Xenograft model

Xenograft experiments were approved by the veterinary office of the Canton of Zurich. Briefly, primary ALL cells were transplanted intrafemorally to NSG mice as previously described\(^1\). For transplantation, 1x10^5 to 5x10^6 viable primary ALL cells were recovered from cryopreserved bone marrow aspirates and transplanted intrafemorally into immunodeficient NOD/LtSz-scid IL2Rnull (NSG) recipients\(^2\). Leukemia progression was monitored in the peripheral blood by flow cytometry using anti-mouse CD45 (eFluor®450, Clone 30-F11, eBioscience), anti-human CD45 (AlexaFlour647, Clone HI30, BioLegend), anti-human CD19 (PE, Clone HIB19, BioLegend) (for BCP-ALL) or anti-human CD7 (PE, Clone 124-1D1, eBioscience) (for T-ALL). Flow cytometry was performed on a BD FACS Canto™ II (BD Biosciences) and data were analysed using FlowJo (version 7.1.6, TreeStar). The human leukemia cell load was calculated as the ratio of mCD45^{neg}hCD45^{pos}hCD7^{pos} cell count and total lymphocyte count. Leukemic cells were harvested from the spleen of the animals when human cells in peripheral blood exceeded 50% of the total lymphocyte count. Mouse spleens were mechanically disrupted and tissue debris was removed with a 45 μM filter. Tissue mass was treated with RBC lyses buffer (4.1 g NH_4Cl, 0.5 g KHCO_3, 0.2 ml of 0.5M EDTA; in 500 ml ddH_2O) to enrich for leukemia cells (>95%) and the engraftment in the splenic compartment was assessed by flow cytometry using the same CD markers as peripheral blood engraftment. Cells were cryopreserved in freezing medium (10% FBS and 90% DMSO) and stored in a biobank in 10-60 million cell aliquots in 1 ml for further use. The identity of xenografts was confirmed by DNA fingerprinting using the commercial AmpFlSTR® NGM SElectT kit containing Amelogenin and 16 autosomal STR loci (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11; D18S51, D19S433, TH01, FGA, SE33, D10S1248; D22S1045, D2441, D1S1656, D12S391). DNA amplification was performed using reaction conditions as described by the manufacturer (life technologies). The amplified products were separated and detected with a 3500 Genetic Analyzer (life technologies). Allele calling was performed using the GeneMapper ID-X Software v1.4 (life technologies).

Genomic characterization of leukemia samples

Sample preparation and hybridization capture for targeted deep sequencing (TDS) was performed using the SureSelect\(^{XT}\) Target Enrichment System for Illumina paired end sequencing following protocol version H0 (G7550-90000). Briefly, 200 ng genomic DNA was sheared with Covaris, ligated to adapters, and amplified by PCR. Samples were hybridized to an Agilent SureSelect\(^{XT}\) custom designed capture library, which includes 52 target genes (Table S3) selected by members of the IntReALL and AEIOP-BFM study groups. Quality
control was performed using a Lab-on-a-Chip protocol. Clustering and DNA sequencing from 17 pm DNA was performed according to service provider operating procedures. Image analysis, base calling and primary quality checks were performed using the Illumina software RTA v1.18.64. All TDS was performed by Genome Scan B.V. (Leiden, Netherlands).

TDS data analyses were based on established pipelines according to the GATK (The Genome Analysis Toolkit) standards (Best practices for variant detection with the GATK v.4, for release 2.0). Reads were initially subjected to quality assessment of the produced reads and adapter removal prior to alignment with BWA-MEM (v. 0.7.15) for paired-end reads to the human genome (GATK repository, build 37 decoy). PCR duplicate removal (Picard v. 2.5.0) followed by realignment around indels and base recalibration using GATK tools (v3.2-2) were performed on BAM files. Single nucleotide and Indel variant calling were performed using samtools mpileup (v. 1.3.1) and bcftools (v. 1.3.1). Variants were annotated with Annovar (v. 2016-Feb-01); variants that have been previously reported as SNPs in the dbSNP and 1000 genomes databases, as well as variants in regions with less than 40 reads were not considered in further analyses. Clusters of events linked with cross-contamination were identified by primer BLAST and confirmed by event frequency distributions in patient and PDX material. All calls were subjected to visual inspection with the IGV viewer.

