Supplemental Data - Herrmann et al.:
Supplement to Manuscript

Delineation of target expression profiles in $CD34^+/CD38^-$ and $CD34^+/CD38^+$ stem- and progenitor cells in acute (AML) and chronic (CML) myeloid leukemia

Patients and Methods

Antibodies and other reagents

A number of fluorochrome-conjugated monoclonal antibodies (mAb) were applied in this study (Table S1). For identification and phenotyping of normal and leukemic stem and progenitor cells ($CD45^+/CD34^+/CD38^-$ and $CD45^+/CD34^+/CD38^+$ cells), CD34 mAb 581, CD38 mAb HIT2, and CD45 mAb 2D1 or HI30 were applied as reported.[1] The screen-panel of mAb was established based on literature data and data obtained by gene array and qPCR screening. A specification of antibodies used in this study (n=93) is provided in Table S1. Most mAb were clustered in international workshops on human leukocyte differentiation antigens.[2,3] RPMI 1640 medium and fetal calf serum (FCS) were purchased from PAA laboratories (Pasching, Austria), ^{3}H-thymidine from Perkin Elmer (Waltham, MA, USA), recombinant human (rh) granulocyte colony-stimulating factor (G-CSF) from Amgen (Thousand Oaks, CA, USA) and from PeproTech (Rocky Hill, NJ, USA), rh interleukin-3 (IL-3) from Novartis (Vienna, Austria), rh stem cell factor (SCF) from PeproTech, and rh IL-2, rh EPO and rh SLIT2 from R&D systems (Minneapolis, MN, USA). The CD33-targeting drug

gemtuzumab/ozogamicin (GO) (Mylotarg®) was kindly provided by Wyeth Austria. Midostaurin (PKC412) was purchased from LC Laboratories (Woburn, MA, USA), alemtuzumab (Mabcampath®) from Genzyme (Cambridge, MA, USA) and gilteritinib from Selleck Chemicals (Houston, TX, USA). The IL-2-diphtheria toxin-conjugate denileukin-diftitox was produced as reported.[4] The human BCR/ABL1+ CML cell line KU812 was kindly provided by Dr. K. Kishi (Niigata University, Niigata, Japan) and was maintained in RPMI 1640 medium, 10% FCS, and antibiotics at 37°C. The identity of the cell line was confirmed in 2016 by the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) using nonaplex-PCR. After confirmation, these cells were passaged once and multiple aliquotes were stored in liquid nitrogen for subsequent experiments. Knock-down studies with shRNAs against CD25 were performed as published previously.[5]

Patients, diagnostic evaluations and follow-up examinations

A total of 274 patients with AML (121 females and 153 males), 97 patients with CML (47 females and 50 males) and 288 control cases were examined. The median age of our AML patients at diagnosis was 63 years (range: 19-92 years) and the median age of our CML patients at diagnosis was 55 years (range: 19-86 years). All patients gave written informed consent before bone marrow (BM) or peripheral blood (PB) samples were obtained. AML patients were classified according to criteria provided by the French-American-British (FAB) cooperative group and the World Health Organization (WHO).[6-11] AML patients in whom no $CD34^+$ blast cells could be detected were excluded from the study. The patients´ characteristics are shown in Table 1 (main text) and Tables S2 (CML) and S3 (AML). In the CML patients examined, diagnoses and

the phase of disease were established according to the WHO proposal.[10-13] Of the 97 CML patients examined, 80 had chronic phase (CP), 9 had accelerated phase (AP), and 8 had blast phase (BP) of CML at the time of sampling. In 239 patients with AML, PB or BM cells (iliac crest or sternum) were analyzed at diagnosis or blast cell persistence after induction chemotherapy. In 42 of these patients, PB or BM samples were analyzed additionally at the time of relapse or disease progression or at the time of complete hematologic remission (CHR). In 35 patients with AML, PB or BM samples were only analyzed at the time of relapse or disease progression. In 82 patients with CML, PB or BM samples were analyzed at diagnosis and in 29 of these patients PB or BM samples were additionally analyzed at the time of disease progression or at CHR. In 15 patients, PB or BM samples were only analyzed at the time of disease progression. In our AML patients, routine BM investigations (morphology, cytogenetics, PCR) were performed at the time of diagnosis, prior to each chemotherapy cycle, and at the time of relapse. In the CML patients examined, karyotype studies were performed in 3-month intervals during the first year; and later, after 12 months, in 6-48 month-intervals, depending on the molecular response. In general, we followed the recommendations of the European Leukemia Net (ELN).[11-13] *BCR-ABL1* mRNA levels were quantified in PB samples in 6-12 month intervals by qPCR and expressed as percent of *ABL* mRNA levels employing the International Scale (IS).[11-13] CML patients were treated with imatinib (400 mg daily per os) according to published guidelines.[12,13] In case of resistance or intolerance against imatinib, patients were treated with a second- or third generation tyrosine kinase inhibitor (TKI): nilotinib, dasatinib or bosutinib.[12,13] AML patients were treated with intensive chemotherapy, including 1-3 induction cycles (containing daunorubicin and

ARA-C in first induction) and up to 4 consolidation cycles with high-dose ARA-C (2x3 g/m^2 on days 1,3,5: age up to 60 years) or intermediate-dose ARA-C (2x1.5 g/m^2 on days 1,3,5: age >60 years) or stem cell transplantation.[14-20] In those who were not eligible for intensive therapy (because of comorbidities or age or a clear pre-phase of a myelodysplastic syndrome, MDS) or refused chemotherapy, 5-azcytidine or experimental drugs were administered. Hydroxyurea (HU) was used as palliative cytoreductive drug. Survival and progression-free survival were captured in the follow up (until death or loss for follow up) in all patients. All patients gave their written informed consent before PB or BM samples were collected. Information about control patients is provided in Table S4 (other bone marrow-derived neoplasms) and Table S5 (lymphoma, normal bone marrow, premalignant conditions). Subgroup analyses were performed in patients with MDS (n=58), myelodysplastic/myeloproliferative overlap neoplasms (MDS/MPN) (n=23), and idiopathic cytopenia of undetermined significance (ICUS, n=40). Normal BM cells were purchased from Lonza (Basel, Switzerland) (n=17) or were obtained from patients with Non-Hodgkin´s lymphoma (NHL) or Hodgkin´s lymphoma (n=98) or patients with suspected BM neoplasm (n=24). Cord blood (CB) samples (n=26) were examined after written informed consent was obtained from mothers. All studies were approved by the ethics committee of the Medical University of Vienna.

Cell sampling and storage

A total number of 878 cell samples, including 381 AML samples, 202 CML samples and 295 control samples were analyzed. All samples were collected during routine investigations. BM aspirate samples were obtained from the iliac crest or sternum after

local anesthesia. Stabilizer-free heparin (Biochrom AG, Berlin, Germany) was used as anticoagulant. Freshly obtained samples (PB, BM, CB) were subjected to flow cytometry analysis and/or isolation of mononuclear cells (MNC) using Ficoll. Isolated MNC were either used immediately or were frozen in liquid nitrogen until used. Frozen MNC and frozen $CD34^+$ MNC were thawed using DNase type I, 100 U/mL (Sigma Aldrich, St. Louis, MO, USA) to prevent cell clumping. CB cells were examined as freshly isolated leukocytes (after erythrocyte lysis) or as MNC after Ficoll-isolation and short-term (18 hours) culture. All cells were stored in a local biobank. Biobanking was approved by the ethics committee of the Medical University of Vienna.

Flow cytometry and cell sorting

Phenotyping of $CD34^+/CD45^+/CD38^-$ SC and $CD34^+/CD45^+/CD38^+$ progenitor cells (in whole BM/PB/CB samples or MNC) was performed by multicolor flow cytometry as described[1] using combinations of fluorochrome-conjugated mAb shown in Table S1. Heparinized BM or PB cells (0.5-1.0 x 10^6 leukocytes per tube) were incubated with various combinations of mAb at room temperature (RT) for 15 minutes. Then, erythrocytes were lysed in FACS-Lysing Solution (BD Biosciences, San José, CA, USA). In a subset of patients, phenotyping was (also) performed on freeze-thawed MNC (BM or PB) derived from patients with CML (n=44) or AML (n=17). No major differences in cell surface marker expression were found when comparing freshly obtained cells with freeze-thawed cells of the same samples (not shown). Flow cytometry was performed on a FACSCalibur or FACSCanto II (BD Biosciences) or Cytoflex S (Beckman Coulter). In our samples, 80,000 to 150,000 cells (thawed MNC)

or 150,000 to 350,000 cells (fresh lysed cells) were acquired and expression of surface markers on $CD34^+/CD45^+/CD38^-$ stem cells (SC) and $CD34^+/CD45^+/CD38^+$ progenitor cells was determined by multi-color flow cytometry. The gating strategy is shown in Figure S1. Antibody-staining results were controlled by isotype-matched control mAb. For detection of biotinylated antibodies, cells were washed in phosphate-buffered saline (PBS) and stained with streptavidin-PE (BD Biosciences). Cells were then washed, and analyzed by flow cytometry using FlowJo software (TreeStar, Ashland, OR, USA) as reported.[1,21] Staining results were expressed as a) percentage of reactive cells and b) as ratio of median fluorescence intensities (MFI) obtained with specific mAb and control mAb (MFI mAb : MFI control mAb) as reported.[1] This MFI ratio (= staining index, SI) was determined to compare expression levels of markers on leukemic stem cells (LSC) and progenitors in various patients´ groups. SI values were graded using the following score: −, 0-1.3; +/−, 1.31-3; +, 3.01-10; ++, >10.

In 6 normal BM samples, 5 cord blood (CB) samples, 21 AML samples and in 7 CML samples (chronic phase, n=4, accelerated phase, n=3), primary $CD34^+/CD38^-$ cells and $CD34^+/CD38^+$ progenitor cells were purified to homogeneity by cell sorting on a FACSAria (Becton Dickinson) as described.[1] The purity of immature, sorted, $CD34^+/CD38^-$ stem cells amounted to >95%. In a subset of patients (n=3), CML cells were pre-enriched as $CD34^+$ cells by magnetic cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). In 6 patients with AML, cells were depleted from $CD3^+$ cells by MACS (Miltenyi Biotec).

Selection of markers and targets for further validation: markers were mainly selected based on their specificity for LSC (expressed on LSC but not on normal stem cells and preferably not on LSC in other forms of leukemia) and targets were expressed

primarily based on i) more or less specific expression on LSC, ii) stability of expression on LSC (on LSC in most patients and also at both, diagnosis and relapse) and iii) the availability of targeted drugs (antibody-type or small molecules).

To estimate numbers of surface molecules per cells, the quantibrite PE fluorescence quantitation kit (BD Biosciences) was applied. In brief, beads were resuspended in 500 µl PBS containing 0.1% bovine serum albumin (Sigma Aldrich) and acquired on a FACSCanto II. Using an appropriate isotype-matched mAb, the SI was calculated for the four beads-populations laden with different amounts of PE molecules in the same way as for leukemic cells. Staining intensities obtained with beads were then correlated with expression levels of surface molecules (in SI) obtained in our staining experiments with LSC. To estimate the numbers of PE-molecules per cell (antibody molecules bound to the cell surface) a PE-molecule to antibody ratio of 1:1 was assumed. The limit of detection was 100 sites per cell corresponding to an SI of 1.6.

Incubation of cells with cytokines and targeted drugs and evaluation of proliferation, apoptosis and cell surface marker expression

In a subset of patients, unfractionated MNC or purified stem- and progenitor cells were incubated with recombinant human (rh) cytokines, including granulocyte colony-stimulating factor (G-CSF, 100 ng/ml), stem cell factor (SCF, 100 ng/ml), interleukin-3 (IL-3, 100 ng/ml) or a cocktail of cytokines (G-CSF+SCF+IL-3; each 100 ng/ml). In select experiments, cells were incubated with the ROBO4 ligand rh SLIT2 (100 ng/ml), IL-2 (100-2,000 ng/ml) or EPO (10 U/ml). In another set of experiments, cells were incubated in the absence or presence of targeted drugs: the IL-2R-targeted drug denileukin-diftitox (KU812 cells: 0.01-10 µg/ml; primary CML cells: 10-100 µg/ml),

the CD33-targeted antibody-conjugate gemtuzumab/ozogamicin (GO, 0.01-5 µg/ml), the multi-targeting FLT3/KIT drug midostaurin (0.01-5 µM), the FLT3-targeting drug gilteritinib (0.1-1 µM) or the CD52-targeted drug alemtuzumab (100-500 µg/ml). After 48 hours, cells were examined for uptake of ^{3}H-thymidine (purified LSC), or the expression of surface markers (CD26 on LSC), or the percentage of apoptotic cells (LSC and KU812) by flow cytometry as published previously.[1,5] Drug effects on apoptosis in LSC were examined by combined staining for surface markers (gating for $CD34^{+}/CD38^{-}$ and $CD34^{+}/CD38^{+}$ cells) and AnnexinV (eBioscience, San Diego, CA, USA). 4′,6-diamidino-2-phenylindole (DAPI, 0.4 µg/ml) (Sigma Aldrich) was used to exclude non-viable cells. Effects of GO and alemtuzumab on absolute numbers of viable LSC were examined using CountBright absolute counting beads (Invitrogen, Carlsbad, CA, USA) essentially as described.[22] For all *in vitro* experiments, cells were incubated in RPMI 1640 medium plus 10% FCS, penicillin/streptomycin and amphotericin B (each 1%). In drug incubation experiments with alemtuzumab, RPMI 1640 medium was supplemented with 30% complement-containing human serum. Effects of denileukin-diftitox on KU812 cells transduced with a random shRNA and KU812 cells transduced with shRNA against CD25 were analyzed after 48 hours of drug incubation by caspase-3 (BD Biosciences) staining and flow cytometry.[5]

Xenotransplantation model

NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice (NSG mice) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). NSG mice were kept under stringent aseptic (sterile) conditions. Twenty-four hours prior to injection, mice were irradiated in flat (sterile) irradiation-cages (2.4 Gy). In typical experiments, T cell-depleted AML cells

or sorted $CD34^+$ CML cells (all samples obtained at diagnosis) were injected into adult NSG mice (5 mice per group). Prior to injection, cells were incubated in control medium (RPMI 1640 medium plus 10% FCS plus 30% human serum plus solvent control), GO (5 µg/ml), alemtuzumab (500 µg/ml), or a combination of GO+alemtuzumab, for 1 hour at 37°C. Then, cells were resuspended in RPMI medium with 10% FCS and injected into the lateral tail vein of adult female NSG mice (1.5-15x10^6 AML cells, 0.5-1.0x10^6 $CD34^+$ CML cells). After injection, mice were inspected daily and sacrificed as soon as they developed disease-symptoms or after a maximum observation period of 15 weeks (AML) or 27 weeks (CML). Then, mice were sacrificed, and the long bones (humeri, tibiae, and femura) were flushed to recover BM cells for flow cytometry essentially as described.[1] Multicolor flow cytometry was performed using mAb against human CD19, CD33, and CD45. TO-PRO-3 (Invitrogen) was used to exclude non-viable cells. Engraftment was defined as a population of at least 0.01% human $CD45^+$ cells in flushed mouse BM samples. Animal studies were approved by the ethics committee of the University of Veterinary Medicine Vienna (Austria), and were carried out in accordance with guidelines for animal care and protection and protocols for experimental animal housing and studies approved by Austrian law (BMWFW-68.205/0050-WF/V/3b/2015).

Gene array studies

To define mRNA expression patterns in $CD34^+$ cell subsets in AML, CML and normal BM samples, sorted cells ($CD34^+/CD38^-$ and $CD34^+/CD38^+$) were subjected to gene array analysis. Total RNA was extracted from sorted cells using RNeasy kit (Qiagen, Hilden, Germany). Total RNA (100 ng) was then used for GeneChip analyses.

Preparation of terminal-labeled cRNA, hybridization to genome-wide human PrimeView GeneChips (Affymetrix, Santa Clara, CA, USA) and scanning of the arrays were carried out according to manufacturer's protocols (https://www.affymetrix.com). Robust Multichip Average (RMA) signal extraction and normalization were performed as described (http://www.bioconductor.org/).[23,24] Changes in mRNA expression levels were calculated as mRNA ratio of $CD34^+/CD38^-$ cells when comparing normal BM hematopoietic stem cells (HSC) with LSC (AML or CML). To calculate differential gene expression between individual sample groups, we performed a statistical comparison using the LIMMA package as described previously.[25] Briefly, LIMMA estimates the ´fold-change´ between predefined sample groups by fitting a linear model and using an empirical Bayes method to define the standard errors of the estimated log-fold changes for each probe set.[26]

Potential SC markers were reviewed and grouped into non-clustered and clustered surface antigens. In a next step, most upregulated surface antigens that have a defined SC-related function or are known as potential targets were selected. These antigens were re-examined in purified LSC and normal stem cells by qPCR and surface phenotyping. The top-upregulated surface antigens (mRNA) in AML LSC and CML LSC compared to normal BM stem cells are shown in Table S13B. The top-upregulated surface antigens in CML LSC compared to more mature CML progenitors are shown in Table S18. A heat map of regulated genes in AML and CML LSC is depicted in Figure 2 in the main text of the manuscript and in Figure S3. A complete list of all expressed mRNA species is available at Gene Expression Omnibus: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138883&token=glmjuauafpqbxij

Quantitative PCR (qPCR)

To confirm marker expression at the mRNA level, qPCR was performed using primers specific for markers and target antigens and control-antigens (huABL). Experiments were performed on mRNA derived from sorted $CD34^+/CD38^-$ and $CD34^+/CD38^+$ cells (AML, n=14; CML, n=4; cord blood, n = 4; normal BM, n=3). Total RNA was extracted using the RNeasy Micro Kit (Qiagen). cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Invitrogen), random primers, dNTPs (2 mM) (both from Invitrogen), and Rnasin (Promega, Madison, WIS, USA) according to the manufacturer`s instructions. PCR was performed using primers (Eurofins, Ebersberg, Germany) shown in Table S6. mRNA levels were quantified on a 7900HT Fast Real-Time PCR System or Quantstudio 3 (both from Applied Biosystem, Foster City, CA, USA) using iTAq SYBR Green Supermix with ROX (Bio-Rad, Hercules, CA, USA), as described.[27] PCR conditions were: hold stage: 2 minutes at 50°C, 2 minutes at 95°C (denaturation); PCR stage: 15 seconds at 95°C, 1 minute annealing and extension at 60°C (40x cycles); melt curve stage: 15 seconds at 95°C, 15 seconds at 60°C, 15 seconds at 95°C. ABL served as a reference gene. Results are expressed as ΔCt values (= Ct_{ABL} - Ct_{GENE}). Samples with undetectable mRNA expression were set to a maximum Ct value of 38.

