Supplemental Material and Methods

Primograft Samples

Primograft samples were derived by passaging individual primary patient samples (Ficoll-gradient purified) through NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice (see below).

Optical Genome Mapping (Bionano)

In a retrospective analysis ultra-high molecular weight genomic DNA was extracted from frozen banked cells (1.5×10^6) using the Bionano Cell Culture DNA Isolation Kit, nicked and labeled following the instructions of the manufacturer (Dokument 30246 Rev.C sowie Dokument 30206 Rev.F; Bionano Genomics Inc.). The Saphyr Genome Imaging Instrument (Part #90023; Bionano Genomics Inc.) using a Saphyr Chip (Part #20366) was disposed for molecular imaging. The throughput target of the run was set to 2000 Gbp (Gigabases). Single-molecule data was collected, filtered for a minimum size of 150 Kbp and *de novo* genome map assembly was performed. Structural variants were called using the Bionano Variant Annotation Pipeline setting hg19 as reference genome.

Isolation and culture of human MSCs from bone marrow

For studies involving human bone marrow ethical approval from the ethical committee of Hannover Medical School was obtained. Samples were collected in accordance with the Declaration of Helsinki after written informed consent of the respective donors. Isolation and culture of human MSCs was performed as described earlier [18]. Briefly, bone marrow samples were obtained during resection of the femoral head for implantation of a total hip arthroplasty in otherwise healthy donors and isolated

from these samples by density gradient centrifugation and subsequent plastic adhesion of mononuclear cells and cultured as described [18]. MSC preparations were routinely frozen in passage 2 with 95 % (v/v) fetal calf serum/ 5 % (v/v) dimethylsulfoxide and stored at – 140 °C.

Cell Culture

B-lineage lymphoid cell lines BV173 (BCR-ABL+) and 697 (BCR-ABL-) (DSMZ, Brunswig, Germany) were cultured in RPMI 1640 supplemented with 10% FCS and 1% penicillin/streptomycin.

Pharmacologic agents

Venetoclax and dexamethasone were purchased from Selleck Chemicals and solubilized in dimethyl sulfoxid (DMSO) or PBS to 10 mM stock, respectively, and then supplemented to the culture medium at the required concentration. Inotuzumab-ozogamicin was purchased from Pfizer and dissolved in 0.9% NaCl at 0.25mg/mL stock concentration.

***Ex vivo* drug treatment and assessment of cytotoxicity**

Primary ALL cells were isolated by Ficoll-gradient density centrifugation and subsequent lysis of erythrocytes by ammonium chloride (0.83%). PDX cells were isolated from bone marrow or spleen of inoculated NSG mice. Primary ALL or PDX cells were co-cultured in flat-bottom 96-well plates (Thermofisher, Darmstadt, Germany) seeded with $0.4x10^4$ MSCs per well in MSC medium (DMEM low glucose, 20% FCS, 25mM HEPES-buffer, 1% penicillin/streptomycin, 2 ng/mL βFGF-2) 24 hours prior to treatment at an ALL seeding density of $0.5x10^6$ cells/ml in SFEM II medium (Stemcell technologies, Cambridge, UK) supplemented with 20% FCS, 20 ng/ml recombinant

IL3, 10 ng/ml recombinant IL7 (both Peprotech, Hamburg, Germany) and 1% penicillin/streptomycin. Cells were treated with increasing concentrations of venetoclax, dexamethasone and inotuzumab-ozogamicin alone or in combination in fixed ratios (for primary samples: 1 nM: 1 nM: 0.0067 nM; for PDX samples: 100 nM: 8.43 nM: 0.0029 nM) for 48 hours and subsequent huCD19 antibody (anti-CD19-APC, BioLegend, San Diego, CA, US) and propidium iodide staining (Serva, Heidelberg, Germany) was performed. Cells were analyzed in a FACS Calibur flow cytometer and data were analyzed with Cell-Quest Pro software (BD Bioscience, Heidelberg, Germany). Drug combination indices were calculated using GraphPad Prism (Version 7.04) and CompuSyn Software.

