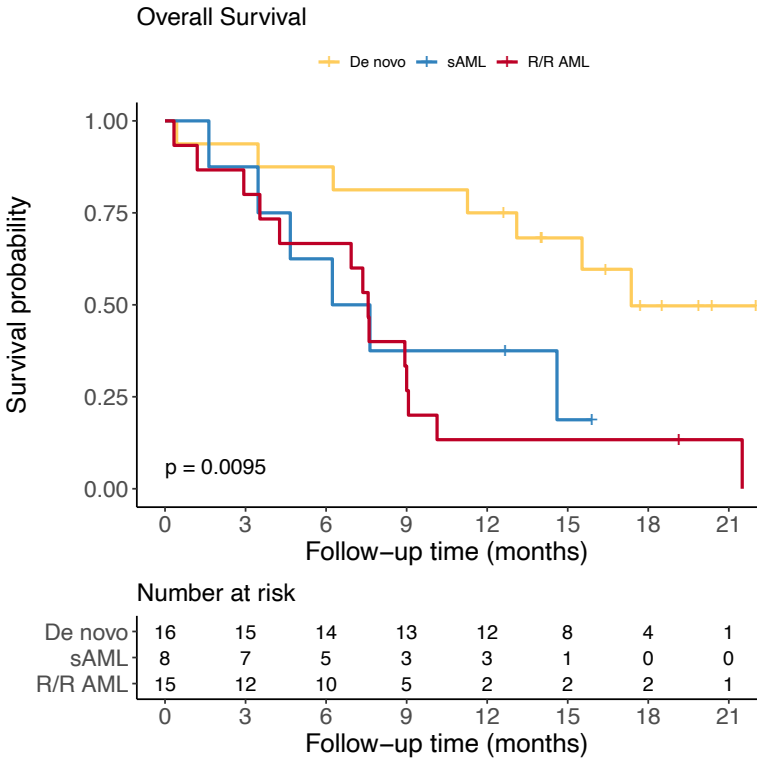


Figure S11. Overall survival in de novo, secondary and R/R AML. Median OS for de novo (17.4 months), sAML (6.9 months) and R/R AML (7.6 months). Participants alive at the data cut-off day were censored. The median follow-up time was 18.6 months.