For the quantification of BCR/ABL1 transcript levels in patient-derived cells (routine PB or BM samples in the follow-up), we performed a qPCR using the BCR/ABL1 Mbcr FusionQuant® Mega Kit (Ipsogen, Marseille, France) and the LightCycler® 2.0-System (Roche, Mannheim, Germany). BCR/ABL1 levels were calculated and expressed according to the international scale (IS).[28-30]

Conventional cytogenetic studies and fluorescence in situ hybridization (FISH)

Conventional cytogenetics was performed in all patients with CML and AML at diagnosis. In patients with CML, cytogenetics was also performed routinely during follow-up at defined time-intervals, namely in 3 month-intervals during the first year; and after the first year in 3-12 month-intervals. In addition, cytogenetics (and FISH if necessary) was performed in all patients with (suspected) relapse. In most samples, at least 20 metaphases were examined in each sample. In case of questionable results or poor growth, FISH analysis was performed. Karyotypes were reported according to available guidelines.[31] In patients with CML, results were expressed as percent Ph-chromosome-positive metaphases (of all metaphases examined). In order to confirm the clonal origin of sorted stem cells ($CD34^+/CD38^-$ cells), FISH was performed on cytospin slides using probes specific for genes located on chromosome 3 or chromosome 16, the long arm of chromosome 5, and AML1 and ETO (RUNX1 and RUNX1T1) (Kreatech, Amsterdam, Netherlands). A detailed description of probes is shown in Table S7. Results obtained by conventional karyotyping in patients´ cells at diagnosis are summarized in Tables S2-S5, and a summary of FISH results obtained with purified (sorted) stem cells is shown in Table S8.

Statistical evaluations

Differences in marker expression or the percentage of LSC in various sample-series were determined by appropriate statistical tests. For comparing surface marker expression on normal versus leukemic ($CD34^+/CD38^-$) stem cells and between subsets of patients (defined by molecular abnormalities such as *FLT3* ITD or cytogenetic

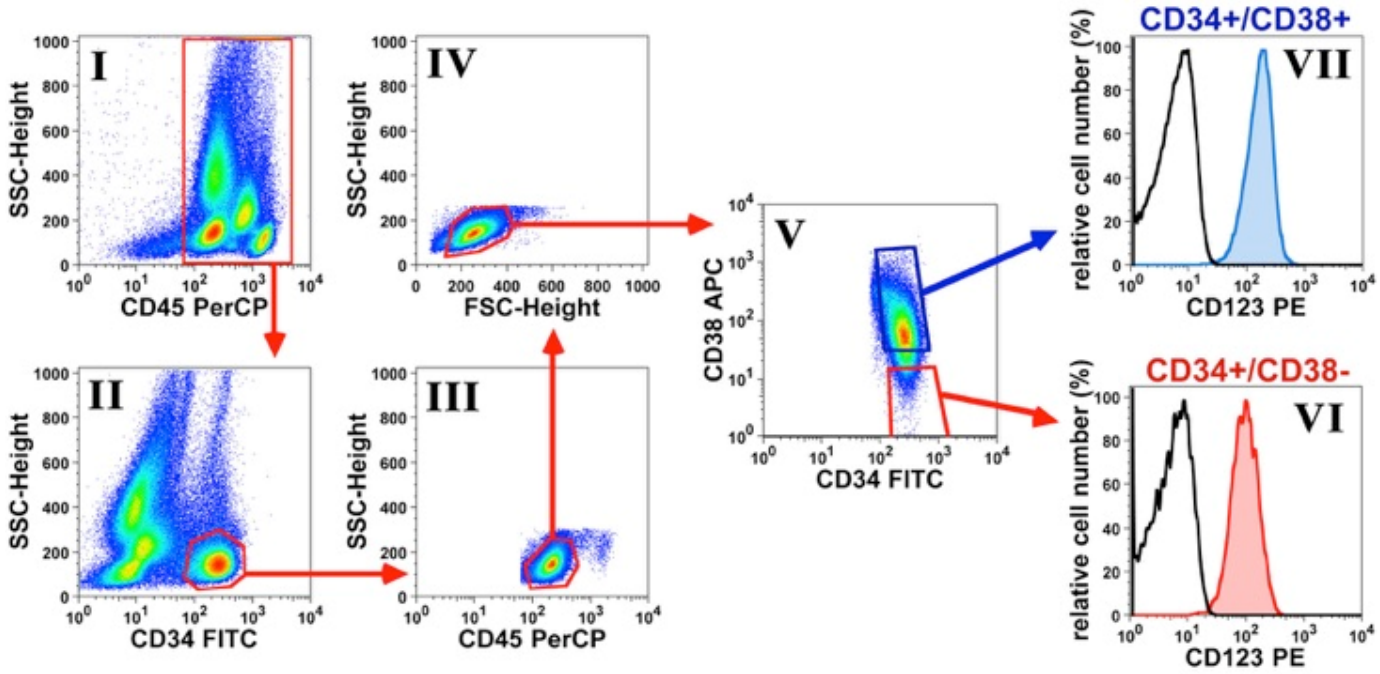
markers), the Kruskal-Wallis test was applied. Statistical post-hoc analyses were performed to confirm significance levels using pair-wise Wilcoxon rank-sum test. Results were adjusted for multiple testing using the Benjamini-Hochberg correction.[32] All statistical analyses were conducted by using R version 3.6.1 (Vienna, Austria).[33] Results were considered significant when the p value was below 0.05.

For determining the level of significance in drug inhibition experiments or differences in engraftment levels of LSC in NSG mice, the Student´s t test was applied. Results were considered significantly different when p was <0.05.

The probability of overall survival (OS) in subsets of patients (AML, CML) defined by marker expression on LSC was calculated by the product limit method of Kaplan and Meier. The patients were split into two groups per marker based on sample size and subpopulation formation. Statistical significance in differences among patient subgroups (defined by divergent surface marker expression on LSC) concerning survival were determined by log rank test.[22]

Supplemental Material – Figures

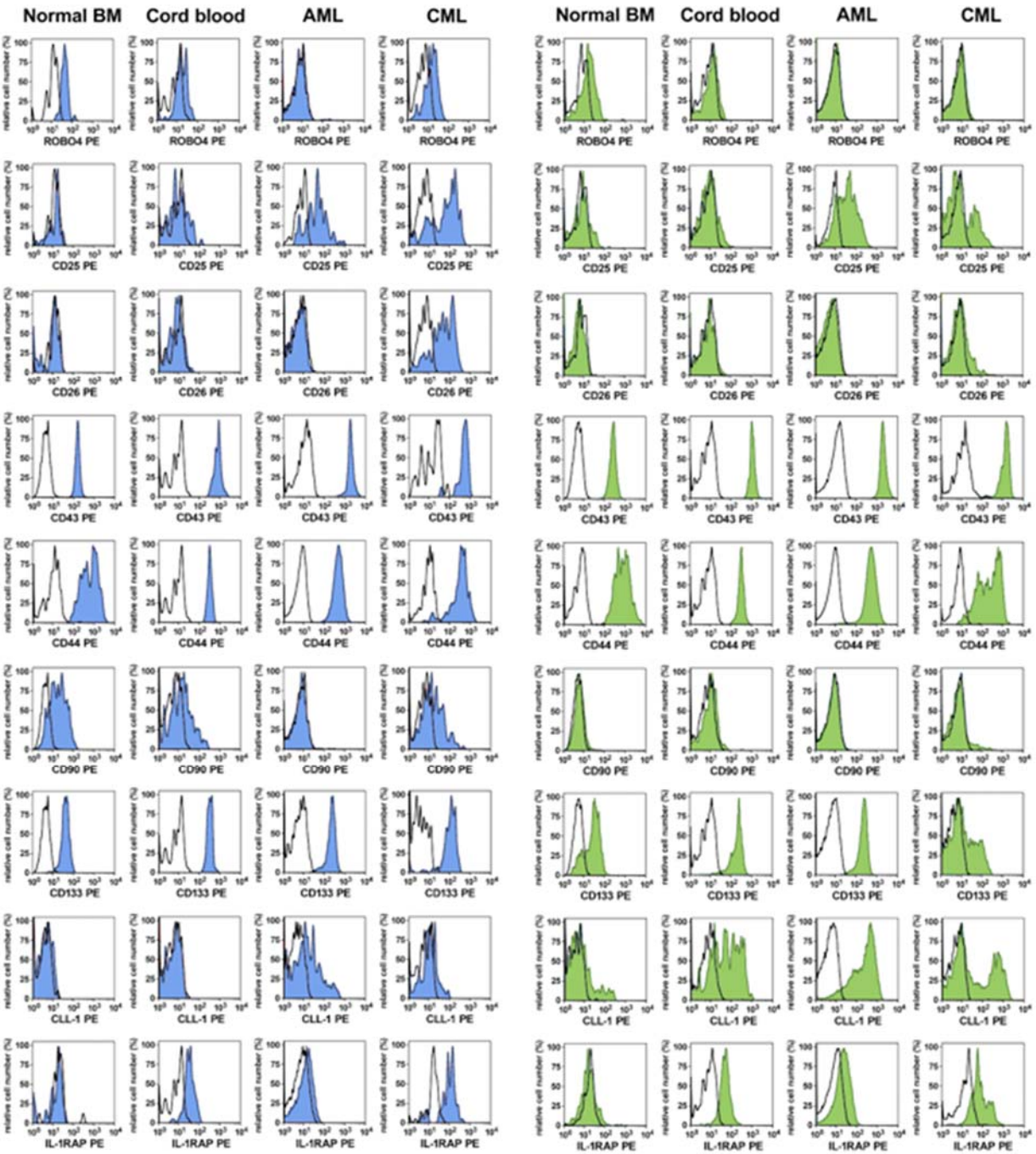
Figure S1



Flow cytometry and gating strategy to define and separate stem- and progenitor cells

In order to define and analyze immature $CD34^+/CD38^-$ stem cells and $CD34^+/CD38^+$ progenitor cells, a sequential gating algorithm (I-VII) was applied using FlowJo software. First, $CD45^+$ cells (I) were gated to exclude erythrocytes and stroma cells, followed by gating of $CD34^+$ stem and progenitor cells (II) and further selection of CD45 dim-positive cells (III). In a next step, dead cells and cell debris was excluded by forward and side-scatter properties (IV). Finally, $CD34^+/CD38^-$ stem cells and $CD34^+/CD38^+$ progenitor cells were analyzed separately for expression of surface markers and targets (V; CD34+/CD38+: blue gate; $CD34^+/CD38^-$: red gate) (VI/VII). The figure shows expression of CD123 on $CD34^+/CD38^-$ stem cells (lower panel, red histogram) and $CD34^+/CD38^+$ progenitor cells (upper panel, blue histogram) in a patient with AML. Antibody reactivity was controlled by isotype-matched antibodies (black open histogram).

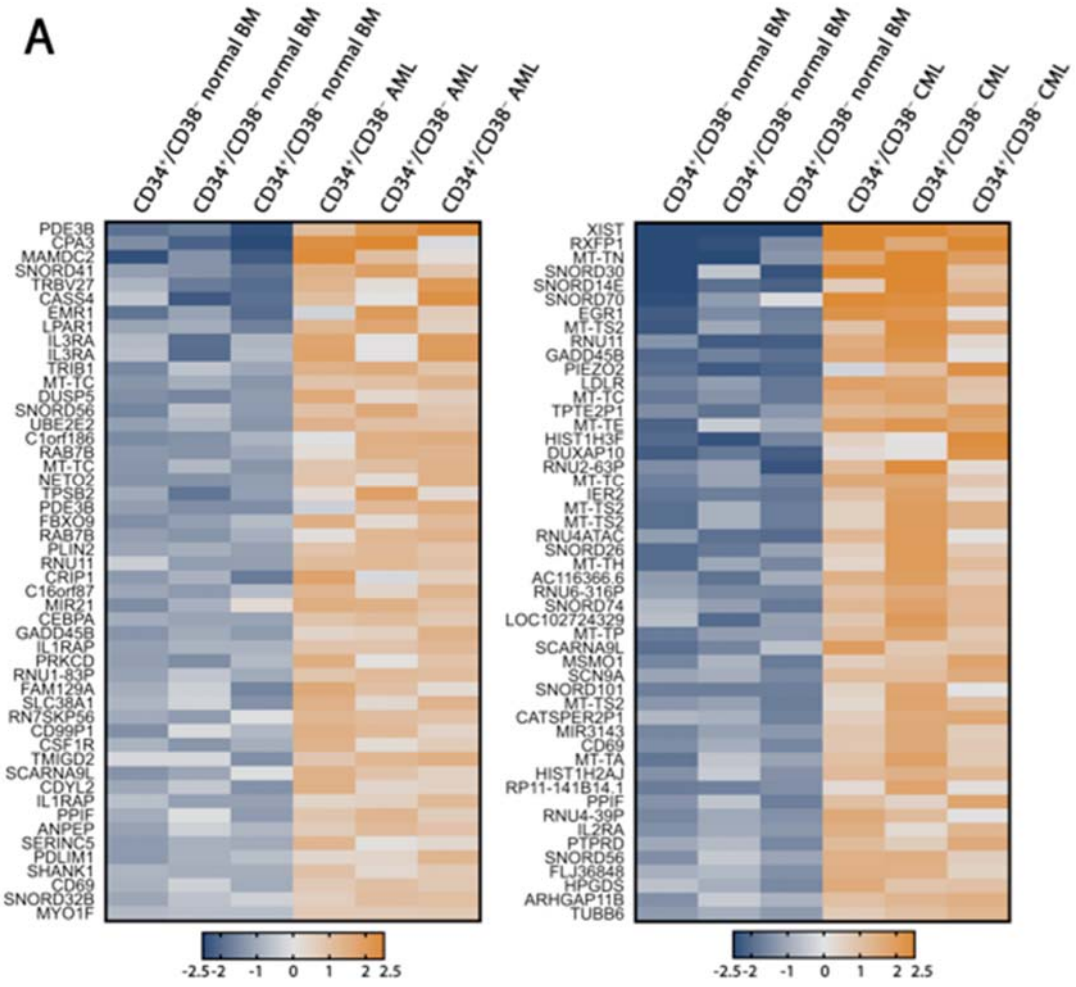
Figure S2



Examples for staining reactions on stem and progenitor cells from normal BM, cord blood and patients with AML and CML

Cells were stained with antibodies against ROBO4, CD25, CD26, CD43, CD44, CD90, CD133, CLL-1 and IL-1RAP. Antibody reactivity with test antibodies is shown in colored histograms ($CD34^+/CD38^-$ stem cells in blue and $CD34^+/CD38^+$ cells in green histograms) and the isotype-matched control antibody in black open histograms. Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CD; cluster of differentiation; CML, chronic myeloid leukemia; IL-1RAP, interleukin-1 receptor accessory protein; ROBO4, roundabout 4.

Figure S3A



Gene chip analysis in CD34$^+$/CD38$^-$ cells in normal BM, AML and CML

Microarray analyses were performed on RNA of highly purified (sorted) CD34$^+$/CD38$^-$ AML and CML LSC as described in the supplement. The heatmaps show the 50 most upregulated genes in CD34$^+$/CD38$^-$ cells in 3 patients with AML (right columns of left subpanel; patients #102, #173, #210) and 3 with CML (right columns of right subpanel; patients #8, #11, #21) (all leukemic samples were obtained at diagnosis). The definition of the color-scoring is indicated in the figures. The 3 left columns of each subpanel show the same genes in normal CD34$^+$/CD38$^-$ BM stem cells. Patients´ numbers (#) refer to the patients´ numbers defined in Table S2 and S3. Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; #, number; LSC, leukemic stem cell; BM, bone marrow.