In 19 BCP-ALL cases without an established abnormality (B-other) or targetable kinase-activating lesions fluorescence in-situ hybridization (FISH) was performed. FISH probes were designed by Cytocell (Cambridge, UK) on request of the BFM trial. The Ph-like panel contained break apart probes for ABL1, ABL2, CRLF2, EPOR, JAK2, CSF1R/PDGFRB and NTRK3. FISH was performed according to the manufacturer’s instructions. Briefly, patient cells were co-denatured with the probe at 82°C for 2 minutes (or 75°C for 5 minutes for fixed cells). Hybridization was done at 37°C overnight, followed by two post hybridization washes in salt solutions at 72°C±1 for 2 minutes and at room temperature for 30 seconds to 1 minute. DAPI (4,6 diamidino-2-phenylindole) was used to counterstain the nuclei. 200 cells were analyzed for each specimen using a fluorescence microscope. Results were interpreted as abnormal if they exceed cutoffs established for the respective probes by the laboratory.

Detection of genomic aberrations in genes frequently deleted in T-ALL were investigated by the Multiplex Ligation-dependent Probe Amplification (MLPA) assay SALSA p335 kit (MRC-Holland, Amsterdam, The Netherlands) using 125 ng of genomic DNA. The assays were performed according to the manufacturer’s protocol. MLPA was performed for 24 primary and PDX T-ALL at diagnosis and/or relapse in the reference laboratories. Selected genes in T-ALL samples were sequenced. An intensity ratio between 0.75 and 1.3 was considered to
represent a normal copy number, a ratio between 0.25 and 0.75 a monoallelic deletion, and a ratio < 0.25 a biallelic deletion. In addition, eight matching primary and PDX T-ALL samples were analysed with HaloPlex™.

RNA-sequencing data was also performed for a subset of T-ALL samples. Quality was assessed by inspecting the reports of FastQC and the alignment statistics of the STAR aligner (v. 2.5.1b). Raw sequencing reads were separated into host- and graft-derived using Xenome (v. 1.0.1) with version hg19 of the human and version mm10 of the mouse reference genome. Only reads which could unambiguously be identified as being of human origin were used for further processing. Reads were aligned to the 1000genomes human reference genome with decoy sequences (hs37d5) using STAR. The aligned reads were subsequently mapped to features with HTSeq-count (v. 0.6.0) in \textit{intersection-nonempty} mode. Differential gene expression analysis was performed using DESeq2 (v. 1.12.2) and batch effects were corrected with the help of the multi-factor design feature of the software. Genes with an absolute log\(_2\)-fold change of at least 1.5 and an FDR-adjusted p-value below 5% were considered as being differentially expressed. Gene fusions were detected using deFuse (v. 0.6.1) and STAR.

\textbf{In vivo drug treatment}

For single agent validation, ALL cells were recovered from cryopreserved xenografts and \(1\times10^6\) cells per mouse were transplanted intravenously in 10-16 mice. After 3 days, randomized cohorts were treated with 100 mg/kg of venetoclax (Activebiochem, dissolved as previously described \(^5\)) or vehicle control, with 5 to 8 mice per treatment arm. Venetoclax or vehicle control were administered orally daily for 14 days. A second block of treatment administered using the same protocol was performed after two weeks of recovery.

For combination experiments, ALL cells were recovered from cryopreserved xenografts and \(1\times10^6\) cells per mouse were transplanted intravenously in 20-24 mice. After 3 days, randomized cohorts were treated with venetoclax, or dexamethasone + vincristine, or venetoclax + dexamethasone + vincristine or vehicle control with 5-8 mice per treatment arm. Venetoclax (Activebiochem) was administrated orally for 14 days at 100 mg/kg. Dexamethasone (Mepha) was injected intraperitoneally for 12 days with a break of two days at the 6\(^{\text{th}}\) and 7\(^{\text{th}}\) day at 10.5 mg/kg. Vincristine (Teva) was administrated by intraperitoneal injection once a week at 0.5 mg/kg.