MOMP induction

PDX cells (L707, P020, P021) and cell lines (697 and BV173) were treated for three hours with venetoclax, dexamethasone or inotuzumab-ozogamicin at a density of 0.5-1x10^6cells/ml. Concentrations were selected to achieve at least 30% PI-positive cells after 24 hours of treatment. Detailed dosing information is provided in the corresponding figure legends. Assessment of mitochondrial outer membrane permeabilization was performed as described earlier[7]. In brief, cells were stained with 50 nM of the mitochondrial dye TMRE (tetramethylrhodamine ethyl ester) (Sigma) for 20 minutes at 37°C. 5 µM FCCP (Carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone) (Sigma) served as a positive destaining control. Cells were analyzed in a FACS Calibur flow cytometer and data were analyzed with Cell-Quest Pro software (BD Bioscience). Reduction of TMRE mean fluorescence of viable cells (FSC/SSC) was analyzed. Untreated controls were set as 100%.

697, BV173 and L707 (in co-culture with MSC, see above) with or without 21 hours of pretreatment with dexamethasone (697:5 µM, BV173:0.2 µM, L707:2 µM) or ino-

tuzumab-ozogamicin (697 and BV173: 100 ng/ml, L707: 500 ng/ml) were treated with 100 nM of venetoclax for 3 hours. TMRE staining was performed as described above. Reduction of TMRE mean fluorescence of viable cells was analyzed. Untreated controls were set as 100%.

DNA-damage response

After 6 hours of drug exposure (venetoclax, dexamethasone, inotuzumab-ozogamicin concentrations indicated in the figure legends) PDX cells (L707, P020 or P021) and cells lines (697, BV173) were fixed with 4% paraformaldehyde for 15 minutes at 4°C. Cells were washed in PBS and cell pellets were resuspended in ice cold methanol and stored overnight in -20°C. Cells were washed twice with PBS followed by staining with anti-pATM (pS1981) (#81292, Abcam), anti-γH2AX (pS139) antibody (#560443, BD Biosciences) or anti-cleaved caspase-3 (#9661, Cell Signaling) for 60 minutes in PBS supplemented with 0.5% FCS. Cells were washed with PBS and incubated for 30 minutes with either an APC-conjugated anti-mouse-antibody (BD Bioscience) or an Alexa Fluor 488 conjugated-anti-rabbit antibody (Jackson Immuno Research, Ely, UK) depending on the origin of the primary antibody. Cells were washed with PBS and cell pellets were resuspended in PBS supplemented with 15 µg/ml propidium iodide (Serva) and 20 µg/ml RNAse (Qiagen) (for γH2AX and pATM staining) or in PBS (for cleaved caspase-3 staining). After 30 minutes of incubation at RT samples were analyzed in a FACS Calibur flow cytometer.

Immunoblotting and co-immunoprecipitations

Whole cell lysates were prepared with lysis buffer (20 mM HEPES, pH 7.5, 0.4 M NaCl; 1 mM EDTA, 1 mM EGTA, 1 mM DTT) supplemented with mini complete protease inhibitor cocktail tablet (Roche Diagnostics, Mannheim, Germany). Immunob-

lotting was performed as described earlier [7] with the following antibodies according to the manufacturer's protocol: anti-p53 (sc-126, Santa Cruz), anti-BIM (cs2933), anti-BAX (cs2772), anti-PUMA (cs4976), anti-GAPDH (cs2118) (all Cell Signaling Technology), and anti-BCL2 (551051, Becton Dickinson). Densitometric analysis was performed using VersaDoc 3000 Imaging system and Image Lab software version 5.0 (both Bio-Rad). The intensity ratio of the protein of interest band to the GAPDH band (loading control) was calculated to measure changes in protein levels.