Figure S12

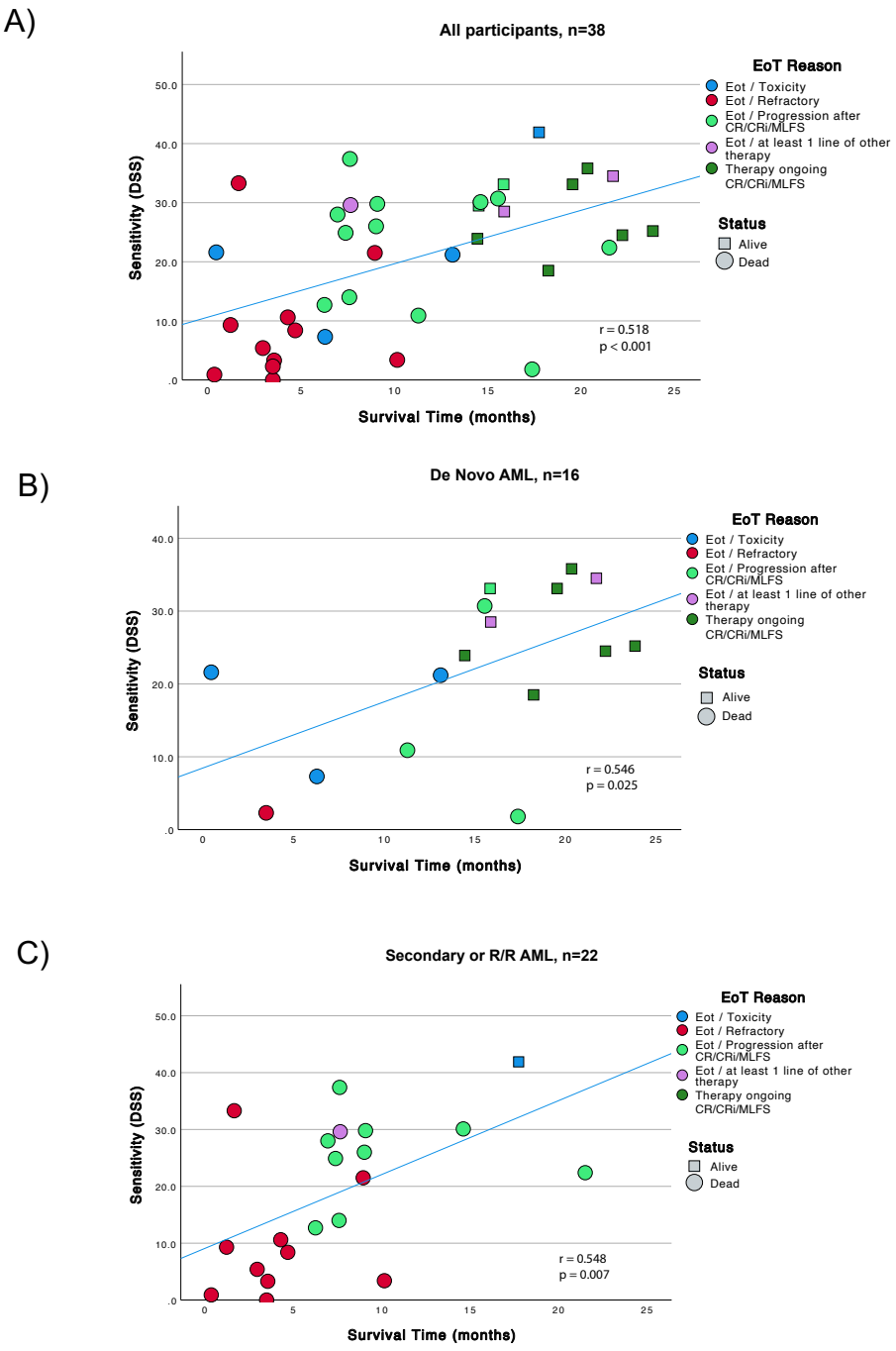


Figure S12. Correlation of ex vivo venetoclax sensitivity (DSS) and survival time (A) in all participants, (B) in de novo AML and (C) in secondary or R/R AML. Drug sensitivity assessed in CM using FC. The end of therapy (EoT) reason for participants continuing in subsequent treatment was either toxicity or relapse after CR/CRi/MLFS response. Toxicity arised in all cases from prolonged cytopenia and/or recurrent infections. One participant in the secondary or R/R AML group and nine participants in the de novo AML group were alive at the time of analysis. Spearman's correlation covariant showed statistically relevant bivariant correlation between ex vivo venetoclax sensitivity and survival time.