Follow up of the circulating leukemia was performed every seven days by flow cytometry as described in xenograft model. Individual mouse event-free survival (EFS) depicted in the Kaplan-Meier graph was calculated as the number of days from transplantation until the mCD45\(^{\text{neg}}\)hCD45\(^{\text{pos}}\)hCD7\(^{\text{pos}}\) (for T-ALL) or mCD45\(^{\text{neg}}\)hCD45\(^{\text{pos}}\)hCD19\(^{\text{pos}}\) (for B-ALL) cell levels reached 25% or termination of the experiment, if 25% of leukemia was not reached. As well
leukemia progression using same populations demonstrated for each mouse separately. The efficacy of drug treatment was evaluated by calculating hazard ratios and p-values as the difference between the median EFS of vehicle control and drug-treated cohorts.

**In vitro drug profiling platform**

The *in vitro* drug response of ALL primary patient samples was assessed in coculture with hTERT-immortalized primary bone marrow mesenchymal stromal cells (MSC) as described previously. hTERT-immortalized MSC cells were obtained from Dario Campana. MSC (2.5x10³ cells/well) were plated in 384-well plates (Greiner, REF781090) in 30µL serum-free medium (AIM-V®, Life Technologies). After 24h incubation at 37°C, 5% CO₂, 2x10⁴-2.3x10⁴ viable leukemia cells suspended in 27.5µL medium were added and incubated for an additional 24h. The mean viability of the recovered ALL cells was 77% after thawing (determined using Trypan blue). A 10mM stock solution compounds (n=104) were prepared in DMSO (stored at -80°C, max. 3 freezing and thawing cycles). Serial dilutions were used in duplicates (from 1x10⁵ nM diluted 10x over four additional points with epMotion 5070 robot, Eppendorf). 29 cases were tested and then 44 compounds were excluded (inactive, IC₅₀>10µM). 60 compounds (Supplementary Table 2) were used with 8-point dilutions. Optimal concentration ranges (Supplementary Table 3) were calculated based on the frequency distribution of IC₅₀ values per each drug. Drug dilutions were performed using epMotion 5070 or Tecan D300 robots. After 72h of incubation with compounds, live cell numbers were evaluated using CyQUANT® (Life Technologies) live cell staining (20µL: CyQUANT (1:300), Repressor (1:20) in medium for well) and incubated for 1h at 37°C, 5 % CO₂. Automated imaging was performed using the ImageXpressMicro microscope (Molecular Devices) equipped with a CoolLSNap HQ camera (Photometrics) and a 10x Plan Fluor objective with 0.3 NA (Nikon) (covering 50% of the well surface). Images were processed using CellProfiler (Broad Institute). Cells were classified and counted using the Advanced Cell Classifier (Peter Horvath, ETH). This software uses the random forest classification method to identify ALL cells from MSCs. More information on the two imaging processing programs used can be found on [http://acc.ethz.ch/](http://acc.ethz.ch/). Drug profiling biological replicates have been performed for T-VHR-03 (5 point vs. 8 point screening), T-MR-09 (5 point vs. 8 point screening) and T-HR-08 (PDX sample vs. patient derived sample) with good reproducibility (pPearson = 0.81, p-value < 2.2e10⁻¹⁶) (Supplementary Table 3).

**Cell viability assay**

2.5x10³ cells hTERT-MSC cells in serum-free AIM-V® medium were seeded in 384-well plates (Greiner, REF781090). After 24h, primary, 2.5x10⁴ ALL cells in AIM-V were seeded as
suspension cultures or in co-culture with MSC. ALL cell viability was assessed after 1, 4 and 7 days of incubation. Cells were collected from monoculture or co-culture by scraping and stained with 7-AAD (BD Pharmingen™). Cell viability (7-AAD) was measured by flow cytometry, gating strategy shown in method figure 1. Counting beads (SPHERO™ Accu Count Blanc Particles, Spherotech Inc.) were used to normalize cell counts; viabilities are reported as averages ± SD (normalized to input) of duplicate wells at day 4.