For co-immunoprecipitation studies, 697 and BV173 cells were treated with 10 ng/ml INO and 5 µM DEX (697) or 5 ng/ml INO and 0.5 µM DEX (BV173), respectively for 24 hours and subsequently lysed in CHAPS-buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% CHAPS) supplemented with mini complete protease inhibitor cocktail tablet on ice for 30 min. Immunoprecipitation was performed as described earlier with anti-BCL2 antibody (AW604, Millipore), and recovered and washed immunoclompexes were subjected to western blot analysis [7]. Quantitative densitometric analysis of co-immunoprecipitations was performed by chemiluminescence imaging of immunoblots using ChemiDoc MP Imaging system (Bio-Rad).

Cytotoxicity

Cytotoxicity was assessed by staining with 10 µg/ml propidium iodide after 24 hours or 48 hours of the indicated drug treatment. Cells were analyzed in a FACS Calibur flow cytometer and data were analyzed with Cell-Quest Pro software (BD Bioscience).

Animal experiments

L707 cells were described earlier [13]. For generation of P020 and P021 PDX models, patient samples were transplanted into the tail vein of NSG mice. Leukemia engraftment was monitored by immunophenotyping of peripheral blood. Mice were sacrificed and cells were isolated from bone marrow and subsequently transduced with Chili-Luc lentiviral vector as described earlier [16]. Cells were washed and further passaged in NSG mice. Chili-Luc transduced PDX cells were sorted for RFP expression (Sorter Facility MHH) and adoptively transplanted in NSG mice.

L707, P021 and P020 PDX cells stably expressing luciferase ($1x10^6$) were intravenously transplanted into recipient NSG mice. Upon confirmed engraftment by IVIS bioluminescence imaging [16], mice were treated with the respective antineoplastic agents. Dexamethasone (1 mg/kg) was applied orally in 0.1 M sodium citrate by oral gavage 5 days per week or in combination with venetoclax in a vehicle consisting of 60% Phosal, 30% PEG 400 and 10% Ethanol with a treatment delay of minimum 2 hours [9]. Inotuzumab-ozogamicin was injected intraperitoneally two days a week for two weeks in a concentration of 100 µg/kg (L707) in 0.9% NaCl. P020 and P021 were treated once a week for three weeks with 10 µg/kg (INO^{low}) and 100 µg/kg (INO^{high}). Detailed dosing information is indicated in the figure legends. Tumor burden was assessed by *in vivo* bioluminescent assay. D-Luciferin (1 mg/mouse) (Appli-Chem, Darmstadt, Germany) was provided intraperitoneally. Life imaging of tumor growth was detected every two weeks by using IVIS Lumina II (Perkin Elmer, Waltham, MA, US). Living Image 4.0 software was used to analyze the bioluminescence radiance. Female mice used in the experiments were 8 to 10 weeks old and were randomly allocated to each group.

All animal studies were in accordance with the German animal protection law and with the European Communities Council Directive 86/609/EEC and 2010/63/EU for the protection of animals used for experimental purposes. All experiments were ap-

proved by the Local Institutional Animal Care and Research Advisory Committee and permitted by the local authority, the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit [No. 33.14-42502-04-16/2217].

Analysis of r/r PDX samples

End of experiments analysis of relapsed mice was performed with PDX cells isolated when termination criteria were fulfilled. For detection of surface CD22 expression, cells were stained with anti-huCD22-APC (BioLegend, clone HIB22) and anti-huCD45-PE or anti-huCD45-FITC (both BioLegend) according to the reporter fluorescence protein of the respective PDX model. Cells were analyzed in a FACS Calibur flow cytometer and data were analyzed with Cell-Quest Pro software (BD Bioscience). For assessment of MOMP induction by venetoclax and DSB-induction by inotuzumab-ozogamicin cells were subjected to the respective assays (see above).

Conversion of murine doses to human equivalent doses

Human equivalent doses were calculated according to FDA guidelines and Nair and Jacobs [19] with mouse to human conversion factor Km = 0.08 and a human mg/kg to mg/m^2 conversion factor of 37. Human body weight between 50 and 100 kg was used for calculation. Human equivalent doses were calculated to 1.6 mg/kg for venetoclax, 4 x 0.3. mg/m^2 for inotuzumab-ozogamicin, and 0.08 mg/kg for dexamethasone all within the range of clinical usage, respectively.