Figure S3B

Gene chip analysis of CD markers in $CD34^+/CD38^-$ cells in normal BM, AML and CML

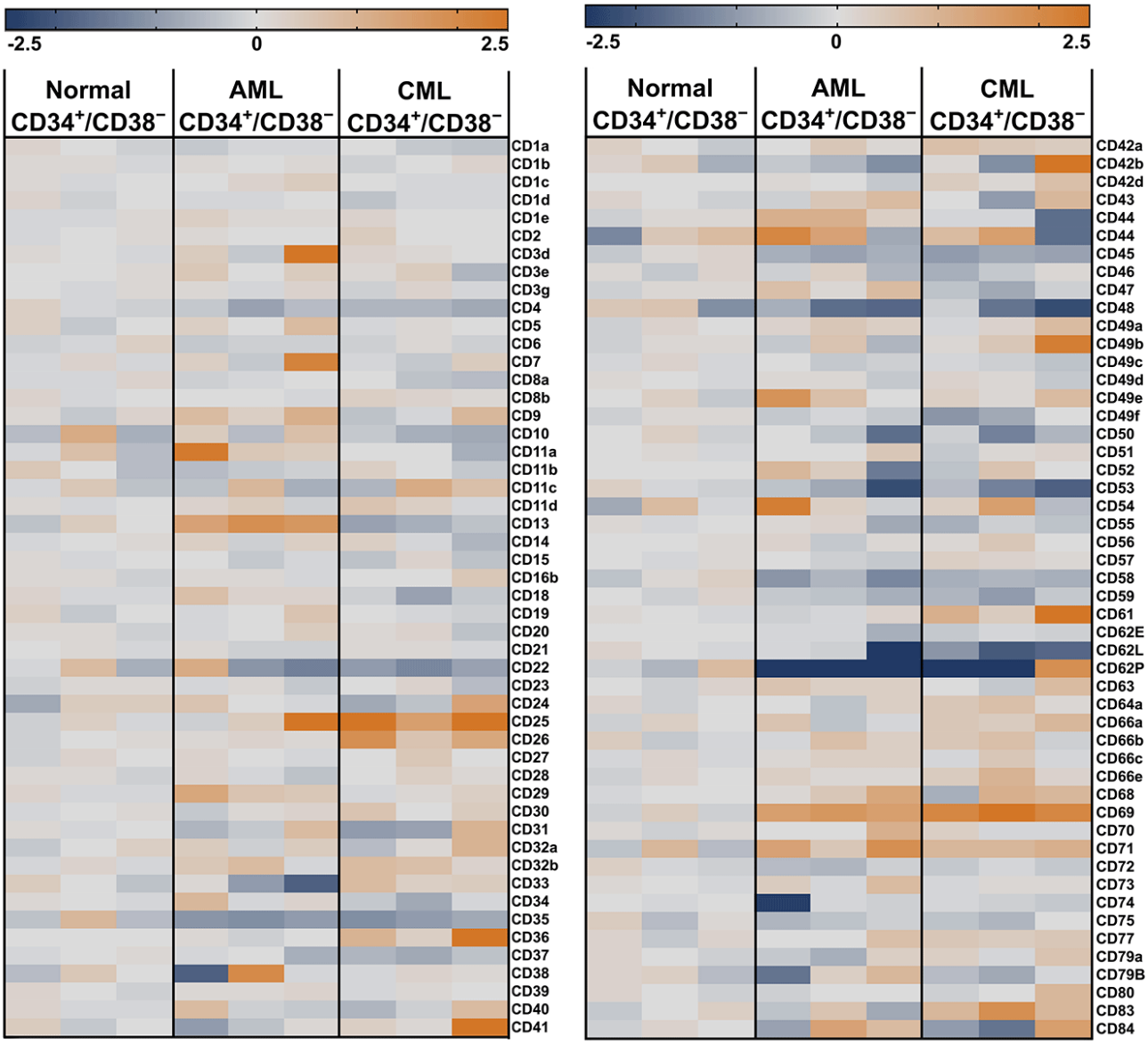


Figure S3B continued

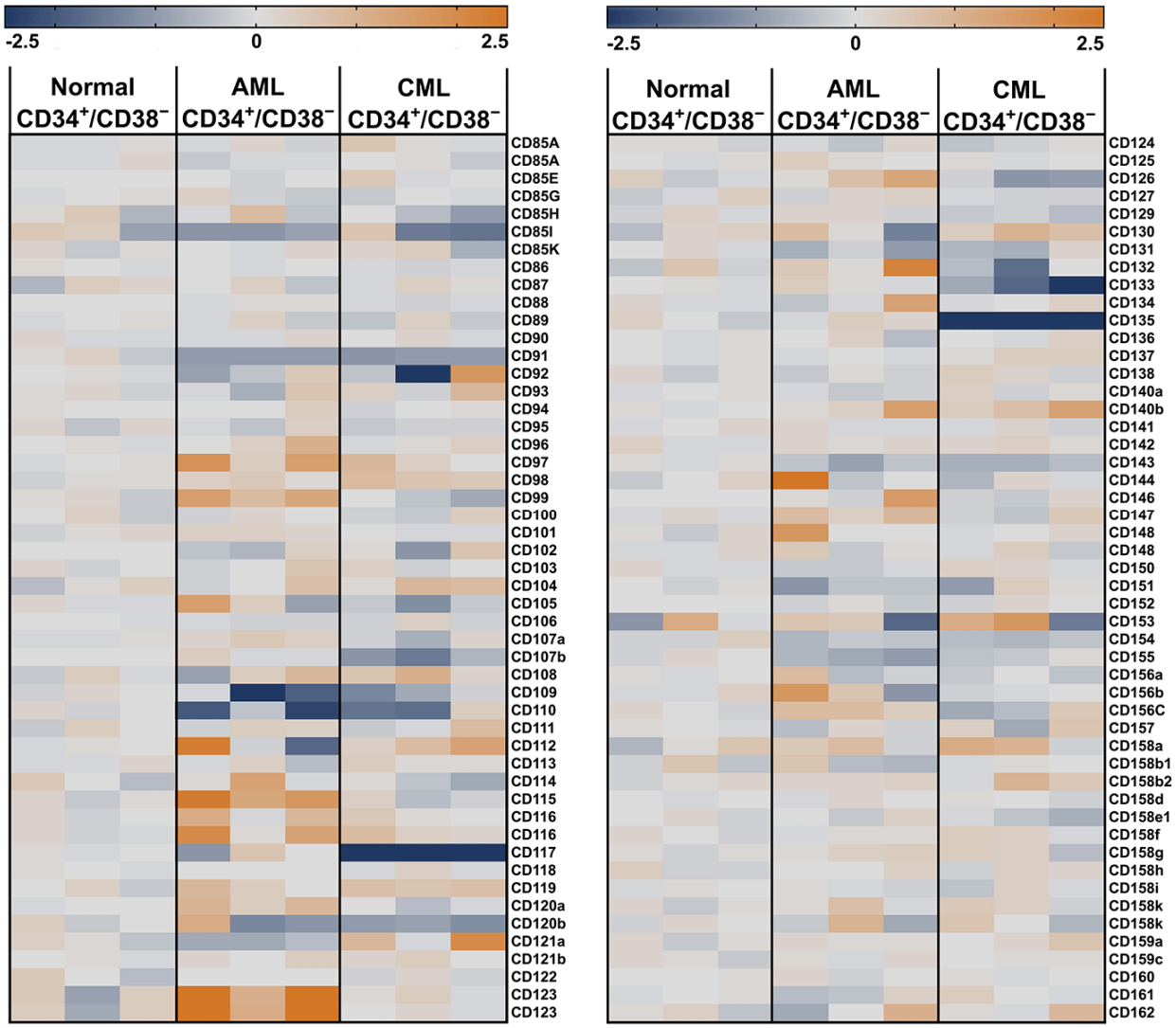


Figure S3B continued

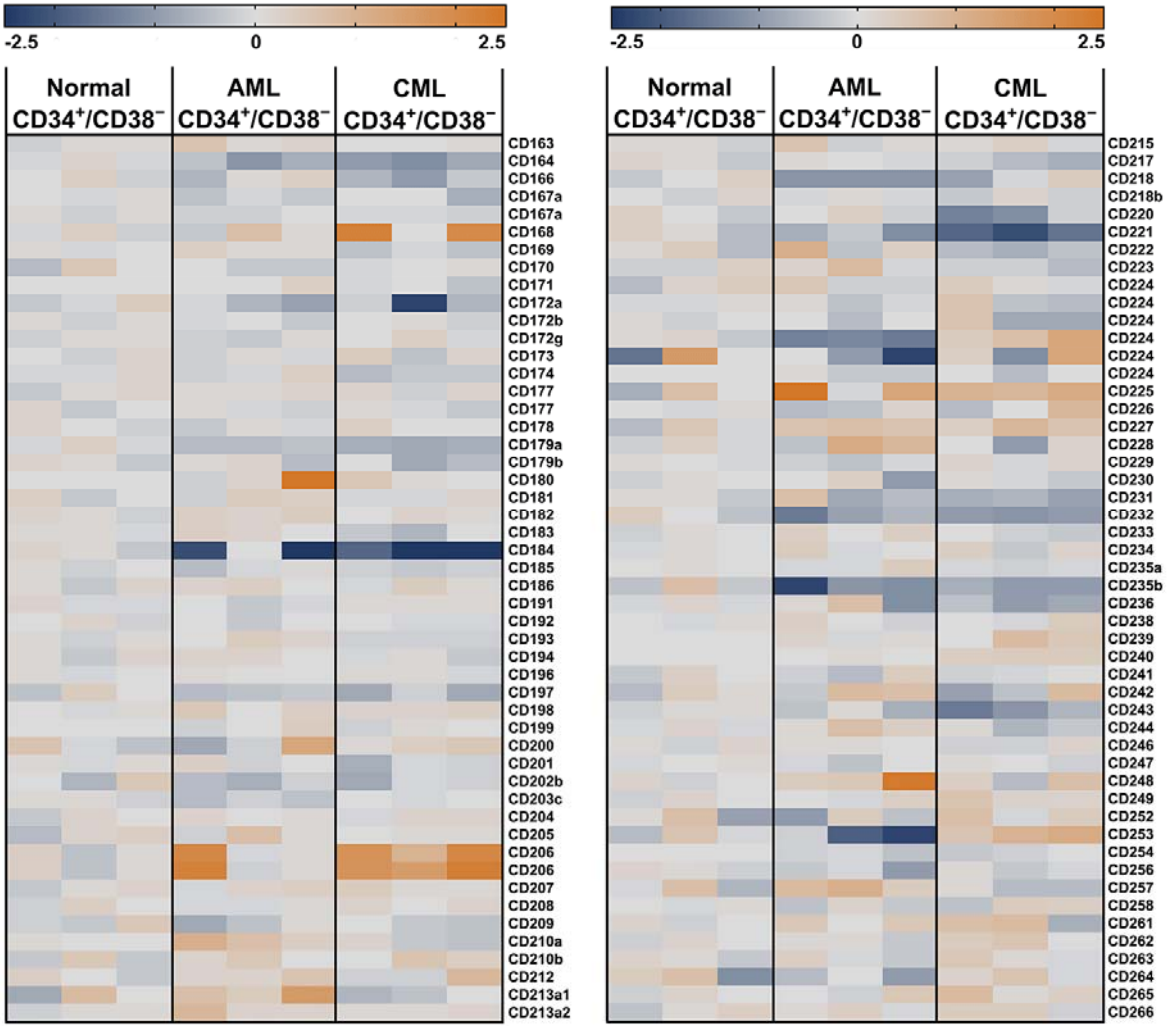
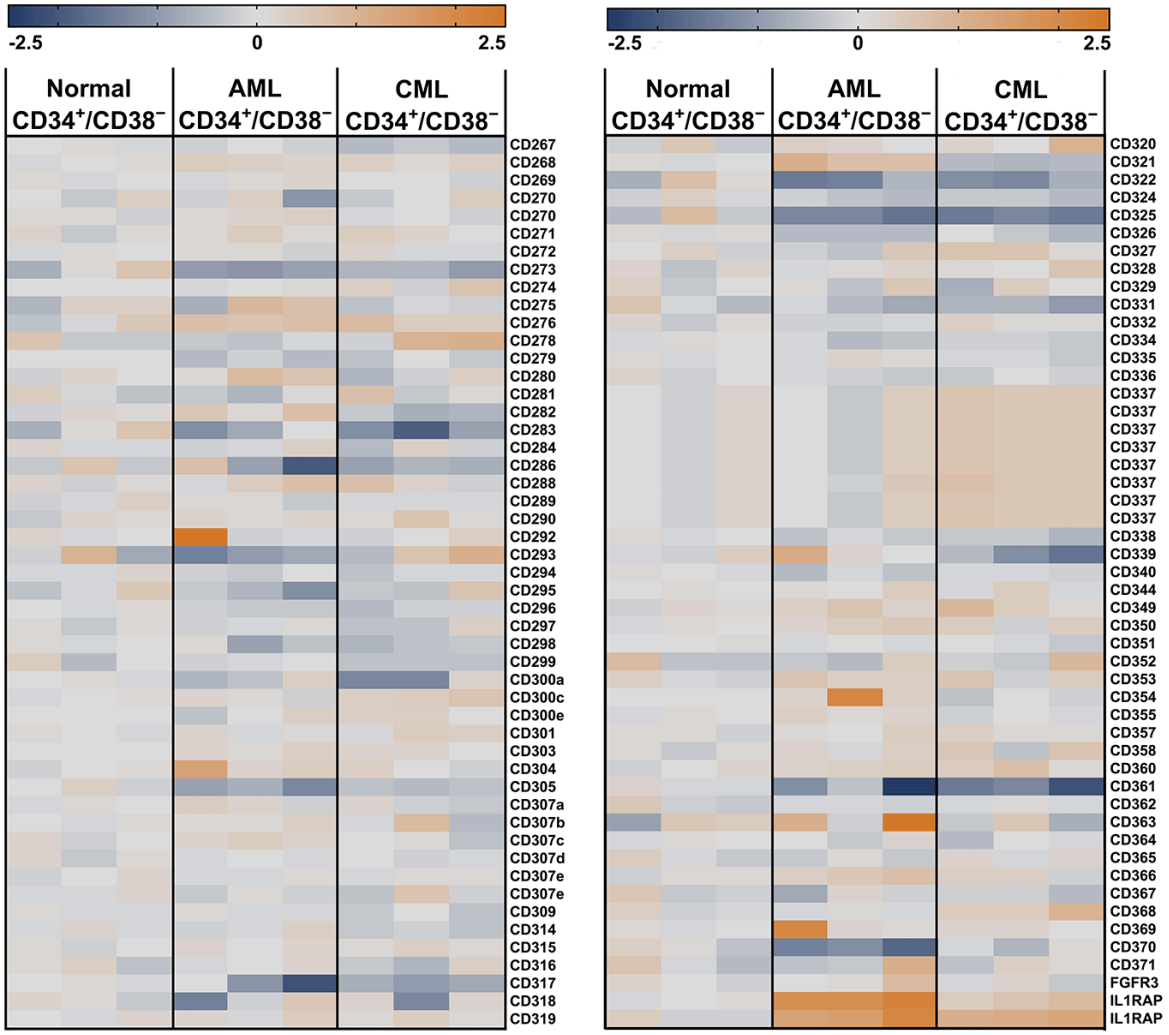


Figure S3B continued



Gene chip analysis of CD markers in CD34$^+$/CD38$^-$ cells in normal BM, AML and CML
Gene array data of sorted CD34$^+$/CD38$^-$ stem cells (SC) obtained from control bone marrow (BM) (n=3; left columns) and patients with AML (n=3; patients #102, #173, #210; middle columns) and CML (n=3; patients #8, #11, #21; right columns) (leukemic samples were obtained at diagnosis). Gene array analyses were performed as described in the supplement. mRNA expression levels were normalized to the mean expression of the control samples and are shown as heatmap. The definition of the color-scoring is indicated in the figures. Patients´ numbers (#) refer to the patients´ numbers defined in Table S2 and S3. Abbreviations: AML, acute myeloid leukemia; CD; cluster of differentiation; CML, chronic myeloid leukemia; IL-1RAP, interleukin-1 receptor accessory protein; FGFR3, fibroblast growth factor receptor 3.

Figure S3C

Gene chip analysis of CD markers in $CD34^+/CD38^+$ cells in normal BM, AML and CML