Figure S13

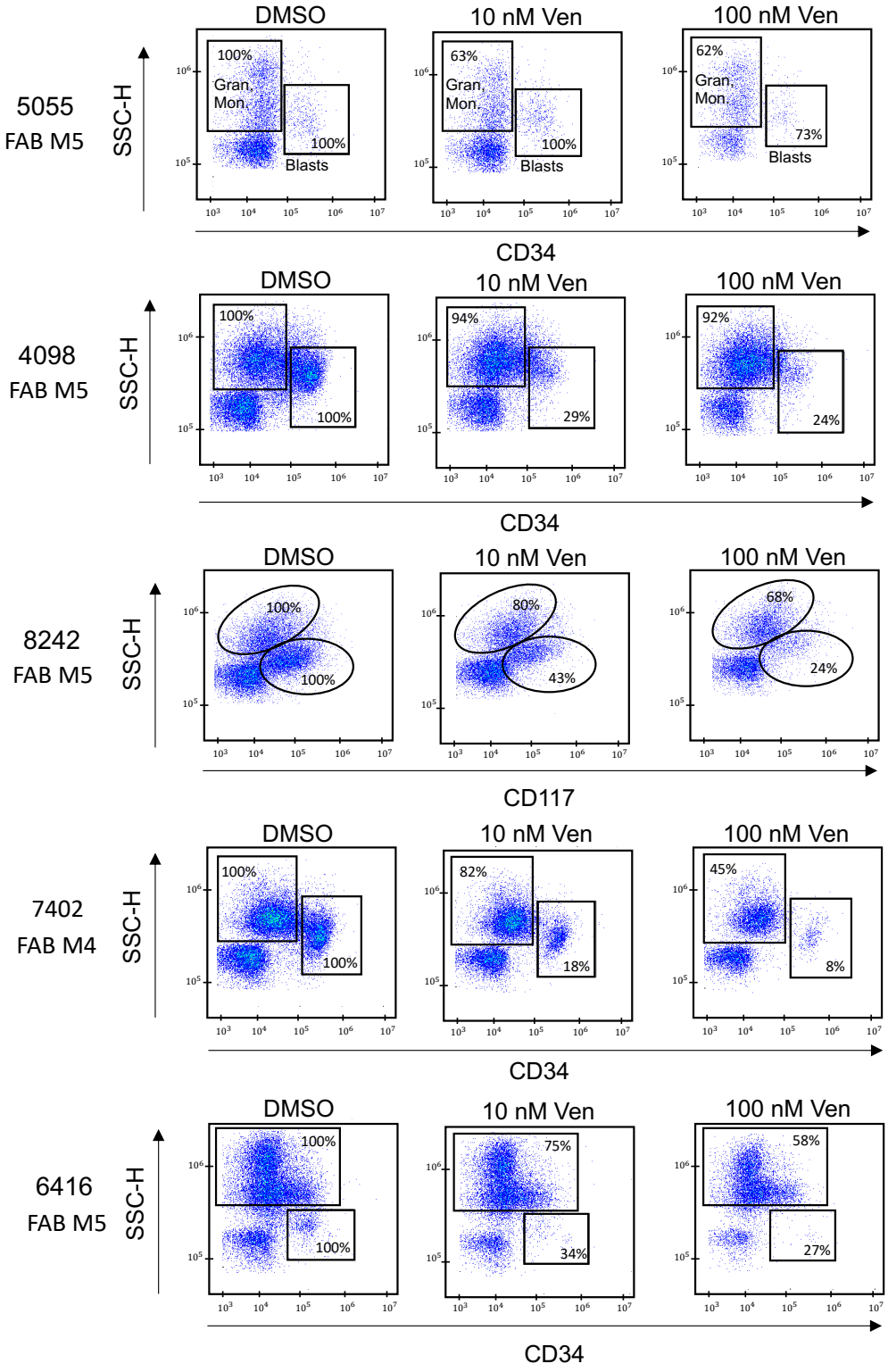


Figure S13. Flow cytometry illustration of monocytic AML. Four samples had FAB M5 and one sample FAB M4 AML. SSC-H/CD34 or SSC-H/CD117 scatter plots illustrate the percentage of different cell populations relative to DMSO control after 2-day treatment of 10 nM and 100 nM venetoclax ex vivo.