Statistical Analysis

Error bars represent SD if not otherwise stated. Statistical analysis was conducted using GraphPad Prism software (Version 7.04) or Microsoft Excel. Student´s t-test or

one-way ANOVA with Bonferroni multiple comparison test was used to determine the statistical significance of the results. Kaplan-Meier statistical analysis was performed using log-rank test. P values <0.05 were considered significant.

References

18. Jungwirth N, Salinas Tejedor L, Jin W, et al. Mesenchymal Stem Cells Form 3D Clusters Following Intraventricular Transplantation. *J Mol Neurosci*. 2018;65(1):60-73.
19. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*. 2016;7(2):27-31.

Table S1: Clinical data for ALL patients: primary cells and primografts. * primograft P020; # primograft P021, n.a. : not available ; Primograft L707 has recently been described [13].

sample	diagnosis	Presentation/ relapse	cytogenetics	CD22 surface expression (%)	age at presentation	gender
019	pro-B-ALL	relapse	n.a.	23.2	39	male
020*	c-ALL	relapse	55,XY,+1,dup(4)(q22.1q22.1),+6,+8,inv(8)(q21.1q24),del(9)(p21.3),+10,+11q,+14,+18,+19,+21,+X	73.9	41	male
021#	pre-B-ALL	relapse	46,XY,der(1)t(1;19)(q23.3;p13.3),dup(1)(q23.3qter),der(6)t(6;7)(q12;q21.3)del(6)(q12qter),dup(7)(q21.3qter)del(9)(p13.2)	59.5	37	male
029	pro-B-ALL	relapse	46 XY, t(11;19)(q23;p13)	24.0	37	male
L05	pro-B-ALL	presentation	46,XX, t(4;11)	n.a.	73	female
L20	c-ALL	presentation	t(9;22) (q34;q11) (p210)	n.a.	59	female
036	c-ALL	presentation	46, XY	28.8	67	male
037	c-ALL	presentation	45,XY,-9, t(9;22)(q34;q11) (p190)	37.9	70	male
L707	ALL	presentation	t(17;19)	92.1	16	female

Supplemental Figure 1: S1A: Representative flow cytometric analysis of TMRE mean fluorescence. BV173 cells were treated for 3 hours with 1000 nM venetoclax (VEN) and were subsequently stained with 50 nM TMRE for 20 minutes. The ionophore FCCP served as positive destaining control. Side scatter (SSC) is plotted against TMRE fluorescence.

S1B: Representative flow cytotmetric analysis of γH2AX and pATM expression. 697 cells were treated for 6 hours with 100 ng/ml INO and subsequently fixed and stained with anti-γH2AX and anti-pATM. γ-irradiation (4 Gy) served as positive control. PI-A is plotted against γH2AX (upper) and pATM (lower) fluorescence, respectively.

S1C: MOMP induction of 697 and BV173 cells treated for 3 hours with 1000 nM VEN. Viable cells were gated based on FSC/SSC profile and MFI of untreated cells was set as 100%. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction. (**p<0.01; ***p<0.001).

S1D and E: Flow cytometric analysis of (**D**) pATM and (**E**) γH2AX-expression of 697 and BV173 cells treated for 6 hours with 250 nM VEN, 5000 nM DEX (697) or 1000 nM DEX (BV173) or 100 ng/ml INO. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction. (**p<0.01; ***p<0.001).

S1F and G: Flow cytometric analysis for (**F**) PI negativity (rel. viability) and (**G**) cleaved-caspase 3 expression of P020, P021 and L707 PDX cells treated for 6 hours with VEN (P020: 250 nM ; P021: 1000 nM ; L707: 250 nM), DEX (P020: 1 µM; P021: 5 µM ; L707: 1 µM) or INO: (P020: 100 ng/ml; P021: 100 ng/ml; L707: 1000 ng/ml), respectively. (**G**) Cells were fixed, permeabilized and stained with an anti-cleaved caspase 3 antibody. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction. (**p<0.01; ***p<0.001).