**Method Figure 1.** Flow cytometry gating strategy for evaluation of cell death using 7-AAD and leukemia-specific cell surface markers. Dead (red) and live (green) leukemia cells could be distinguished by SSC and FSC parameters as in co-culture and monoculture (upper and middle panel) and just few MSC cells would be mixed in dead cell population (lower panel).

**Cell proliferation and apoptosis assays**

1x10^5 of ALL cells were seeded in co-culture with 1x10^5 of MSC in 190µL AIM-V medium in the 96-well flat bottom plate. To label proliferating cells 10µM EdU (5-ethyl-2'-deoxyuridine a modified thymidine analogue, Click-iT EdU Imaging Kit, C10337, life technologies) was added at different time points (4h, 66h and 148h) and fixed after 20h. To detect EdU-labeled cells samples were processed based on manufacturer instructions and labelled with Alexa Fluor 488 azide. Additional 0.1µg of PI (Propidium Iodide Staining solution, cat. no. 51-66211E (556463), BD Pharmingen™) was added before the reading to mark proliferating cells and analyse using 2D approach (EdU vs. PI) as described^{10}. For live/dead ALL cell assessment, cells were collected at the same time points and labelled with anti-human CD7 (PE, Clone
124-1D1, eBioscience) or CD19 (PE, Clone HIB19, BioLegend). Apoptotic cells were labelled using 2µM final concentration of Cell Event™ Caspase-3/7 Green (C10423, life technologies) and incubated for 30 minutes before reading with flow cytometry. Apoptotic cells were counted as the ratio of hCD7orCD19pos-CellEventpos:hCD7orCD19pos-CellEventneg. The cut off for proliferative (S-phase cells ≥40%) and non-proliferative (S-phase cells <40%) groups of cells was defined by fitting an EM mixture model (R package mixtools) to the distribution of all cells in S-phase, and obtaining the intersection of the two Gaussian curves from the mixture model fit.

**Flow cytometry.** For intracellular flow cytometry, 5 to 10x10^6 cells were fixed in 1 ml freshly prepared 2% paraformaldehyde in PBS for 10min at room temperature (RT). Cells were washed twice with 1ml of PBS and centrifuged at 650g for 5min. Pellets were resuspended in 100µL of PBS and permeabilized by slowly adding ice cold pure methanol and stored at -20ºC overnight. The cells were washed two times with 2ml PBS and centrifuged at 1500g for 5min and resuspended in 450µL FACS buffer (HBSS, Gibco, ref: 14170-088, 4% FBS) with Fc blocker (anti-mouse CD16/CD32, eBioscience, ref 14-0161-85, final dilution: 1/200) and incubated at RT for 30min. 50µL aliquot were then incubated with primary BCL-2 (monoclonal [E17] rabbit anti-human, Abcam, ref ab32124, final dilution: 1/600), BCL-XL (monoclonal rabbit anti human, Cell Signaling, ref 2764, final dilution: 1/200), MCL1 (monoclonal [Y37] rabbit anti-human, Abcam, ref ab32087, final dilution: 1/200), BAK (monoclonal [Y164] rabbit anti-human, Abcam, ref ab32371, final dilution: 1/600), BIM (monoclonal rabbit anti-human, Cell Signaling, ref 2933, final dilution: 1/200), pSRC (monoclonal rabbit anti-human, Cell Signaling, ref 2109, final dilution: 1/200) or pSRC(Tyr416) (monoclonal rabbit anti-human, Cell Signaling, ref 6943, final dilution: 1/100). After 45min incubation at RT, the samples were washed once with 150µL FACS buffer and centrifuged at 1500g for 5min. Cells were resuspended in 100µL FACS buffer containing FITC-labeled anti-rabbit IgG secondary antibody (Jackson ImmunoResearch, ref 711-096-152, final dilution: 1/400) and 2µL anti-human CD19 (Pacific Blue, Clone LT19, AbD serotec, ref: MCA1940PB) for BCP-ALL) or anti-human CD7 (APC, Clone 124-1D1, eBioscience, ref: 17-0079-42) for T-ALL) and incubated for 20min at 4ºC, followed by three washes with 150µL PBS and centrifugation at 1500g for 5min. Each sample was resuspended in 200µL of PBS and analysed by flow cytometry on a BD FACS Canto™ II (BD Biosciences). The data were analysed using FlowJo (version 7.1.6, TreeStar).