Figure S14

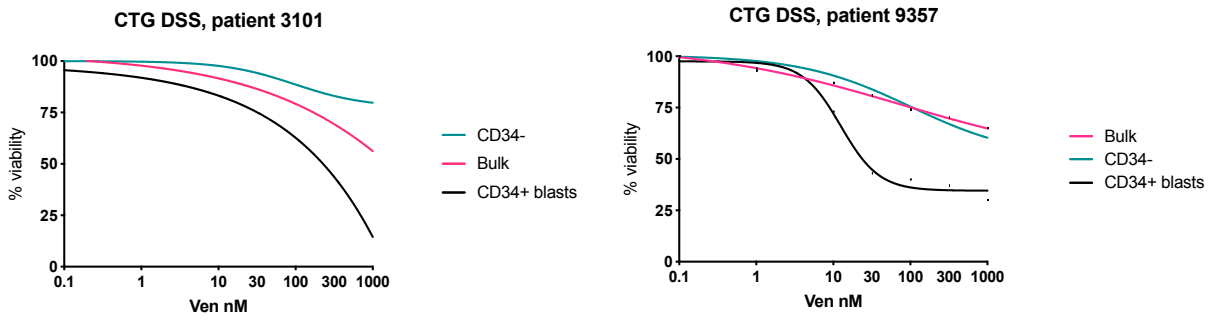


Figure S14. CTG measurement of CD34 enriched blasts vs. bulk sample vs. CD34- cell fraction. CD34 blasts were enriched from two samples with low blast counts using CD34 magnetic beads after thawing. Venetoclax dose response curves for CD34- cell fraction, CD34+ blasts, and bulk sample (all cell compartments) were measured using CTG viability assay. CD34+ blasts were more sensitive to venetoclax compared to other fractions (CD34- fraction or bulk sample) in both cases.