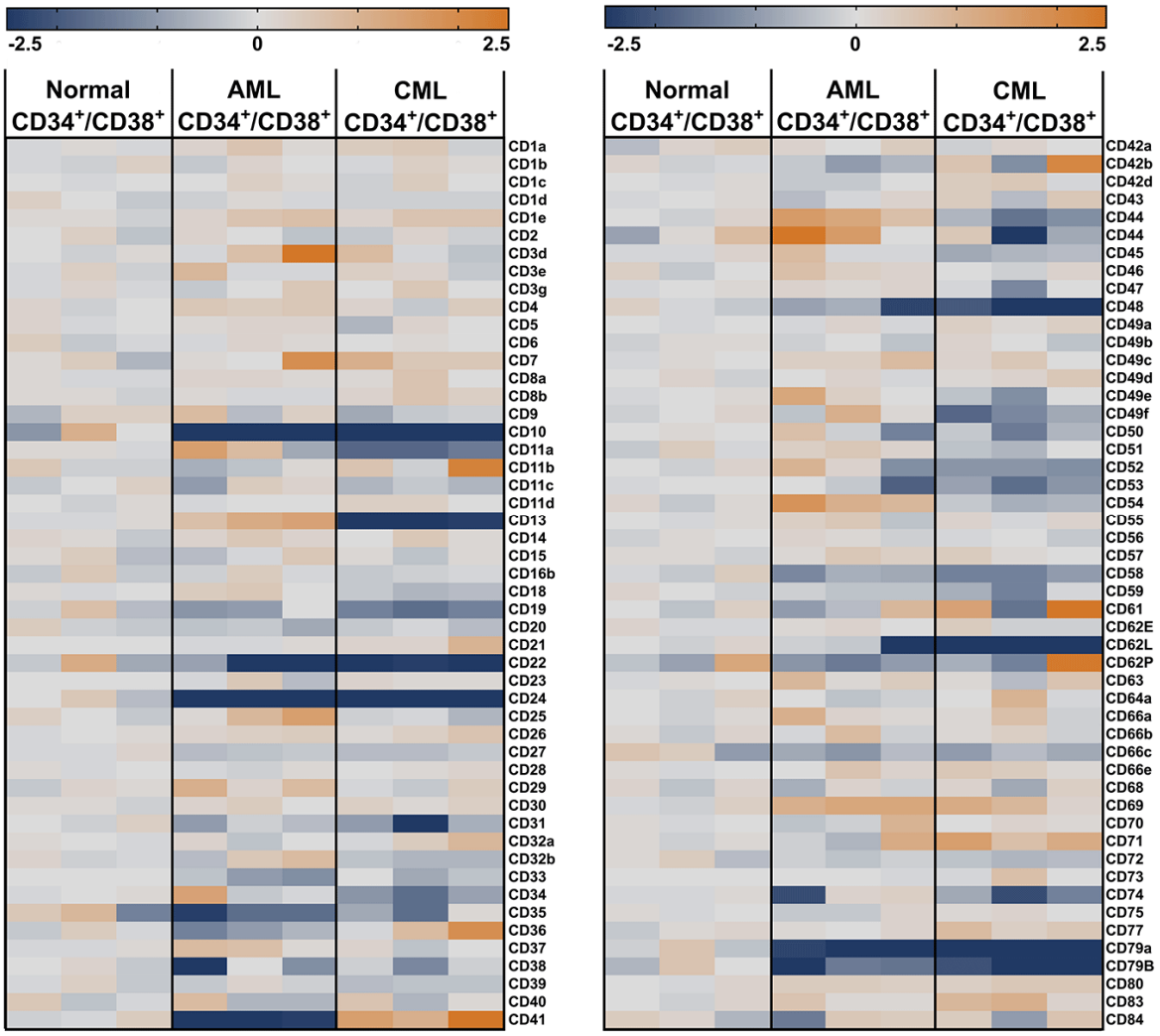


Figure S3C continued

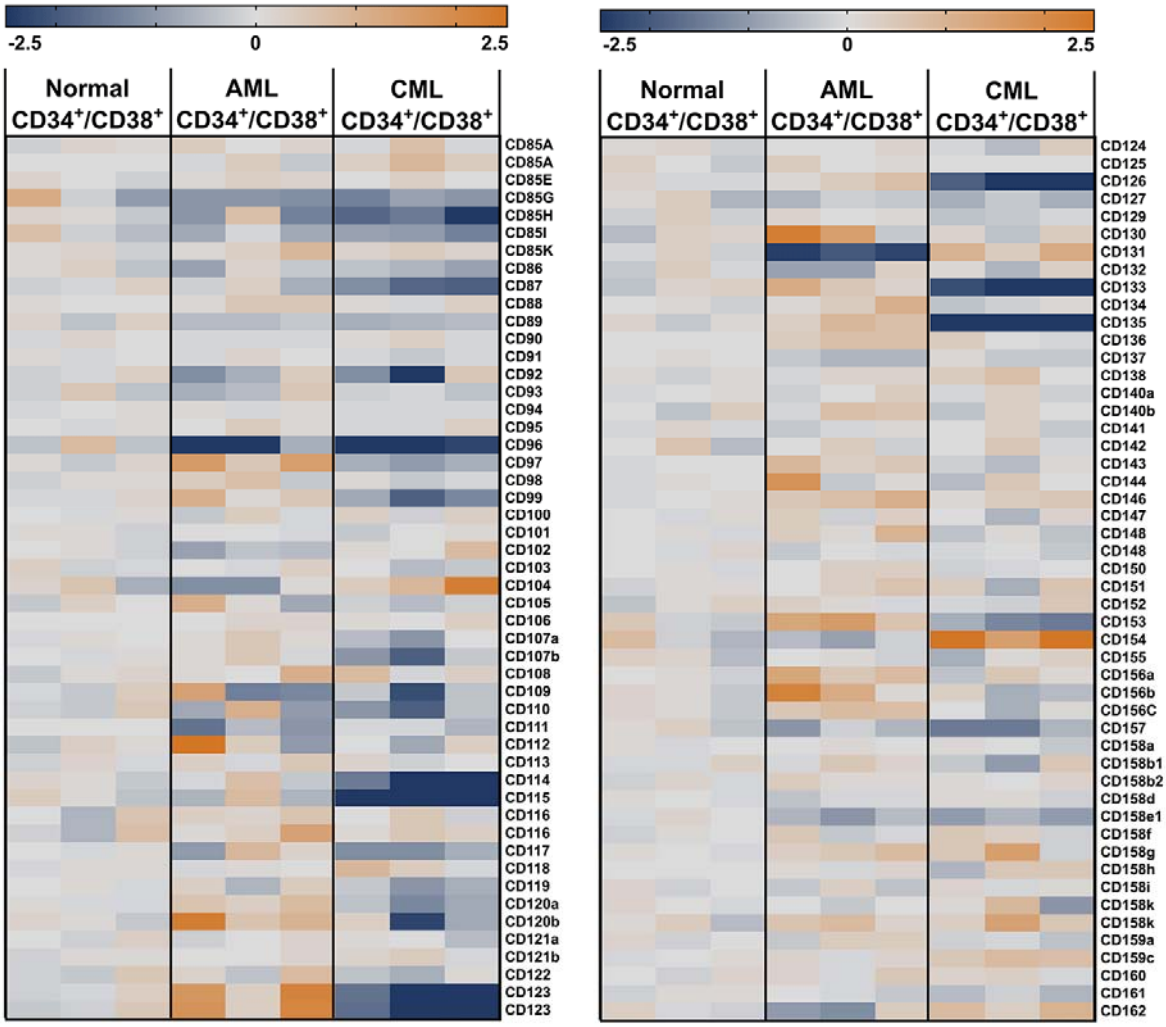


Figure S3C continued

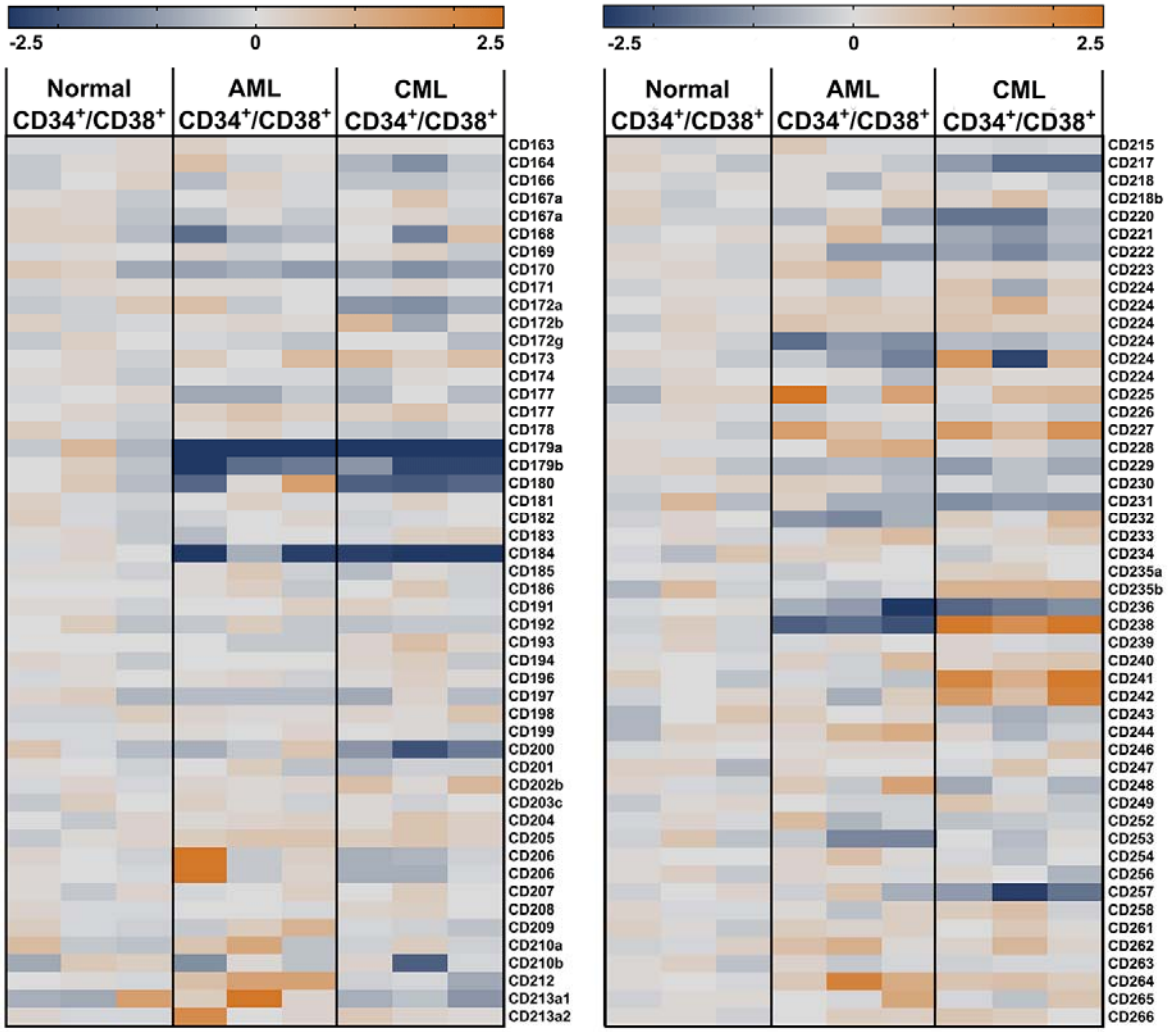
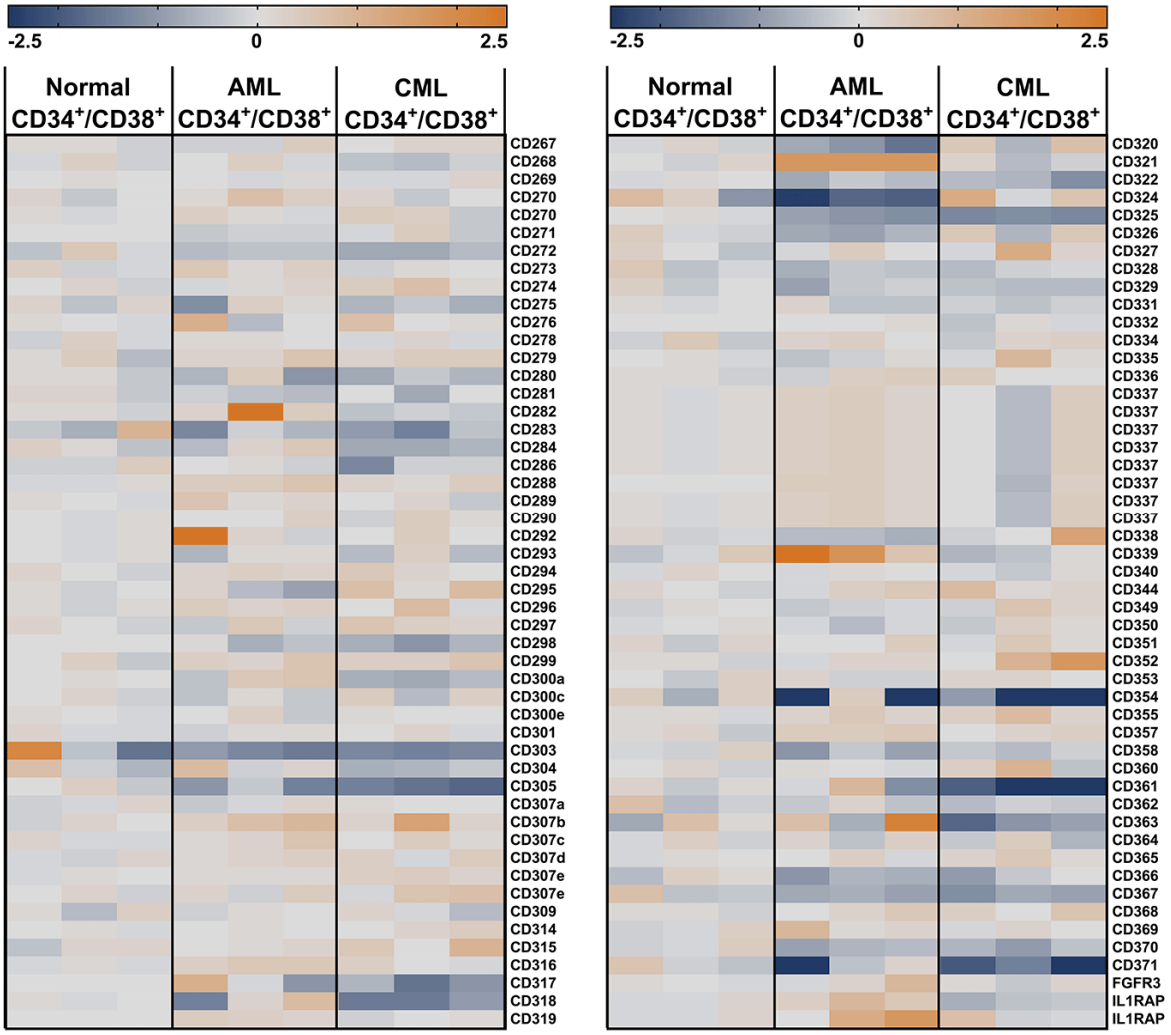
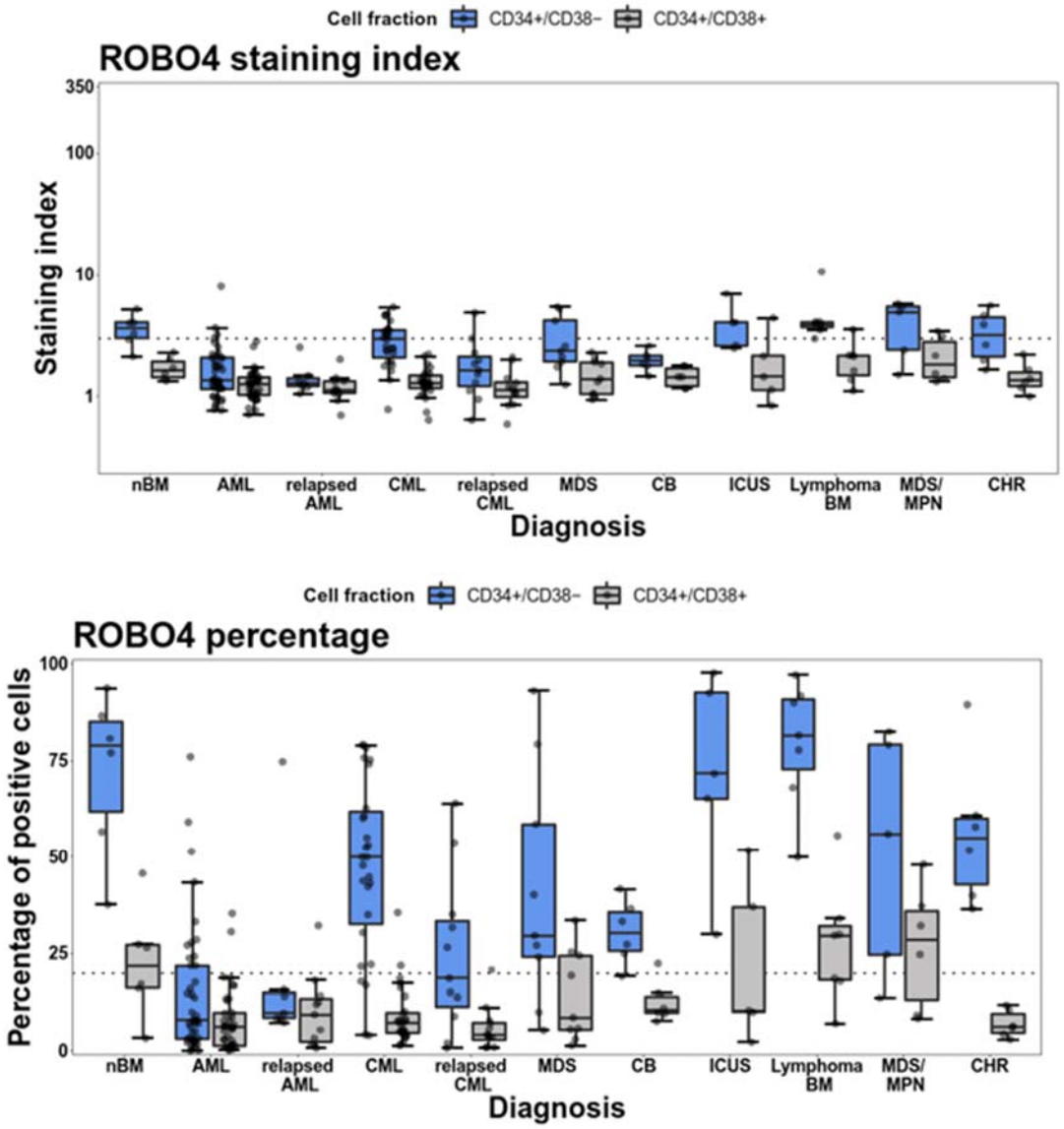


Figure S3C continued



Gene chip analysis of CD markers in CD34$^+$/CD38$^+$ cells in normal BM, AML and CML
Gene array data of sorted CD34$^+$/CD38$^+$ progenitor cells obtained from control bone marrow (BM) (n=3; left columns) and patients with AML (n=3; patients #102, #173, #210; middle columns) and CML (n=3; patients #8, #11, #21; right columns) (leukemic samples were obtained at diagnosis). Gene array analyses were performed as described in the supplement. mRNA expression levels were normalized to the mean expression of the control samples and are shown as heatmap. The definition of the color-scoring is indicated in the figures. Patients´ numbers (#) refer to the patients´ numbers defined in Table S2 and S3. Abbreviations: AML, acute myeloid leukemia; CD; cluster of differentiation; CML, chronic myeloid leukemia; IL-1RAP, interleukin-1 receptor accessory protein; FGFR3, fibroblast growth factor receptor 3.