**Western blot**
Whole-cell extracts were prepared from 3-5x10^6 cells using radioimmunoprecipitation assay (RIPA) buffer (20mM Tris-Cl pH 7.5, 150mM NaCl, 1% NP-40,1mM EDTA pH 8.0, 0.1% SDS) supplemented with complete mini protease inhibitor cocktail (Roche Applied Sciences) and
were sonicated prior to dilution with SDS loading buffer (250mM Tris pH 6.8, 4% SDS, 0.02% Bromophenol Blue, 40% Glycerol, 4% (v/v) β-mercaptoethanol). After SDS-PAGE (Criterion™ XT Precast Gels, BIO-RAD, 4-12% Bis-Tris, REF 345-0125), proteins were blotted onto nitrocellulose membranes (Trans-Blot Turbo transfer pack, BIO-RAD, REF 170-4159). Membranes were blocked in 5% non-fat dry milk and incubated with primary Bcl2 (monoclonal mouse anti-human BCL2, Clone 124, ref M0887), BCL-XL (monoclonal rabbit anti human, Cell Signaling, ref 2762), MCL-1 (polyclonal rabbit anti human, S-19 Santa Cruz biotechnology, ref sc-819), BAX (monoclonal rabbit anti human, D2E11, Cell Signaling, ref 5023), BAK (monoclonal rabbit anti human,D2D3, Cell Signaling, ref 6947), BID (polyclonal rabbit anti human, Cell Signaling, ref 2002), BIM (monoclonal rabbit anti human, Cell Signaling, ref 2933) and Tubulin (monoclonal mouse anti-tubulin, Sigma, ref 081M4861) antibodies diluted in milk 1:1000. Horseradish peroxidase-labeled anti-mouse antibodies were used for signal detection with a chemiluminescence substrate (SuperSignal®, 34095, Thermo Scientific). Signals were detected by direct scanning (Fujifilm LAS-3000).
References


Figure S1

A
Supplementary Figure S1.
Mutational landscape of BCP-ALL and T-ALL based on targeted deep sequencing (A) and T-ALL based on haloplex (B) and MLPA and targeted deep sequencing (C).
Supplementary Figure S2. Mean plasma concentration (Cmax) achieved at maximal tolerated and/or recommended dose. Available Cmax data for the compounds included in the library as published. Data collected from studies performed in pediatric patient cohorts, if not available – in adult cohorts. Drugs organized into the same groups as in Figure 2.

*, p<0.05 (Two-sided t test).
Supplementary Figure S3. Drug responses in BCP- and T-ALL. Statistically significant differences in drug responses of diagnostic BCP-ALL (n=29) and T-ALL (n=18) samples are given as IC50 values.