Figure S15

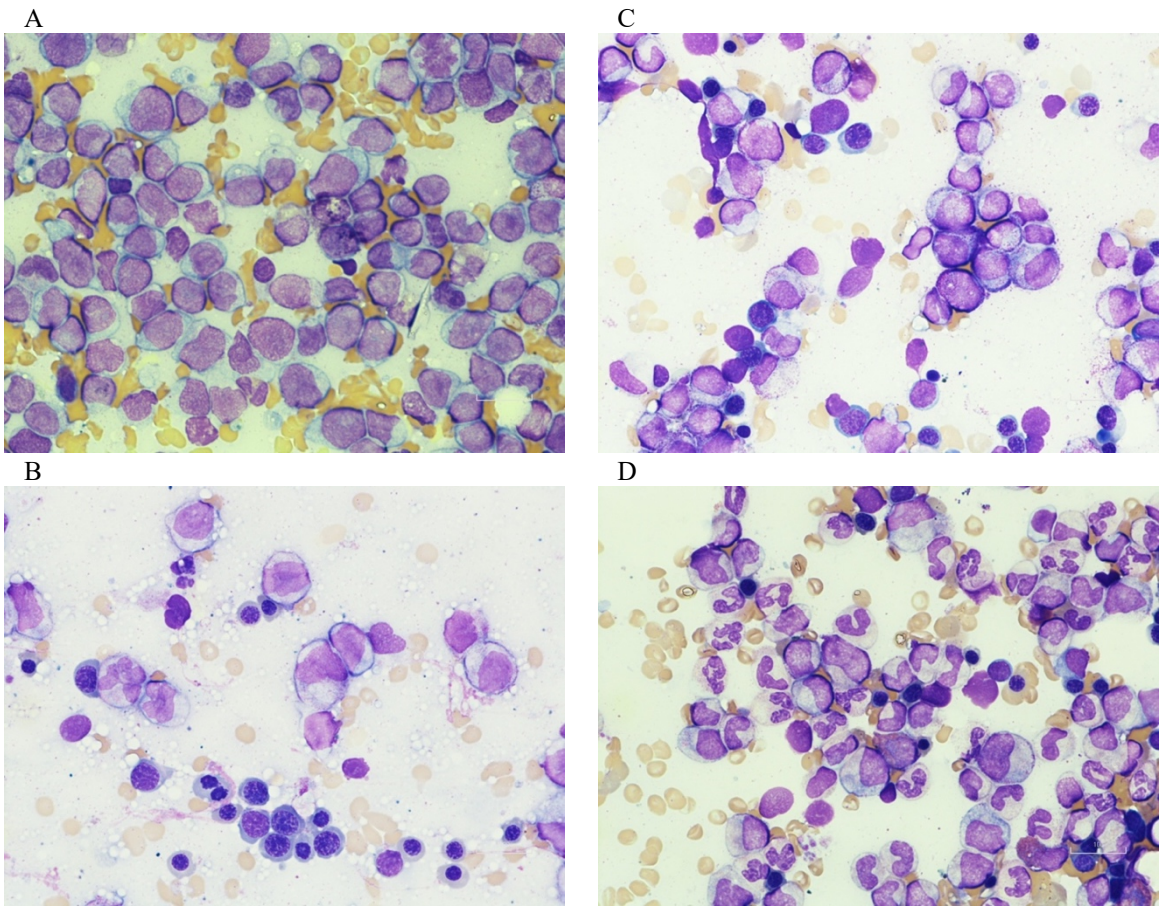


Fig. S15. Bone marrow aspirate morphology of de novo AML patient (FAB M5, 6416) in four timepoints before and during study therapy. At screening **(A)**, after C1 **(B)**, after C2 **(C)**, and after C3 **(D).** Combined blast and promonocyte percentage of 25% persisted after C1. Complete remission was maintained after C2 with combined blast and promonocyte percentage within normal range (<5%) and recovery from cytopenia. Elevated amount of monocyte lineage cells persisted after achieving complete remission until disappearing after C6.

Table S1. Compounds

	Compound	Supplier	Supplier ref.	Concentration range (nM)
Single compound				
	Venetoclax	ChemieTek	CT-A199-2	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
	Azacitidine	Medchem Express	HY-10586	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
	Navitoclax	Selleck	S1001-3	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
	A-1331852	Chemietek	CT-A133	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
	S-63845	ChemieTek	CT-S63845	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
Combination 1				
	Venetoclax	ChemieTek	CT-A199-2	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
	Azacitidine	Medchem Express	HY-10586	300
Combination 2				
	Venetoclax	ChemieTek	CT-A199-2	0.1-1000 (0.1; 1; 10; 30; 100; 300; 1000)
	Azacitidine	Medchem Express	HY-10586	1000

Table S2. Media composition

Media	Supplements	Cytokines
RPMI	**RPMI:** *Corning, cat. 15-040-CV* **10% FBS**, *Gibco, cat. 10270-106* **2 mM L-glutamine**, *Lonza, cat. 17-605E* **100 U/ml penicillin**, *Lonza, cat. DE17-602E* **100 µg/ml streptomycin**, *Lonza, cat. DE17-602E*	None
CM (conditioned medium)	**Complete RPMI 87,5% +** **12,5% conditioned medium** (collected from HS-5 mesenchymal stem cell cultures) **HS-5 cells**: *American Type Culture Collection (Manassas, VA)*	**Olink Target 48 cytokine panel (pg/ml):** **CM 12.5%:** **CSF3** 41 591, **IL6** >10 000, **CXCL8** > 10 000, **CCL2** > 10 000, **CSF2** 4 595, **VEGFA** 2 614, **MMP1** 1 744, **MMP12** 1 366, **CCL7** 824, **CCL8** 530, **IL1B** 107, **CCL3** 47.8, **CXCL12** 25.1, **CSF1** 11.5, **CXCL10** 7.3, **TGFA** 4.6, **IL15** 3.1, **TNFSF1**2 2.7, **FLT3LG** 1.7, **TNF** 1.3, **CCL11** 1.0, **CCL4** 1.1, **IL10** 0.5, **IL13** 0.4, **IL17C** 0.4, IL33 0.4, **IL17F** 0.4, **CCL13** 0.4, **TSLP** 0.3, **HGF** 0.3, **CCL19** 0.2, **IL17A** 0.2, **IL27** 0.2, **IL7** 0.1, **LTA** 0.1, **CXCL11** 0.1, **IL18** 0.1, **EGF** 0.1, **IL2** 0.0, **IFNG** 0.0, **IL4** 0.0, **OSM** 0.0, **OLR1** 0.0, **CXCL9** 0.0, **TNFSF**10 0.0
SPM (StemSpan, SFEM II) Serum-Free medium	**SFEM II**, *STEMMCELL technologies, ref. #09655* **FLT3L**, *Peprotech, cat. 300-07* **TPO,** *Peprotech, cat. 300-18* **SCF,** *Peprotech, cat. 300-19*	**FLT3L** (20ng/ml), **SCF** (20ng/ml), and **TPO** (20ng/ml)