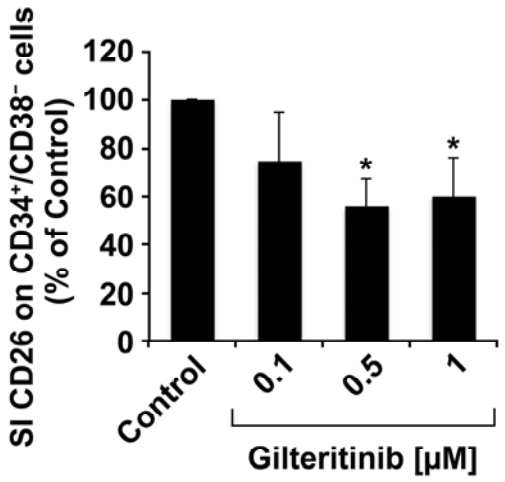
Figure S4



Expression of ROBO4 on leukemic and normal hematopoietic stem cells

The expression of ROBO4 on $CD34^{+}/CD38^{-}$ stem cells (blue boxes) and $CD34^{+}/CD38^{+}$ progenitor cells (grey boxes) in normal bone marrow, patients with CML, MDS, AML and other disease categories as indicated is shown. Samples were examined by multi-color flow cytometry using an antibody against ROBO4. The figure shows the staining ratio compared to an unspecific isotype control antibody (staining index, upper panel) and the percentage of reactive cells in each sample (lower panel). The dotted line represents clear expression (staining index is higher than 3 or more than 20% positive cells). The range of each box represents the 25^{th} (lower box margin) to 75^{th} (upper box margin) percentile. The middle lines inside the boxes represent the median expression level. Abbreviations: BM, bone marrow; n, normal; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; CB, cord blood; ICUS, idiopathic cytopenia of undetermined significance; CHR, AML and/or CML in complete hematologic remission; ROBO4, roundabout 4.

Figure S5

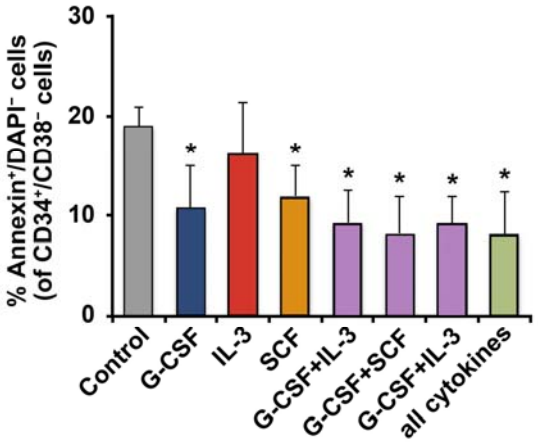


Expression of CD26 on $CD34^+/CD38^-$ AML stem cells from *FLT3* ITD+ patients after incubation with the FLT3 inhibitor gilteritinib

Mononuclear cells from 3 patients with AML (samples obtained at diagnosis) were incubated in medium (Control) or gilteritinib (0.1-1 µM) for 24 hours at 37°C. Thereafter, CD26 expression was analyzed on $CD34^+/CD38^-$ cells using multi-color flow cytometry. Results are expressed as staining index (median fluorescence intensity of CD26 divided through matched isotype control) and are presented as % of control and show the mean±S.D. from 3 experiments. Asterisk (*): $p<0.05$ compared to control. Abbreviations: SI, staining index; AML, acute myeloid leukemia.

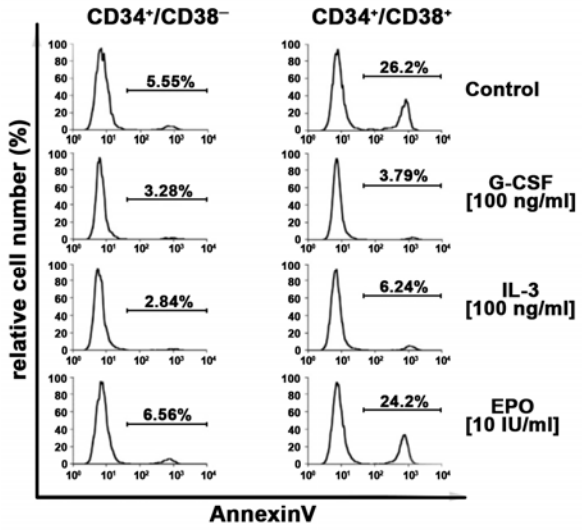
Figure S6

A



		Staining-index score		
patient #	sample at	CD114 G-CSFR	CD117 SCFR	CD123 IL-3RA
210	D	+/−	++	+
1	D	+	++	++
183	D	+	++	++
141	D	+/−	++	++

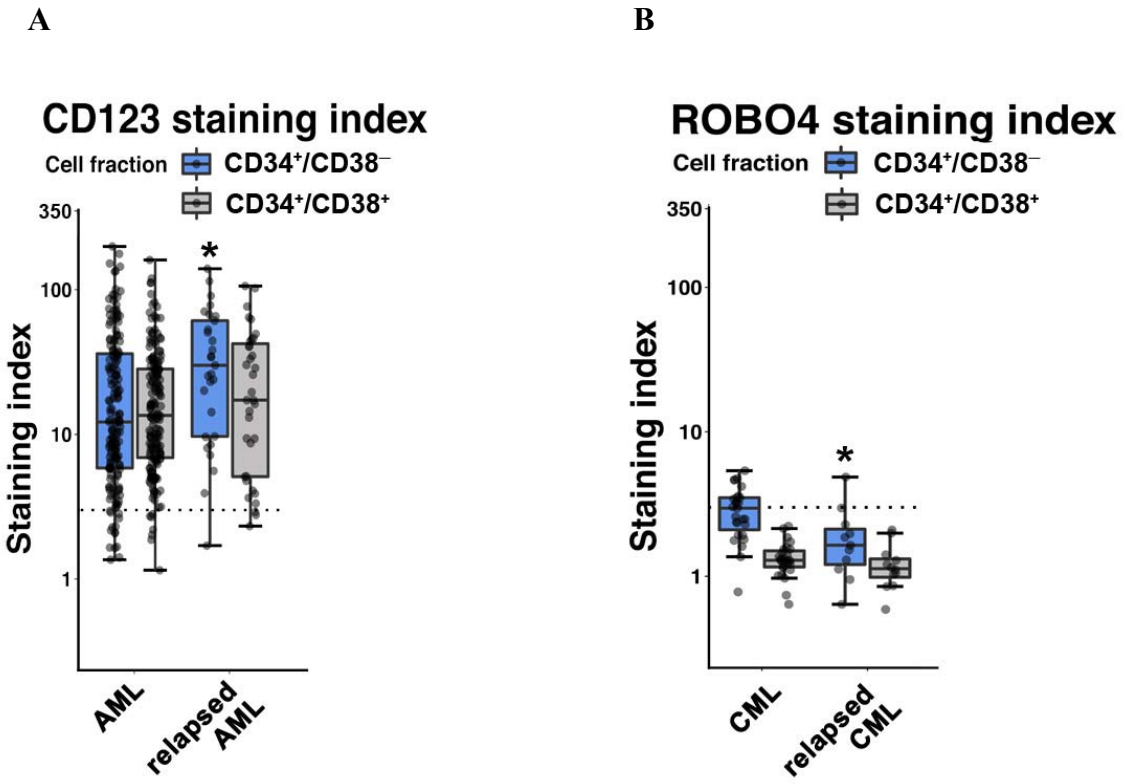
B



Effects of various cytokines on viability of CD34$^+$/CD38$^-$AML LSC

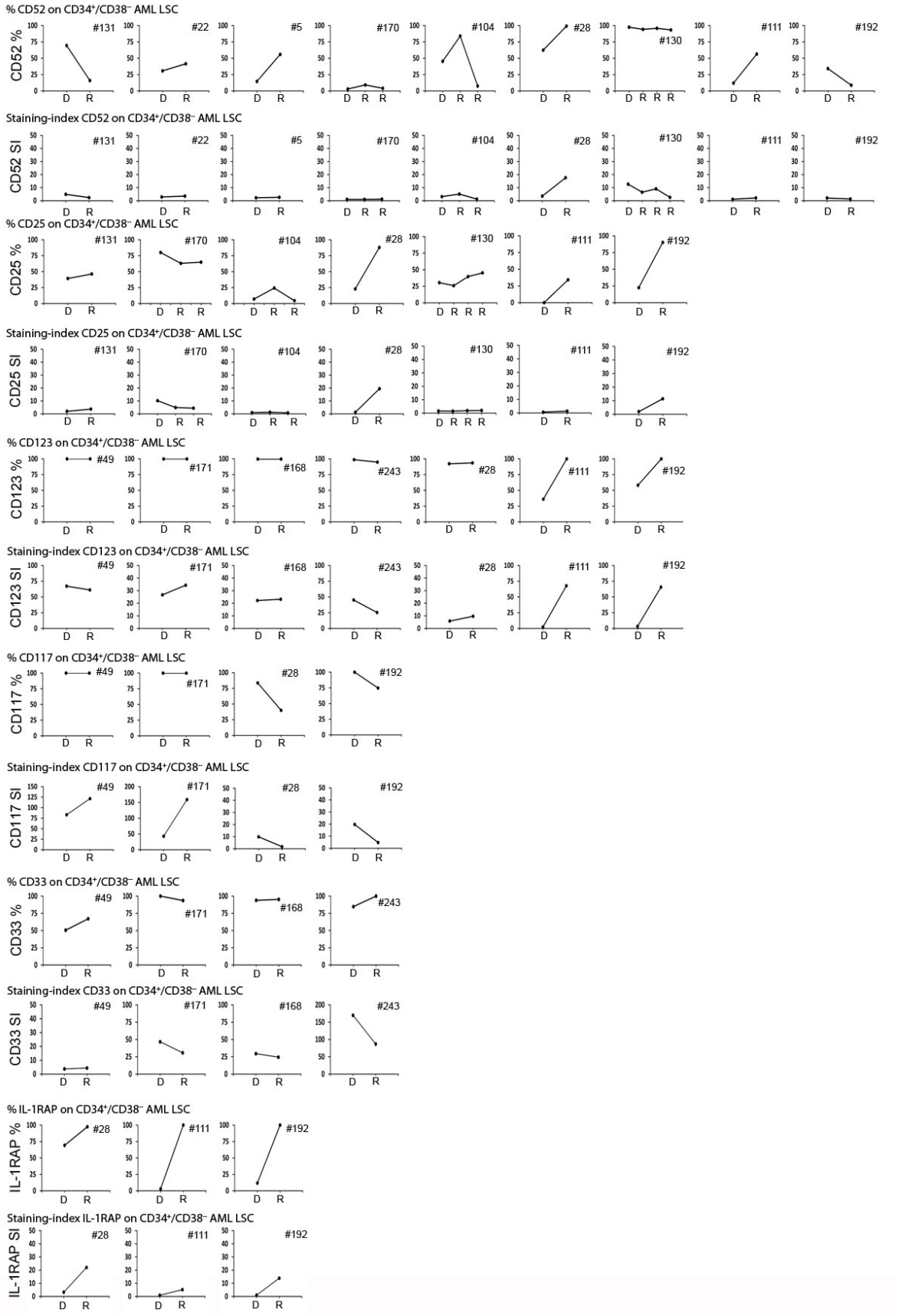
(A) Mononuclear cells from 4 patients with AML (samples obtained at diagnosis) were incubated separately in RPMI 1640 medium and 10% FCS in the absence (control, grey bar) or presence of G-CSF (blue bar), IL-3 (red bar), SCF (yellow bar), or a combination of these cytokines (violet bars for double combinations and green bar for the combination of all cytokines) as indicated (each 100 ng/ml) at 37°C for 48 hours. Thereafter, cells were examined for apoptosis induction in CD34$^+$/CD38$^-$ using flow cytometry. Results are expressed as percent Annexin$^+$/DAPI$^-$ CD34$^+$/CD38$^-$ cells and show the mean±S.D. of 4 independent experiments. Asterisk (*): $p<0.05$ compared to control. Lower left panel: The table shows the expression of the cytokine receptors G-CSFR (CD114), IL-3RA (CD123) and SCFR (CD117) on CD34$^+$/CD38$^-$ cells from 4 patients with AML used in these experiments. Patients´ numbers (#) refer to the patients´ numbers defined in Table S3. Results are obtained using multi-color flow cytometry and are expressed as staining index as described in the supplement and were graded using the following score: −, 0-1.3; +/−, 1.31-3; +, 3.01-10; ++, >10. **(B)** Mononuclear cells from 1 patient with AML were incubated in RPMI 1640 medium and 10% FCS in the absence or presence of G-CSF, IL-3, or EPO at 37°C for 48 hours. Thereafter, cells were examined for apoptosis induction in CD34$^+$/CD38$^-$ (left panel) and CD34$^+$/CD38$^+$ cells (right panel) using AnnexinV/DAPI staining and flow cytometry. Results are expressed as histograms and show the percent of AnnexinV$^+$/DAPI$^-$ cells. Abbreviations: AML, acute myeloid leukemia; DAPI, 4′,6-diamidino-2-phenylindole; EPO, Erythropoetin; G-CSF, granulocyte colony stimulating factor; IL, interleukin; SCF, stem cell factor; D, diagnosis.

Figure S7



(A, B) Expression of surface antigens on $CD34^+/CD38^-$ cells (blue boxes) and $CD34^+/CD38^+$ cells (grey boxes) in patients with **(A)** acute myeloid leukemia (AML) and **(B)** chronic myeloid leukemia (CML) (both at diagnosis and at relapse). Cells were stained with antibodies against CD123 (IL-3RA) and ROBO4. Expression of these surface antigens on $CD34^+/CD38^-$ and $CD34^+/CD38^+$ cells was determined by multi-color flow cytometry as described in the supplement. The dotted line represents clear expression (staining index >3). The range of each box represents the 25th (lower box margin) to 75th (upper box margin) percentile. The middle line inside the boxes represents the median expression level. Asterisk (*): $p<0.05$ compared to data obtained with $CD34^+/CD38^-$ BM cells in diagnostic samples. Pairwise Wilcoxon rank-sum test and p values were adjusted for multiple comparison according to Benjamini-Hochberg.

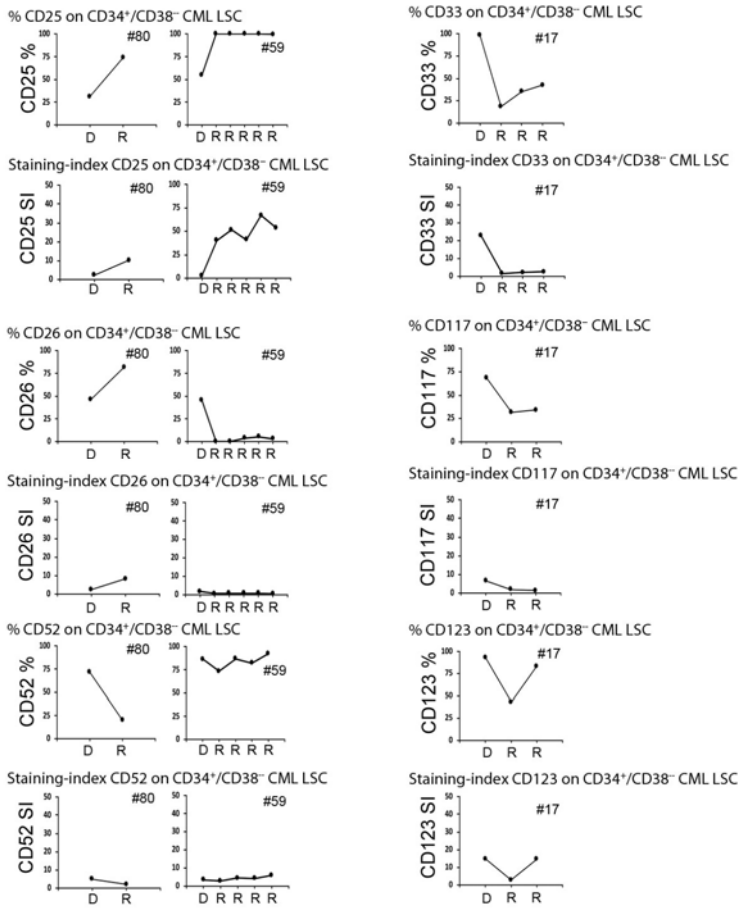
Figure S8A


% CD52 on CD34+/CD38- AML LSC
CD52 %
#131
#22
#5
#170
#104
#28
#130
#111
#192
Staining-index CD52 on CD34+/CD38- AML LSC
CD52 SI
#131
#22
#5
#170
#104
#28
#130
#111
#192
% CD25 on CD34+/CD38- AML LSC
CD25 %
#131
#170
#104
#28
#130
#111
#192
Staining-index CD25 on CD34+/CD38- AML LSC
CD25 SI
#131
#170
#104
#28
#130
#111
#192
% CD123 on CD34+/CD38- AML LSC
CD123 %
#49
#171
#168
#243
#28
#111
#192
Staining-index CD123 on CD34+/CD38- AML LSC
CD123 SI
#49
#171
#168
#243
#28
#111
#192
% CD117 on CD34+/CD38- AML LSC
CD117 %
#49
#171
#28
#192
Staining-index CD117 on CD34+/CD38- AML LSC
CD117 SI
#49
#171
#28
#192
% CD33 on CD34+/CD38- AML LSC
CD33 %
#49
#171
#168
#243
Staining-index CD33 on CD34+/CD38- AML LSC
CD33 SI
#49
#171
#168
#243
% IL-1RAP on CD34+/CD38- AML LSC
IL-1RAP %
#28
#111
#192
Staining-index IL-1RAP on CD34+/CD38- AML LSC
IL-1RAP SI
#28
#111
#192
D
R

Expression of surface markers on CD34$^+$/CD38$^-$ AML LSC at the time of diagnosis (D) and relapse (R)

Multi-color flow cytometry was used to analyze the expression of various surface markers on CD34$^+$/CD38$^-$ AML LSC at the time of diagnosis and relapse samples. Each diagram represents a single patient. Expression levels for each marker analyzed are shown in percent (upper row) and as SI (lower row). Patients´ numbers (#) refer to the patients´ numbers defined in Table S3. Abbreviations: AML, acute myeloid leukemia; LSC, leukemic stem cells; SI, staining index; D, diagnosis; R, relapse.