*, p<0.05; **, p<0.005 (Two-sided t test).
Supplementary Figure S4. *In vitro* sensitivity to drugs targeting cell cycle activity correlates with the fraction of proliferating cells. Drug response profiles of BCP-ALL (N=13) and T-ALL (N=16) were compared between non-proliferative (number of cells in S-phase $\leq 40\%$) and proliferative (number of cells in S-phase $>40\%$) samples; the cut-off was defined by a mixture model fit of the distribution of S-phase cells at 24 hours. Drugs with significant differences (p-value $\leq 0.05$, one-tailed Mann-Whitney U-test) in log[nM] IC50 are shown.
Supplementary Figure S5. Primary ALL cells (n=27) are supported better in co-culture with MSCs than in monoculture at day 1 (A) and day 4 (B). ***: p-value < 0.005; **: p-value < 0.01 (A-B); Correlation plots for drugs tested in both monoculture and coculture, stratified by drug. While the global correlation of responses is significant (Spearman $r = 0.64$, p-value < 2.2e-16), some discrepancies in drugs of interest, including docetaxel, cytarabine, venetoclax and dasatinib, can be observed (C). Correlation plots of venetoclax coculture and monoculture IC50 values against survival prolongation compared to the untreated arm reveal a significant correlation between coculture (Spearman $r = -0.86$, p-value = 0.02), but not monoculture (Spearman $r = -0.17$, p-value = 0.71), results (D). Boxplots comparing % ALL in various organs with response prediction under coculture (E) or monoculture (F) conditions again indicate a better correlation between coculture and in vivo results. ***: p-value < 0.01; ***: p-value < 0.005; *: p-value < 0.06 (E-F).
Supplementary Figure S6. Distinct drug activity patterns can be detected for MLL-AF4 rearranged ALL. MLL-AF4 rearranged ALL (green dots, N=3) samples exhibit individual sensitivity patterns to a number of experimental and clinical compounds, compared to all other BCP-ALL samples (gray dots).
Supplementary Figure S7. Validation of viability analyses obtained on our platform by 7-AAD and flow cytometry. *In vitro* response of ALL samples to venetoclax (A., N=32) and dasatinib (B., N=12) in co-culture on MSC (left panel). Cell viability was measured by flow cytometry using 7-AAD after 72h of treatment and normalized to DMSO-treated controls. Calculated response parameters (IC50) were compared to the results obtained on the automated imaging platform (right panel).
Supplementary Figure S8. Venetoclax reduces leukemia burden in mice with established leukemia.

Treatment of mice with overt leukemia (75% human leukemia cells in the peripheral blood) for two weeks with 100 mg/kg venetoclax administered orally decreased leukemia burden to less than 5%. 
Supplementary Figure S9. Analysis of in vitro activity of venetoclax in combination with dexamethasone and vincristine. In vitro combination by co-titrating venetoclax and dexamethasone or vincristine in a concentration range with single agent activity in two different MLL-AF4 ALL samples. Responses to single compounds are colored in blue, and combinations are given in shades of red for selected venetoclax concentrations. The combination index was calculated by the Chou-Talalay method and is indicated in the table.
Supplementary Figure S10. BCL2 family protein expression levels in ALL.

Levels of BCL2 family proteins in selected ALL cases with high (IC50<100 nM, in red) and low (IC50>1 µM, blue) sensitivity to venetoclax are given. Samples are ordered by immunophenotype and the numbers indicate IC50 values in logarithmic scale.
Supplementary Figure S11. Correlation of BCL2, BCL-XL and MCL1 protein expression levels with the IC50 values of the response to venetoclax.

Protein levels were determined by flow cytometry and the mean fluorescence intensity (MFI) was calculated after normalization to isotype control. 36 ALL samples were compared, and no significant correlation was detected.
Supplementary Figure S12. SRC family gene expression and dasatinib sensitivity. SRC gene expression levels are significantly higher in dasatinib sensitive cases than in dasatinib resistant samples. Higher LYN expression, in contrast, occurs in dasatinib-resistant cases. Expression of other SRC family members do not correlate with dasatinib sensitivity.
Figure S13

(A) Unsupervised hierarchical clustering of the *in vitro* response of T-ALL (n=18) cases based on IC50 values to dasatinib together with EGFR, SRC and BCR-ABL1 inhibitors. Dasatinib sensitivities were determined based on *in vivo* and *in vitro* response correlations.

(B) IC50 distribution of dasatinib sensitive (IC50<100 nM, pink dots) and resistant (IC50>500 nM, blue dots) T-ALL cases. *, p<0.05; **, p<0.005 (*Two-sided t test*).

(C) Significant correlation (adj.p-value <= 0.05, Spearman correlation) between dasatinib response and responses to other SRC kinase inhibitors.

Supplementary Figure S13. Sensitivity to dasatinib in T-ALL correlates with response to SRC and RTK inhibitors, but not to BCR-ABL1 inhibitors.