S1H and I: Flow cytometric analysis of (**H**) PI-negativity (rel. viability) and (**I**) cleaved caspase 3- expression of 697 and BV173 cells treated for 6 hours with 250 nM VEN, 5000 nM DEX (697) or 1000 nM DEX (BV173) or 100 ng/ml INO, respectively. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction. (**p<0.01; ***p<0.001).

S1J: BCL2 immunoprecipitations from lysates from 697 and BV173 cells treated with or without DEX and INO for 24 hours. Ratios of BIM/BCL2 were calculated for three experiments with the BIM/BCL2 ratio of 1 for untreated cells. P-values were calculated by student´s t-test (*p<0.05)

S1K: IC50 of INO, VEN and DEX in combination treatment. Primary ALL samples and PDX cells were treated with INO, VEN and DEX alone or in combination in fixed ratios (for primary samples: 1 nM: 1 nM: 0.0067 nM; for PDX samples: 100 nM: 8.43 nM: 0.0029 nM) for 48 hours followed by CD19 antibody and PI staining. IC50s of INO, VEN and DEX in the triple agent combination treatment (VEN/DEX/INO) were determined by CompuSyn software.

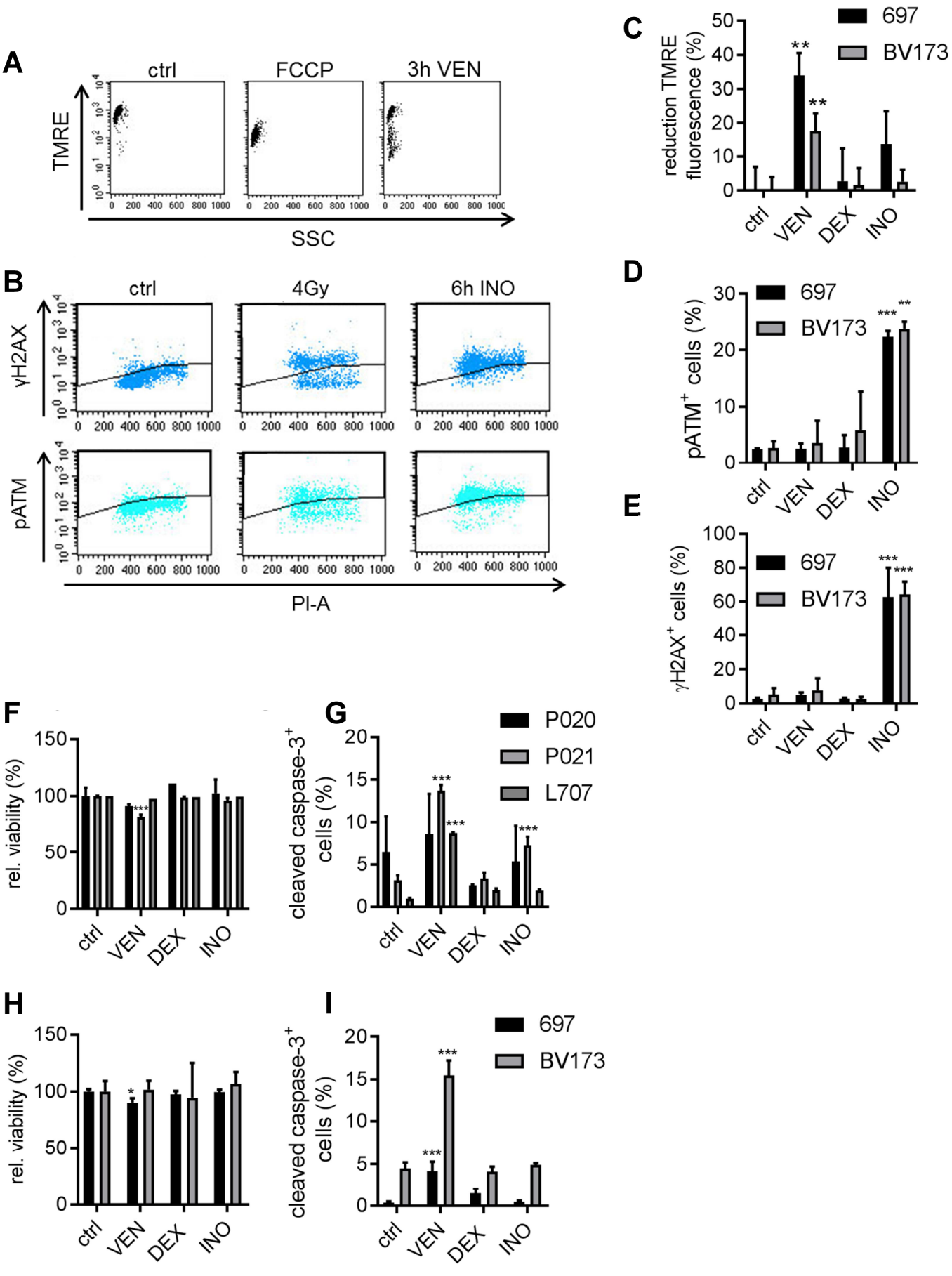
S1L: PBMNCs from healthy donors (n=3) in co-culture with MSCs were incubated with 1000 nM VEN, 1000 nM DEX, and 1000 ng/ml INO or as triple combination (VEN/DEX/INO) for 48 hours followed by PI staining.