Table S3. Treatment outcome in all patients and ex vivo sensitive participants

	CR/CRi	ORR (CR/CRi/MLFS)
	De Novo AML n = 16	
All	12/16 (**75 %**)	14/16 (**88 %**)
Ex vivo sensitive	11/12 (**92 %**)	12/12 (**100 %**)
	R/R/s AML n = 23	
All	9/23 (**39 %**)	12/23 (**52 %**)
Ex vivo sensitive	8/14 (**57 %**)	11/14 (**79 %**)
	De Novo + R/R/s AML n = 39	
All	21/39 (**54 %**)	26/39 (**67 %**)
Ex vivo sensitive	19/26 (**73 %**)	23/26 (**88 %**)

Table S4. Ex vivo venetoclax sensitivity in different genotypic/phenotypic subgroups

A) RPMI

#	Geno/Phenotype	Culture medium	DSS mean Mut/Phen (1)	DSS mean WT (0)	DSS Mean dif (1-0)	P-value	Adj pvalue
1	CD34+CD38-	Ven_RPMI	14.1	29.3	-15.2	0.0056	0.1014
2	SRSF2	Ven_RPMI	33.6	23.8	9.8	0.0085	0.1014
3	TP53_complex	Ven_RPMI	17.7	27.8	-10.1	0.0533	0.4181
4	IDH2	Ven_RPMI	32.2	24.3	7.9	0.0841	0.4181
5	CD123	Ven_RPMI	29.3	23.1	6.2	0.0871	0.4181
6	IDH1	Ven_RPMI	31.5	25.5	6.1	0.1943	0.7548
7	PTPN11	Ven_RPMI	20.2	27.0	-6.8	0.2540	0.7548
8	EZH2	Ven_RPMI	32.3	25.7	6.5	0.2711	0.7548
9	CD71	Ven_RPMI	24.8	28.7	-3.9	0.2939	0.7548
10	M1	Ven_RPMI	29.3	25.1	4.2	0.3145	0.7548
11	RUNX1	Ven_RPMI	29.8	25.7	4.2	0.3724	0.8125
12	ASXL1	Ven_RPMI	30.6	25.9	4.6	0.4483	0.8809
13	NPM1	Ven_RPMI	23.7	26.9	-3.2	0.4772	0.8809
14	M2	Ven_RPMI	27.1	26.3	0.9	0.5674	0.8816
15	TET2	Ven_RPMI	26.9	26.3	0.6	0.6418	0.8816
16	CD34	Ven_RPMI	26.0	29.8	-3.8	0.6422	0.8816
17	DNMT3A	Ven_RPMI	28.2	25.5	2.7	0.6446	0.8816
18	M0	Ven_RPMI	23.5	26.7	-3.2	0.6764	0.8816
19	CD7	Ven_RPMI	27.1	26.8	0.3	0.7804	0.8816
20	CEBPA	Ven_RPMI	25.6	26.6	-1,0	0.7888	0.8816
21	M4/M5	Ven_RPMI	27.3	26.3	1.0	0.8242	0.8816
22	CD64	Ven_RPMI	26.2	27.0	-0.8	0.8366	0.8816
23	RAS	Ven_RPMI	23.9	26.7	-2.8	0.8449	0.8816
24	FLT3	Ven_RPMI	26.6	26.4	0.2	0.9228	0.9228