Figure S8B

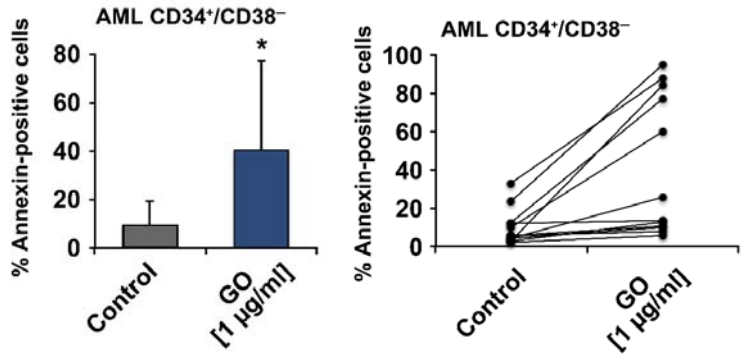


Expression of surface markers on CD34$^+$/CD38$^-$ CML LSC at the time of diagnosis (D) and relapse (R)

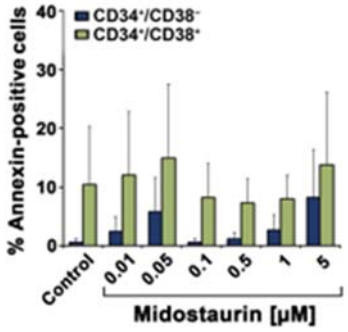
Multi-color flow cytometry was used to analyze the expression of various surface markers on CD34$^+$/CD38$^-$ CML LSC at the time of diagnosis and relapse samples. Each diagram represents a single patient. Expression levels for each marker analyzed are shown in percent (upper row) and as SI (lower row). Patients´ numbers (#) refer to the patients´ numbers defined in Table S2. Abbreviations: CML, chronic myeloid leukemia; LSC, leukemic stem cells; SI, staining index; D, diagnosis; R, relapse.

Figure S9

A



B

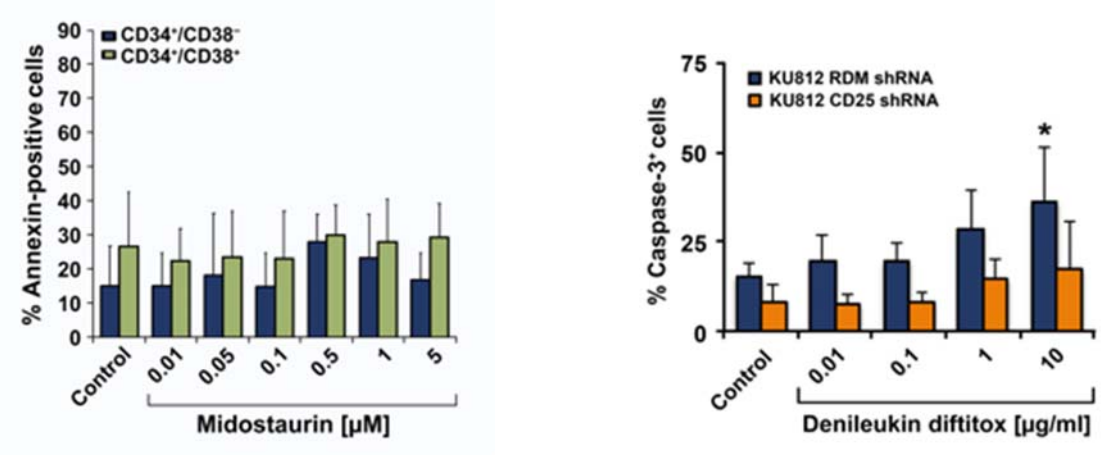


Effects of targeted drugs on survival of leukemic stem cells (LSC) in AML

(A) Mononuclear cells (MNC) from 12 patients with AML (samples obtained at diagnosis or relapse) were incubated in RPMI 1640 medium and 10% fetal calf serum (FCS) in the absence (control, grey bar) or presence of GO, 1 µg/ml (blue bar) at 37°C for 48 hours. Thereafter, apoptosis was analyzed in $CD34^+/CD38^-$ AML LSC using AnnexinV/DAPI staining and flow cytometry. Results are expressed as percent of $AnnexinV^+/DAPI^-$ cells and represent the mean±S.D. from 12 experiments. Asterisk (*): $p<0.05$ compared to control. Results are expressed as bar plot (left panel) or line plot (right panel). **(B)** MNC from 3 patients with AML (sample obtained at diagnosis) were incubated with various concentrations of midostaurin at 37°C for 48 hours. Then, apoptosis was analyzed in $CD34^+/CD38^-$ AML LSC (blue bars) and $CD34^+/CD38^+$ progenitor cells (green bars) using AnnexinV/DAPI staining and flow cytometry. Results are expressed as percent of $AnnexinV^+/DAPI^-$ cells and represent the mean±S.D. from 3 experiments. Abbreviations: AML, acute myeloid leukemia; GO, gemtuzumab/ozogamicin.

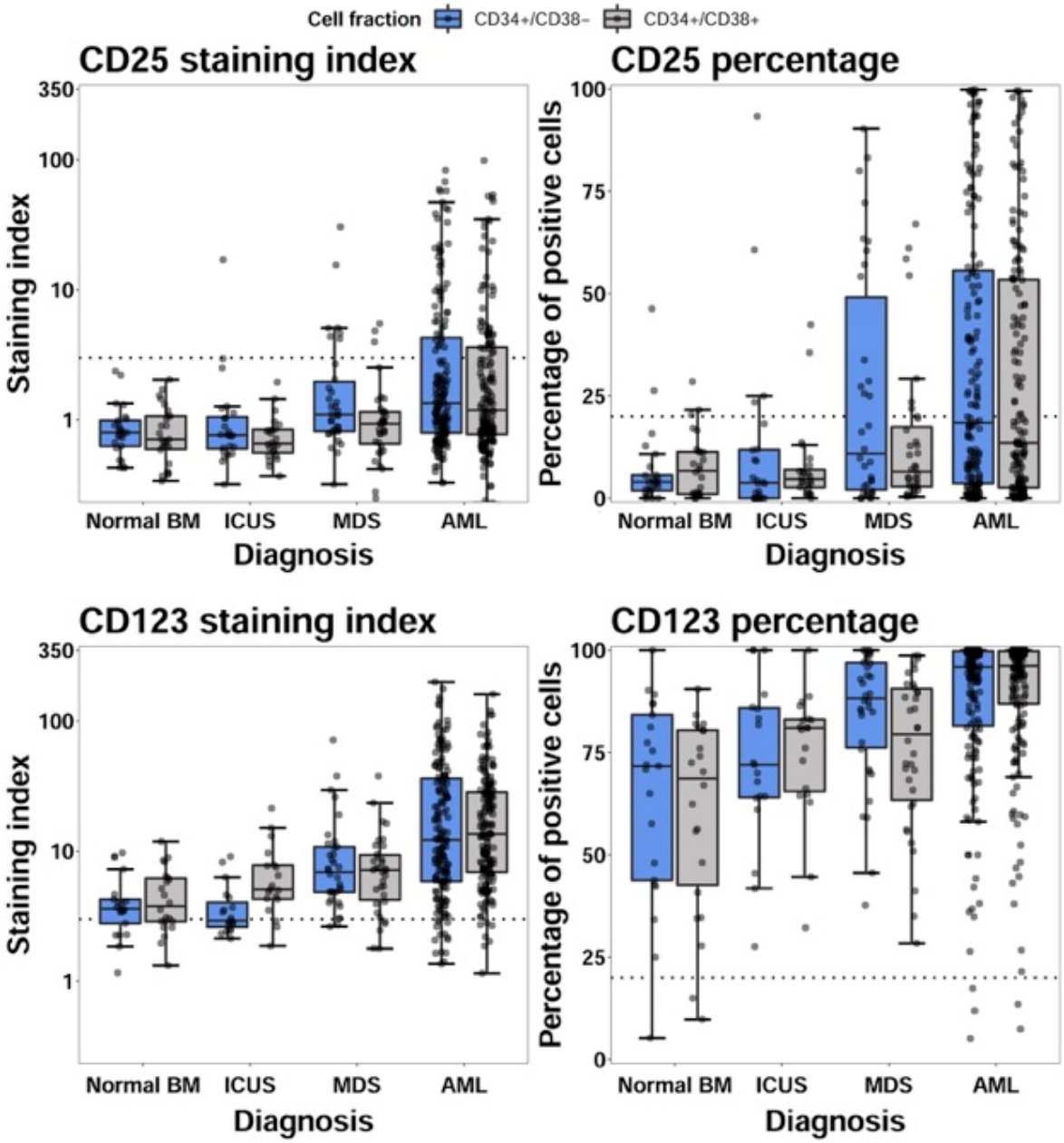
Figure S10

A **B**



Effects of target drugs on apoptosis induction in CML LSC and KU812 cells
(A) Mononuclear cells (MNC) from 5 patients with CML (samples obtained at diagnosis or relapse) were incubated with various concentrations of midostaurin at 37°C for 48 hours. Then, apoptosis was analyzed in $CD34^+/CD38^-$ AML LSC (blue bars) and CD34+/$CD38^+$ progenitor cells (green bars) using AnnexinV/DAPI staining and flow cytometry. Results are expressed as percent of $AnnexinV^+/DAPI^-$ cells and represent the mean±S.D. from 5 experiments. **(B)** KU812 cells transduced with a random control (RDM) shRNA (blue bars) or a CD25 shRNA (orange bars) were incubated in control medium (control) or in various concentrations of denileukin-diftitox at 37°C for 48 hours. Thereafter, cells were analyzed for apoptosis induction by caspase-3 staining and flow cytometry. Results are expressed as percent of caspase-3^+ cells and represent the mean±S.D. from 4 experiments. Asterisk (*): $p<0.05$ compared to control. Abbreviations: shRNA, short hairpin RNA; LSC, leukemic stem cells.

Figure S11



Expression of CD25 and CD123 on preleukemic stem cells in MDS

Boxblots show the expression of CD25 (upper panels) and CD123 (lower panels) on $CD34^{+}/CD38^{-}$ (blue boxes) and $CD34^{+}/CD38^{+}$ (grey boxes) in normal BM, ICUS, MDS and AML. Results are expressed as staining index (left panels) or percentage of positive cells (right panels). The dotted line represents clear expression (staining index is higher than 3 or more than 20% positive cells). The range of each box represents the 25^{th} (lower box margin) to 75^{th} (upper box margin) percentile. The middle line inside the boxes represent the median expression level. Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; ICUS, idiopathic cytopenia of undetermined significance; MDS, myelodysplastic syndrome.

Figure S12

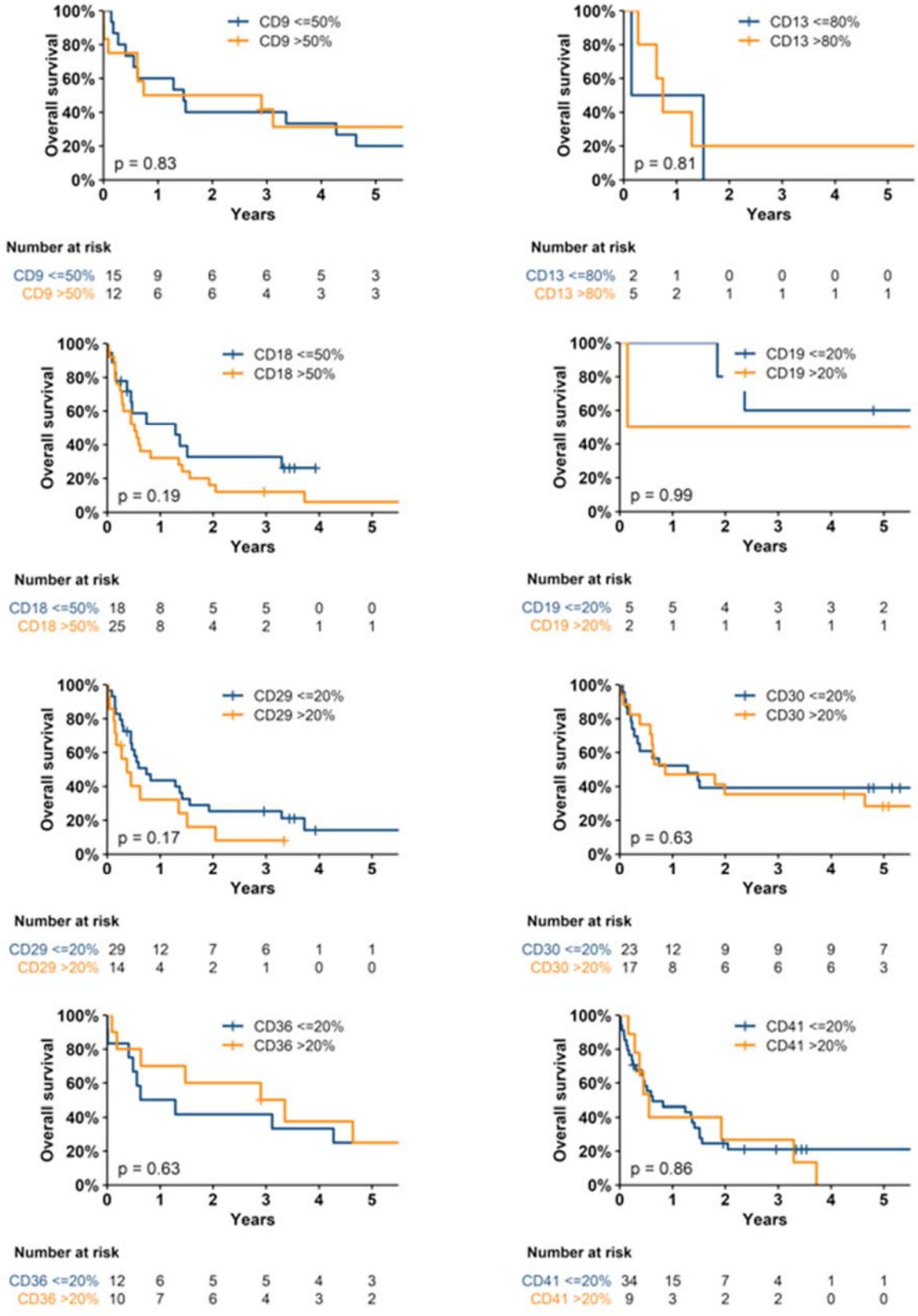
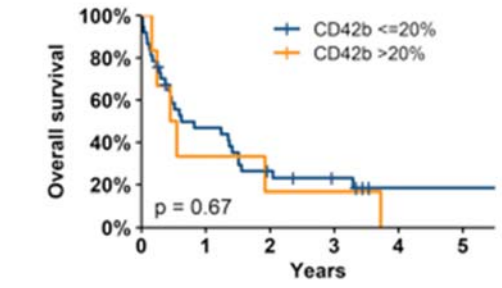
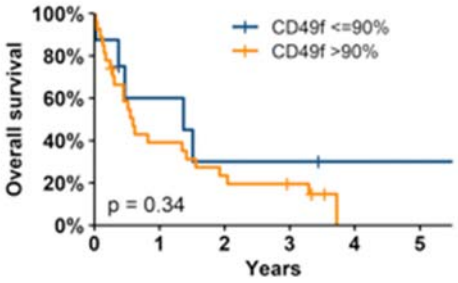

CD9 <=50%
CD9 >50%
Overall survival
p = 0.83
Years
Number at risk
CD9 <=50% 15 9 6 6 5 3
CD9 >50% 12 6 6 4 3 3
CD13 <=80%
CD13 >80%
p = 0.81
CD13 <=80% 2 1 0 0 0 0
CD13 >80% 5 2 1 1 1 1
CD18 <=50%
CD18 >50%
p = 0.19
CD18 <=50% 18 8 5 5 0 0
CD18 >50% 25 8 4 2 1 1
CD19 <=20%
CD19 >20%
p = 0.99
CD19 <=20% 5 5 4 3 3 2
CD19 >20% 2 1 1 1 1 1
CD29 <=20%
CD29 >20%
p = 0.17
CD29 <=20% 29 12 7 6 1 1
CD29 >20% 14 4 2 1 0 0
CD30 <=20%
CD30 >20%
p = 0.63
CD30 <=20% 23 12 9 9 9 7
CD30 >20% 17 8 6 6 6 3
CD36 <=20%
CD36 >20%
p = 0.63
CD36 <=20% 12 6 5 5 4 3
CD36 >20% 10 7 6 4 3 2
CD41 <=20%
CD41 >20%
p = 0.86
CD41 <=20% 34 15 7 4 1 1
CD41 >20% 9 3 2 2 0 0