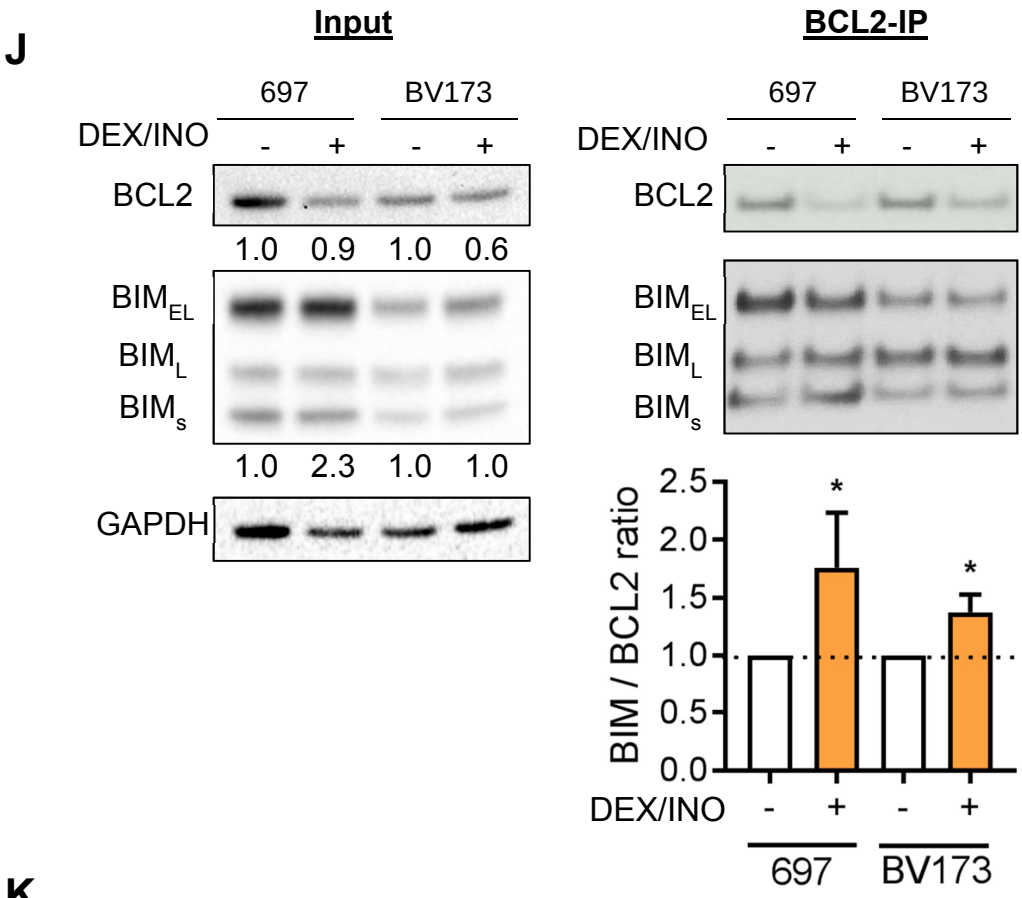
S2A-C: Surface CD22 expression in human $CD45^+$ cell population of relapsed/refractory (**A**) L707, (**B**) P020 and (**C**) P021 PDX cells. R/r ALL mice were sacrificed at the end of experiment with severe leukemia when termination criteria were fulfilled, and r/r ALL cells were harvested. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction. (**p<0.01; ***p<0.001).