B) CM

#	Geno/Phenotype	Culture medium	DSS mean Mut/Phen (1)	DSS mean WT (0)	DSS Mean dif (1-0)	P-value	Adj pvalue
1	SRSF2	Ven_CM	30.4	16.6	13.8	0.0033	0.0784
2	CD34+CD38-	Ven_CM	8.7	23.1	-14.3	0.0138	0.1655
3	TP53_complex	Ven_CM	10.9	21.8	-10.9	0.1199	0.5999
4	IDH1	Ven_CM	25.8	19.3	6.5	0.1273	0.5999
5	IDH2	Ven_CM	25.8	18.3	7.5	0.1713	0.5999
6	CD71	Ven_CM	17.6	23.6	-6.0	0.1766	0.5999
7	EZH2	Ven_CM	27.2	19.5	7.6	0.1786	0.5999
8	M1	Ven_CM	24.1	18.6	5.5	0.2000	0.5999
9	RUNX1	Ven_CM	25.9	19.1	6.8	0.2369	0.6317
10	NPM1	Ven_CM	16.1	21.0	-4.9	0.2764	0.6633
11	CD123	Ven_CM	22.9	18.7	4.1	0.3482	0.6659
12	DNMT3A	Ven_CM	22.7	19.1	3.6	0.3562	0.6659
13	ASXL1	Ven_CM	27.0	19.6	7.4	0.3655	0.6659
14	PTPN11	Ven_CM	13.7	21.0	-7.2	0.3884	0.6659
15	FLT3	Ven_CM	18.8	20.7	-1.9	0.6278	0.9115
16	CEBPA	Ven_CM	18.3	20.8	-2.5	0.6955	0.9115
17	RAS	Ven_CM	19.7	20.5	-0.8	0.7137	0.9155
18	TET2	Ven_CM	21.4	20.0	1.4	0.7522	0.9115
19	M2	Ven_CM	22.0	19.9	2.1	0.7538	0.9155
20	M0	Ven_CM	17.8	20.6	-2.8	0.7596	0.9115
21	CD64	Ven_CM	20.8	21.1	-0.3	0.9296	1.0000
22	CD34	Ven_CM	20.2	21.4	-1.2	0.9805	1.0000
23	M4/M5	Ven_CM	21.2	20.2	0.9	1.0000	1.0000
24	CD7	Ven_CM	20.8	21.1	-0.3	1.0000	1.0000

C) SPM

#	Geno Phenotype	Culture medium	DSS mean Mut/Phen (1)	DSS mean WT (0)	DSS Mean dif (1-0)	P-value	Adj pvalue
1	CD34+CD38-	Ven_SPM	2.3	9.8	-7.5	0.0169	0.2912
2	PTPN11	Ven_SPM	1.3	9.2	-7.8	0.0362	0.2912
3	SRSF2	Ven_SPM	12.5	7.1	5.3	0.0516	0.2912
4	TP53_complex	Ven_SPM	3.7	9.3	-5.7	0.0622	0.2912
5	IDH1	Ven_SPM	12.9	7.6	5.3	0.0689	0.2912
6	CD71	Ven_SPM	6.3	10.5	-4.2	0.0728	0.2912
7	CD7	Ven_SPM	12.3	7.1	5.2	0.0972	0.3331
8	TET2	Ven_SPM	13.0	6.9	6.1	0.1677	0.4694
9	IDH2	Ven_SPM	10.4	7.7	2.7	0.1760	0.4694
10	EZH2	Ven_SPM	12.7	8.0	4.7	0.2871	0.6210
11	RAS	Ven_SPM	10.6	8.3	2.3	0.3017	0.6210
12	M1	Ven_SPM	9.2	8.2	1.1	0.3105	0.6210
13	M2	Ven_SPM	11.6	7.7	3.8	0.3746	0.6915
14	CD34	Ven_SPM	8.9	5.1	3.9	0.4209	0.7216
15	M0	Ven_SPM	9.7	8.4	1.3	0.4607	0.7372
16	DNMT3A	Ven_SPM	9.7	7.8	1.8	0.5844	0.8076
17	NPM1	Ven_SPM	5.7	9.0	-3.2	0.5872	0.8076
18	FLT3	Ven_SPM	8.4	8.5	-0.1	0.6057	0.8076
19	CD123	Ven_SPM	8.7	9.4	-0.7	0.6708	0.8473
20	RUNX1	Ven_SPM	10.7	8.0	2.7	0.7423	0.8674
21	ASXL1	Ven_SPM	9.0	8.4	0.6	0.7752	0.8674
22	M4/M5	Ven_SPM	8.5	8.5	0.0	0.7951	0.8674
23	CEBPA	Ven_SPM	8.9	8.4	0.5	0.8608	0.8982
24	CD64	Ven_SPM	8.6	8.6	0.0	1.0000	1.0000