Figure S12 continued



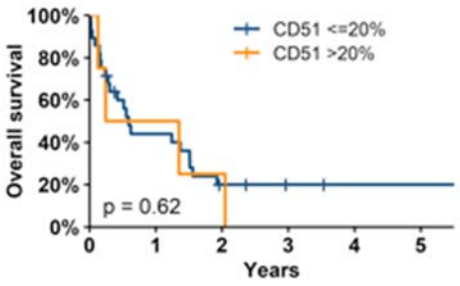
Number at risk

CD42b <=20%	37	16	8	5	1	1
CD42b >20%	6	2	1	1	0	0



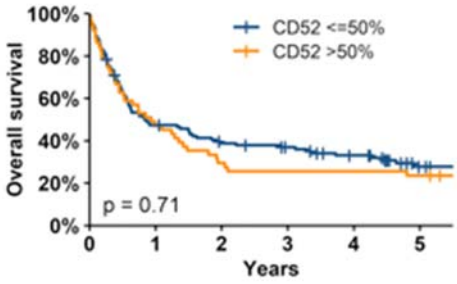
Number at risk

CD49f <=90%	8	4	2	2	1	1
CD49f >90%	27	10	6	4	0	0



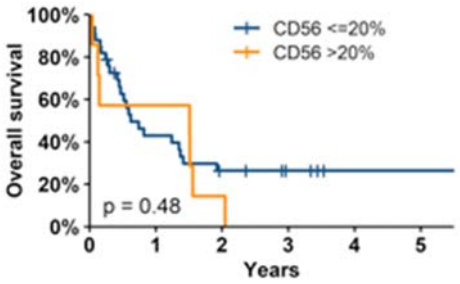
Number at risk

CD51 <=20%	28	11	4	2	1	1
CD51 >20%	4	2	1	0	0	0



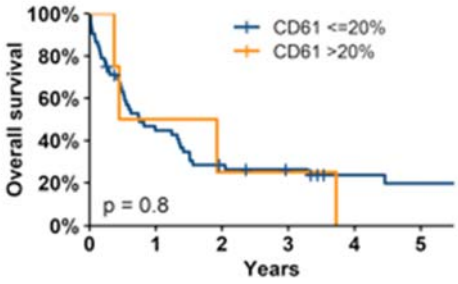
Number at risk

CD52 <=50%	121	56	44	39	31	16
CD52 >50%	51	24	15	13	13	12



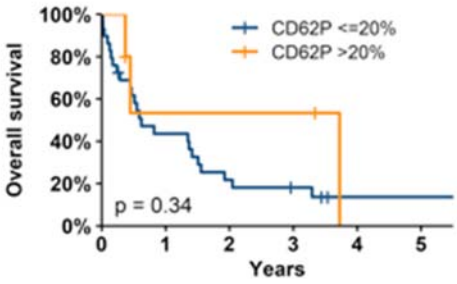
Number at risk

CD56 <=20%	33	13	7	4	1	1
CD56 >20%	7	4	1	0	0	0



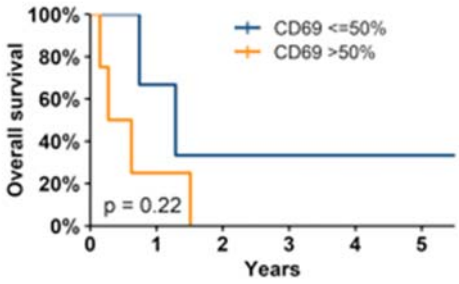
Number at risk

CD61 <=20%	52	22	13	10	6	5
CD61 >20%	4	2	1	1	0	0



Number at risk

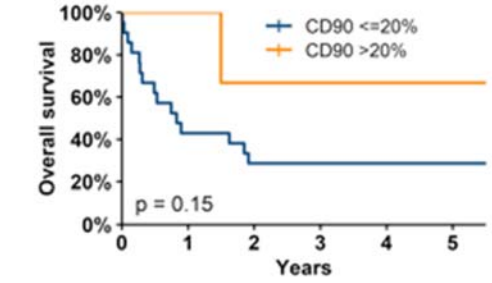
CD62P <=20%	29	12	6	4	1	1
CD62P >20%	5	2	2	2	0	0



Number at risk

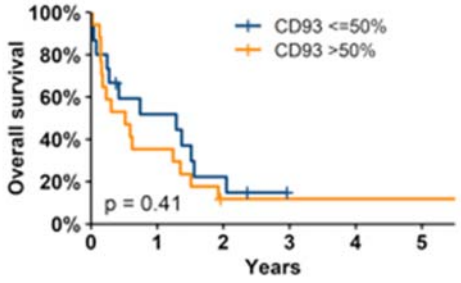
CD69 <=50%	3	2	1	1	1	1
CD69 >50%	4	1	0	0	0	0

Figure S12 continued



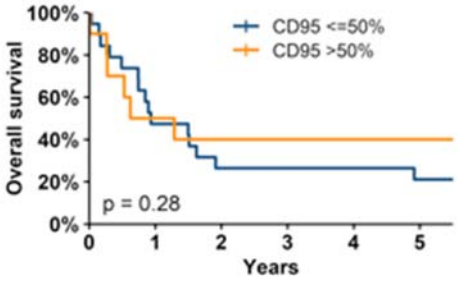
Number at risk

CD90 <=20%	21	9	6	6	6	6
CD90 >20%	3	3	2	2	2	2



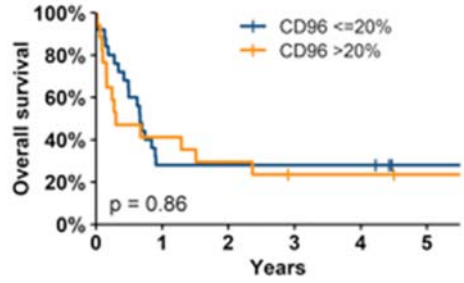
Number at risk

CD93 <=50%	15	7	3	0	0	0
CD93 >50%	17	6	1	1	1	1



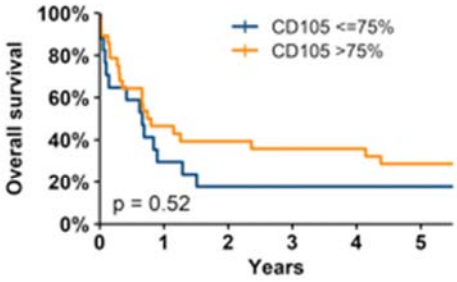
Number at risk

CD95 <=50%	19	9	5	5	5	4
CD95 >50%	10	5	4	4	4	4



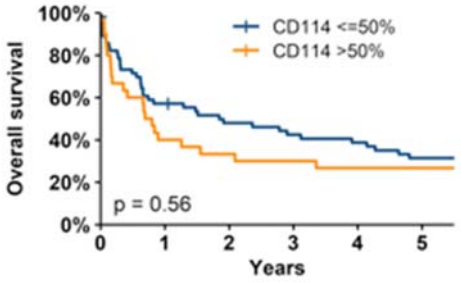
Number at risk

CD96 <=20%	25	7	7	7	7	4
CD96 >20%	17	7	5	3	3	2



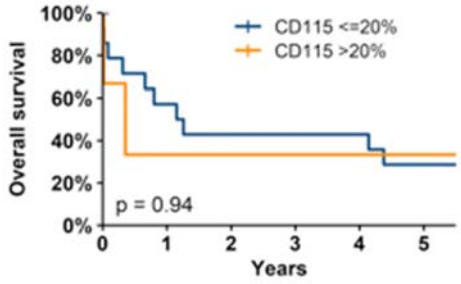
Number at risk

CD105 <=75%	17	5	3	3	3	3
CD105 >75%	28	13	11	10	10	8



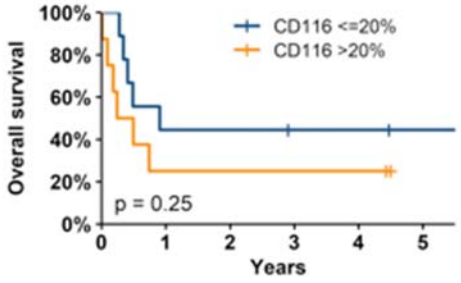
Number at risk

CD114 <=50%	57	32	26	23	21	17
CD114 >50%	30	12	10	9	8	8



Number at risk

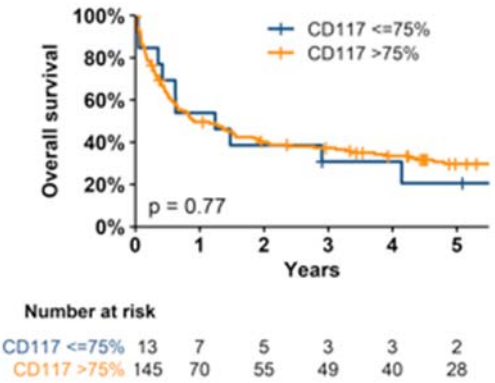
CD115 <=20%	15	8	6	6	6	4
CD115 >20%	3	1	1	1	1	1

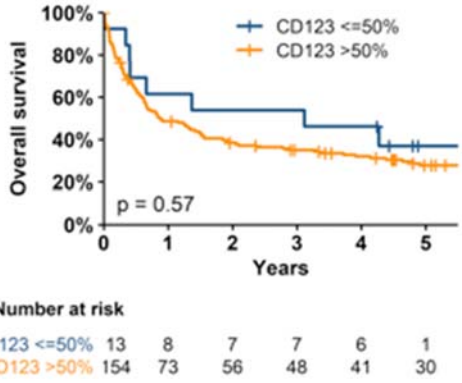


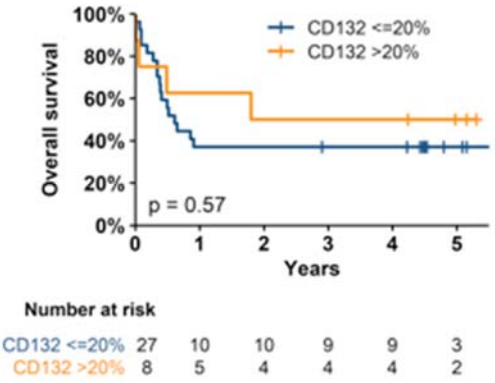
Number at risk

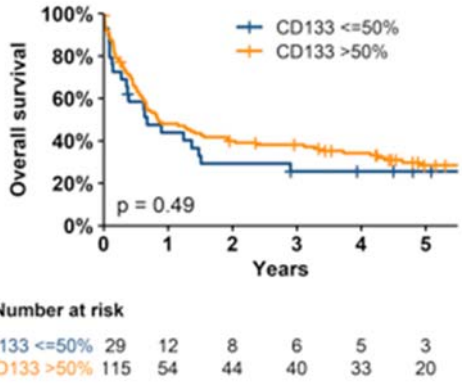
CD116 <=20%	9	4	4	3	3	2
CD116 >20%	8	2	2	2	2	0

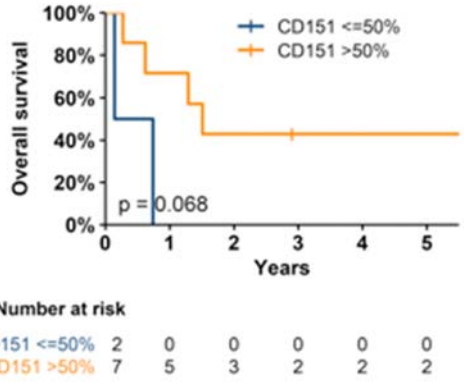
Figure S12 continued

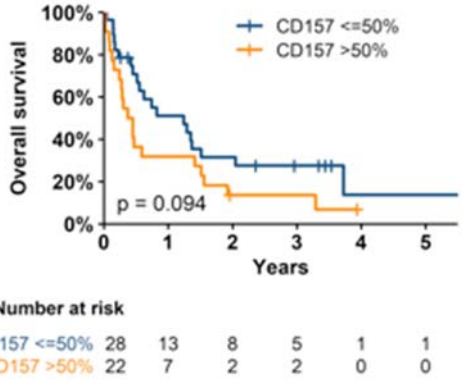


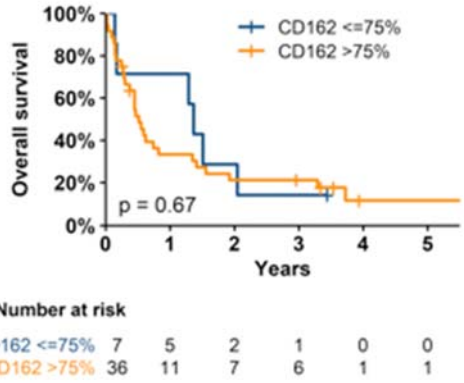












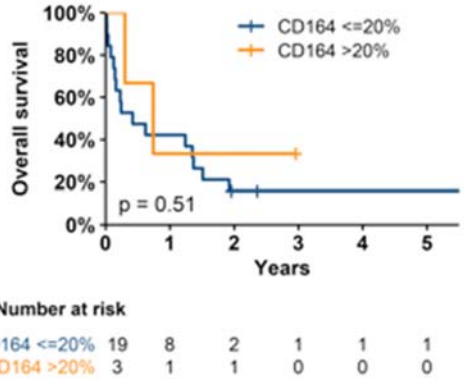


Figure S12 continued

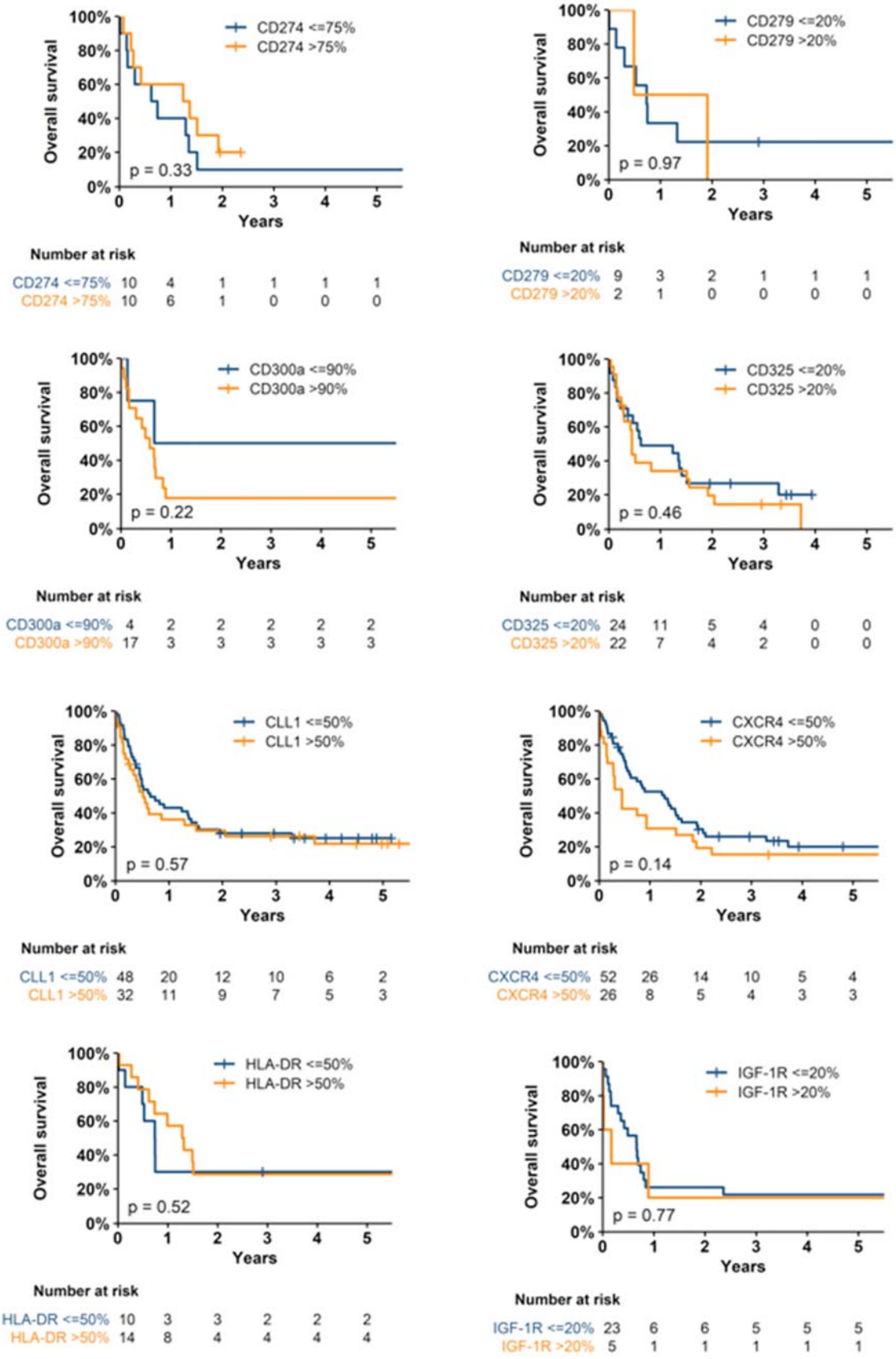
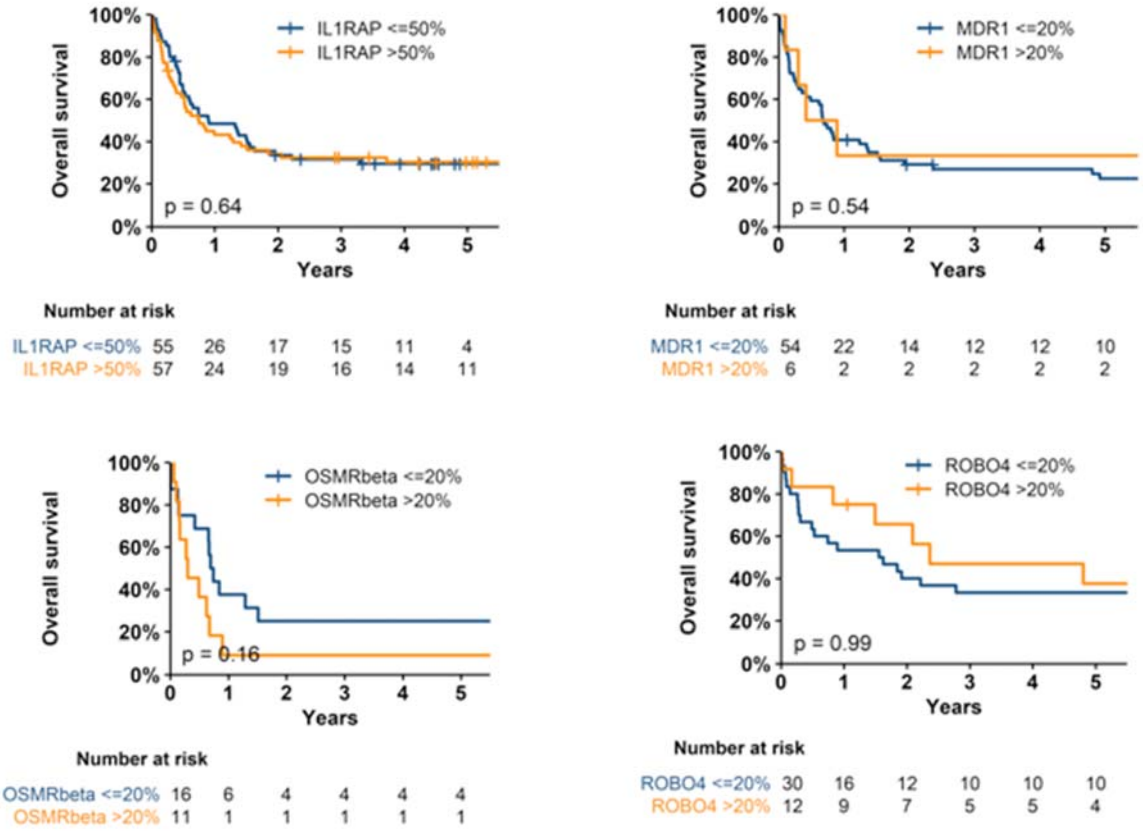