S2D and E: MOMP induction in r/r L707 and P021 cells. Cells were treated for 3 hours with 1000 nM VEN. Viable cells were gated based on FSC/SSC profile and MFI of untreated cells was set as 100%. Cells were >95% positive for human CD45. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction.

S2F and G: Flow cytometric analysis of γH2AX expression of r/r L707 and P021 PDX cells treated for 6 hours with 1000 ng/ml INO. Values are expressed as means ± SD. p-values were calculated by one-way ANOVA with Bonferroni correction.

Supplemental Figure 1




J
Input
BCL2-IP
697
BV173
DEX/INO
- + - +
BCL2
1.0 0.9 1.0 0.6
BIM_EL
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1.0 2.3 1.0 1.0
GAPDH
BIM / BCL2 ratio
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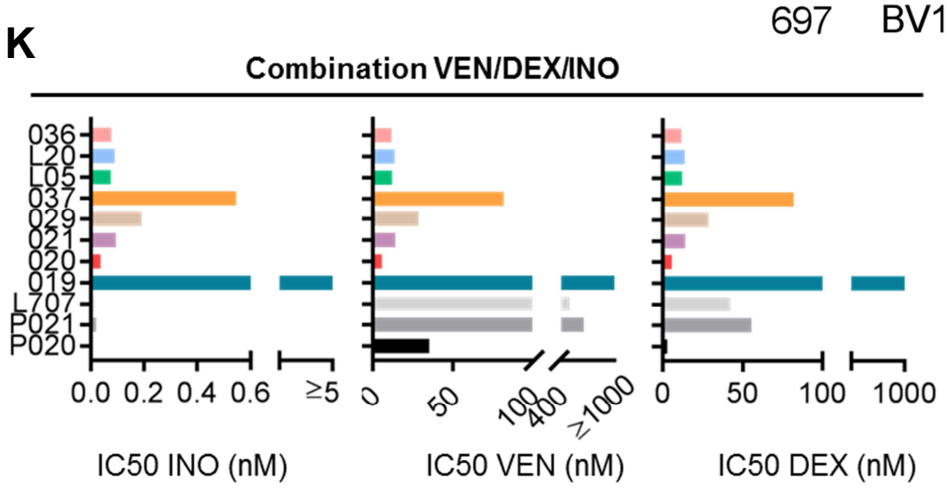
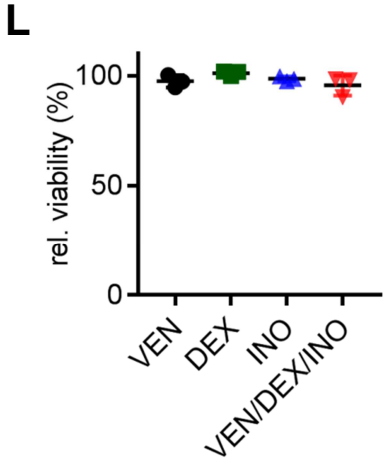

K
Combination VEN/DEX/INO
036
L20
L05
037
029
021
020
019
L707
P021
P020
0.0 0.2 0.4 0.6 ≥5
IC50 INO (nM)
0 50 100 400 ≥1000
IC50 VEN (nM)
0 50 100 1000
IC50 DEX (nM)


L
rel. viability (%)
100
50
0
VEN
DEX
INO
VEN/DEX/INO

Supplemental Figure 2