Table S5. Comparison of AUROC, median DSS and EC50 between different methods

Assay	Medium	Drug	Median DSS	Median EC50 (nM)	Area under ROC curve	*P*-value
Flow cytometry	CM	Venetoclax	23.9	5.8	0.820	0.0018
Flow cytometry	CM	Ven + Aza 300nM	22.6	7.4	0.812	0.0024
Flow cytometry	RPMI	Venetoclax	27.6	2.9	0.783	0.0058
Flow cytometry	SPM	Venetoclax	7.7	>1000	0.781	0.0066
Flow cytometry	CM	Ven + Aza 1 000nM	24.9	5.5	0.727	0.0274
CTG	CM	Venetoclax	4.1	>1000	0.704	0.0545

Table S6. Patient characteristics and treatment responses in AML FAB M4/5

Patient	FAB	Disease stage	Previous therapy	Mutations	Karyotype	Cell composition after 1st cycle	Cell composition at relapse	Blast-spesific sensitivity (FC, CM)	Bulk sensitivity (CTG, CM)	Relapse (DOR)	Additional information
5055	M5	Relapsed disese	Chemotherapy, midostaurin	FLT3-TKD, CEBPA, RUNX1, CUX1, U2AF1	Normal	RD	N/A	Res (DSS 3.3)	Res (DSS 0.1)	RD	
4098	M5	Diagnosis		CEBPAx2, DNMT3A, TET2, CSF3R	Trisomy 21	CD34+ blasts 0.8%, monocytes 6%	CD34+ blasts 14%, monocytes 1%	Sens (DSS 30.7)	Res (DSS 1.6)	10.5 months	At remission blast and monocyte clerance. Relapse of CD34+ cells
8242	M5	Diagnosis		NPM1, FLT3-TKD, TET2	Normal	Blasts + promonocytes 6.1%	In remission, no monocytosis	Sens (DSS 18.5)	Res (DSS 1.0)	in remission 14.0 months	Blast and promonocyte clerance by Cycle 3
7402	M4	Secondary AML (post-CMML)	Trial therapy incl. azacitidine	EZH2, NRAS	Normal	Blasts < 5%, monocytic phenotype persists	CD34+CD117+ blasts 7%, monocytes 15%	Sens (DSS 29.5)	Res (DSS 0.6)	9.8 months	Blast clerance after Cycle 1, monocytosis clearance by Cycle 4
6416	M5	Diagnosis		NPM1, KRAS	Normal	Blasts + promonocytes 25%	In remission, no monocytosis	Sens (DSS 23.9)	Res (DSS 0.0)	in remission 12.4 months	Blast and promonosyte clearace by Cycle 3, monocytosis clearance by Cycle 6

Table S7. Antibodies used in flow cytometry

Marker	Fluorophore	iQue PLUS Channel	Clone	Dilution	Manufacturer	Ref. Number
CD14	FITC	BL1	MφP9	1:100	BD Biosciences	345784
CD11b	PE-Cy7	BL5	ICRF44	1:100	BD Biosciences	557743
CD34	APC	RL1	8G12	1:100	BD Biosciences	345804
CD64	APC-H7	RL2	10,1	1:100	BD Biosciences	561190
CD38	BV421	VL1	HIT2	1:100	BD Biosciences	562444
CD117	BV605	VL4	104D2	1:100	BD Biosciences	562687
CD45	BV786	VL6	HI30	1:100	BD Biosciences	563716
Annexin V	PE	BL2	-	1:50	BD Biosciences	559763
7-AAD	-	BL4	-	1:50	BD Biosciences	559763