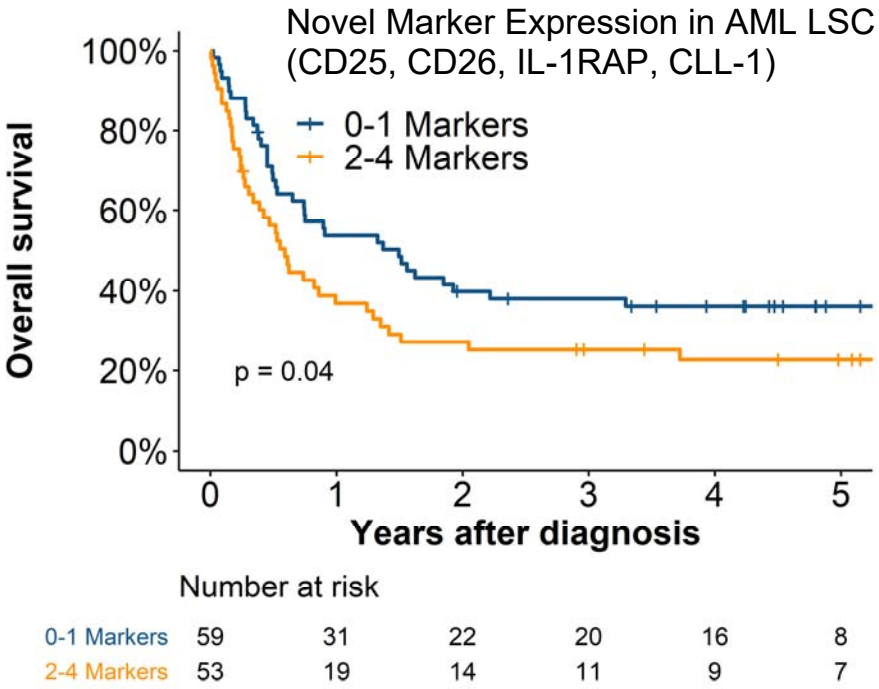
Figure S12 continued



Overall survival of patients with AML

Patients were split into two groups per marker based on higher (yellow graphs) or lower (blue graphs) expression of surface antigens on $CD34^+/CD38^-$ cells as indicated in each subpanel. The thresholds of expression were selected based on patient distribution and formation of subsets in flow cytometry experiments (see supplement). Overall survival of patients with AML was calculated according to the method of Kaplan and Meier. p values were calculated by log-rank test.

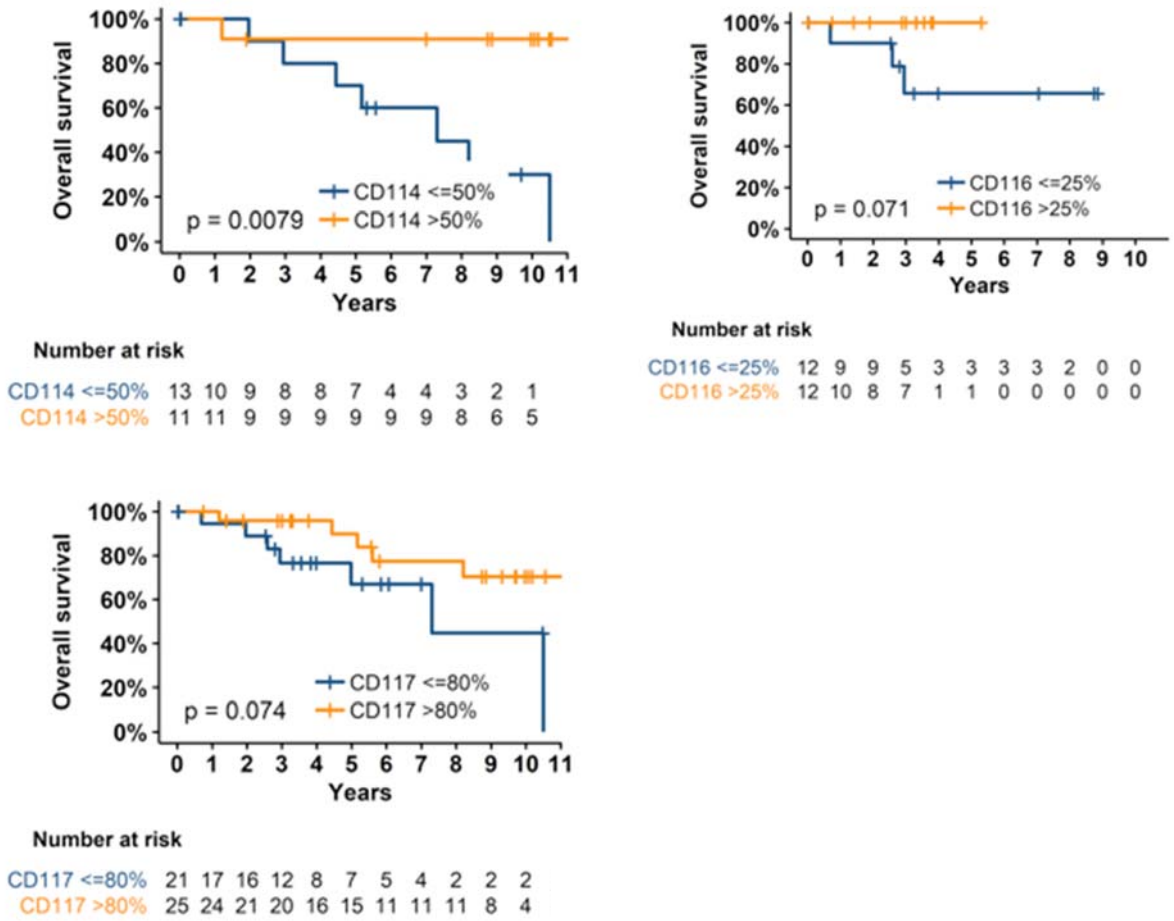
Figure S13



Influence of the numbers of aberrantly expressed surface markers in LSC on overall survival in patients with AML

A total number of 112 patients with AML were included. Patients were split into two groups based on aberrant expression of surface markers on $CD34^{+}/CD38^{-}$ LSC: group A (blue graph: one or no marker detected on LSC) and B (yellow graph: two to four markers detected on LSC). The following aberrantly expressed markers were included in these analyses: CD25 (IL-2RA), CD26 (DPPIV), CD371 (CLL-1), and IL1-RAP. Expression thresholds of markers selected to define subsets was based on patient distribution and formation of sizable subsets. Only patients in whom at least 3 markers were analyzed were included. Overall survival was calculated according to the method of Kaplan and Meier. The p value was calculated by log-rank test.

Figure S14



Overall survival of patients with CML

Patients were split into two groups per marker based on higher (yellow graphs) or lower (blue graphs) expression of surface antigens on $CD34^{+}/CD38^{-}$ cells as indicated in each subpanel. The thresholds of expression were selected based on patient distribution and formation of subsets in flow cytometry experiments (see supplement). Samples were obtained from patients with CML in CP, AP and BP. Overall survival of patients with CML was calculated according to the method of Kaplan and Meier. p values were calculated by log-rank test. Abbreviations: CML, chronic myeloid leukemia; CP, chronic phase; AP, accelerated phase; BP, blast phase.

Supplemental (S) Tables

Table S1

Specification of antibodies used in multicolor flow cytometry experiments

CD	Antigen	Clone	Conjugate	Species, Isotype	Manufacturer
n.c.	Isotype control	MOPC-21	PE	Mouse, IgG1	BD Biosciences
n.c.	Isotype control	20102	PE	Mouse, IgG2A	R&D Systems
n.c.	Isotype control	X39	FITC	Mouse, IgG2A	BD Biosciences
n.c.	Isotype control	133303	PE	Mouse, IgG2B	R&D Systems
n.c.	Isotype control	polyclonal	biotinylated	Sheep, IgG	R&D Systems
CD3	TcR	UCHT1	APC	Mouse, IgG1	BD Biosciences
CD9	Tetraspanin 29	M-L13	PE	Mouse, IgG1	BD Biosciences
CD11a	Integrin α-L	G43-25B	PE	Mouse, IgG2A	BD Biosciences
CD13	ANPEP	WM15	PE	Mouse, IgG1	BD Biosciences
CD14	LPSR	TÜK4	FITC	Mouse, IgG2A	Dako
CD18	Integrin β2	6.7	PE	Mouse, IgG1	BD Biosciences
CD19	B4	4G7	FITC	Mouse, IgG1	BD Biosciences
CD19	B4	4G7	PE	Mouse, IgG1	BD Biosciences
CD20	MS4A1	L27	PE	Mouse, IgG1	BD Biosciences
CD23	FcεR2	M-L233	PE	Mouse, IgG1	BD Biosciences
CD25	IL2Rα	2A3	PE	Mouse, IgG1	BD Biosciences
CD26	DPPIV	M-A261	PE	Mouse, IgG1	BD Biosciences
CD29	Integrin β1	419217	PE	Mouse, IgG2B	R&D Systems
CD30	TNFRSF8	BerH8	PE	Mouse, IgG1	BD Biosciences
CD33	Siglec-3	WM53	PE	Mouse, IgG1	BD Biosciences
CD34	HPCA-1	581	FITC	Mouse, IgG1	BD Biosciences
CD34	HPCA-1	581	FITC	Mouse, IgG1	BioLegend
CD34	HPCA-1	581	PE	Mouse, IgG1	BD Biosciences
CD34	HPCA-1	581	pacific blue	Mouse, IgG1	BioLegend
CD36	TSPR	5-271	PE	Mouse, IgG2A	BioLegend
CD38	T10	HIT2	APC	Mouse, IgG1	BD Biosciences
CD41	Integrin α2b	HIP8	PE	Mouse, IgG1	BioLegend
CD42b	GP1Bα	HIP1	PE	Mouse, IgG1	BioLegend
CD43	Sialophorin	1G10	PE	Mouse, IgG1	BD Biosciences
CD44	Hermes	515	PE	Mouse, IgG1	BD Biosciences
CD45	LCA	2D1	APC-H7	Mouse, IgG1	BD Biosciences
CD45	LCA	2D1	PerCP	Mouse, IgG1	BD Biosciences
CD45	LCA	HI30	V500	Mouse, IgG1	BD Biosciences
CD47	MER6	B6H12	PE	Mouse, IgG1	BD Biosciences
CD49f	Integrin α6	GoH3	PE	Rat, IgG2a	BioLegend

CD	Antigen	Clone	Conjugate	Species, Isotype	Manufacturer
CD51	Integrin αV	NKI-M9	PE	Mouse, IgG2A	BioLegend
CD51/61	Integrin αV/β3	23C6	PE	Mouse, IgG1	BioLegend
CD52	CAMPATH-1	HI186	PE	Mouse, IgG2B	BioLegend
CD56	NCAM1	NCAM16.2	PE	Mouse, IgG2B	BD Biosciences
CD61	Integrin β3	VI-PL2	PE	Mouse, IgG1	BioLegend
CD62P	SELP	AK-4	PE	Mouse, IgG1	eBioscience
CD69	CLEC2C	FN50	PE	Mouse, IgG1	BioLegend
CD90	Thy-1	5E10	PE	Mouse, IgG1	BD Biosciences
CD93	MXRA4	VIMD2	PE	Mouse, IgG1	BioLegend
CD95	FAS	DX2	PE	Mouse, IgG1	BD Biosciences
CD96	Tactile	NK92.39	PE	Mouse, IgG1	eBioscience
CD97	ADGRE5	VIM3b	PE	Mouse, IgG1	BioLegend
CD99	MIC2	3B2/TA8	PE	Mouse, IgG2A	eBiosciences
CD105	Endoglin	166707	PE	Mouse, IgG1	R&D Systems
CD110	TPOR	167639	PE	Mouse, IgG2A	R&D Systems
CD114	G-CSFR	LMM741	PE	Mouse, IgG1	BD Biosciences
CD115	M-CSFR	61708	PE	Mouse, IgG1	R&D Systems
CD116	GM-CSFRα	hGMCSFR-M1	PE	Mouse, IgG1	BD Biosciences
CD117	SCFR/KIT	104D2	PE	Mouse, IgG1	BD Biosciences
CD122	IL-2Rβ	Mik-β3	PE	Mouse, IgG1	BD Biosciences
CD123	IL-3Rα	32703	PE	Mouse, IgG1	R&D Systems
CD123	IL-3Rα	7G3	PE	Mouse, IgG2A	BD Biosciences
CD123	IL-3Rα	AC145	APC	Mouse, IgG2A	Miltenyi Biotec
CD129	IL-9R	33423	PE	Mouse, IgG1	R&D Systems
CD131	IL-3Rβ	1C1	PE	Mouse, IgG1	Abcam
CD132	IL-2Rγ	AG184	PE	Mouse, IgG1	BD Biosciences
CD133	Prominin 1	AC133	PE	Mouse, IgG1	Miltenyi Biotec
CD134	OX40	ACT35	PE	Mouse, IgG1	BD Biosciences
CD135	FLT3	BV10A4H2	PE	Mouse, IgG1	Biolegend
CD150	SLAMF1	7D4	PE	Mouse, IgG1	BioLegend
CD151	RAPH	PETA-3	PE	Mouse, IgG1	BD Biosciences
CD157	BST1	SY/11B5	PE	Mouse, IgG1	BD Biosciences
CD162	Selectin P ligand	KPL-1	PE	Mouse, IgG1	BD Biosciences
CD164	Sialomucin	67D2	PE	Mouse, IgG1	BioLegend
CD167a	DDR1	51D6	PE	Mouse, IgG3	BioLegend
CD184	CXCR4	12G5	PE	Mouse, IgG2A	BD Biosciences
CD203c	ENPP3	97A6	PE	Mouse, IgG1	Immunotech
CD206	MMR	15-2	PE	Mouse, IgG1	BioLegend
CD221	IGF-1R	33255	PE	Mouse, IgG1	R&D Systems
CD243	MDR1	15D3	PE	Mouse, IgG1	BD Biosciences
CD252	OX40L	ik-1	PE	Mouse, IgG1	BD Biosciences
CD271	NGF-R	C40-1457	PE	Mouse, IgG1	BD Biosciences
CD273	PD-L2	MIH18	PE	Mouse, IgG1	BioLegend

CD	Antigen	Clone	Conjugate	Species, Isotype	Manufacturer
CD274	PD-L1	29E.2A3	PE	Mouse, IgG2B	BioLegend
CD279	PD1	EH12.2H7	PE	Mouse, IgG1	BioLegend
CD300a	CMRF-35-H9	MEM-260	PE	Mouse, IgG1	Abcam
CD309	KDR/VEGFR-2	89106	PE	Mouse, IgG1	R&D Systems
CD321	JAM-1	M.Ab.F11	PE	Mouse, IgG1	BD Biosciences
CD325	Cadherin 2	8C11	PE	Mouse, IgG1	BD Biosciences
CD332	FGFR2	98725	PE	Mouse, IgG1	R&D Systems
CD371	CLL-1/CLEC12A	50C1	PE	Mouse, IgG2A	BD Biosciences
n.c.	EPOR	38409	PE	Mouse, IgG2B	R&D Systems
n.c.	HLA-DR	G46-6	PE	Mouse, IgG2A	BD Biosciences
n.c.	IL-1RAP	89412	PE	Mouse, IgG1	R&D Systems
n.c.	MET/HGFR	95106	PE	Mouse, IgG1	R&D Systems
n.c.	NPDC1	polyclonal	biotinylated	Sheep, IgG	R&D Systems
n.c.	OSMRβ	AN-U2	PE	Mouse, IgG1	Biotechnology
n.c.	ROBO4	265703	PE	Mouse, IgG2A	R&D Systems

Abbreviations: CD, cluster of differentiation; PE, phycoerythrin; FITC, fluorescein isothiocyanate; PerCP, peridinin chlorophyll protein; APC, allophycocyanin; n.c., not (yet) clustered; Ig, immunoglobulin; TcR, T cell receptor; LPSR, lipopolysaccharide-related antigen; IL-2RA, interleukin-2 receptor alpha chain; DPPIV, dipeptidylpeptidase IV; HPCA-1, human precursor cell antigen-1; LCA, leukocyte common antigen; G-CSF, granulocyte colony-stimulating factor; SCF, stem cell factor; NGF, nerve growth factor; CLL-1, C-type lectin-like molecule-1; IL-1RAP, interleukin-1 receptor accessory protein; ROBO4, roundabout 4; EPOR, erythropoietin receptor; CXCR, C-X-C chemokine receptor; FGFR, fibroblast growth factor receptor; HGFR, hepatocyte growth factor receptor; HLA, human leucocyte antigen; NPDC1, neural proliferation differentiation and control protein 1; VEGFR, vascular endothelial growth factor receptors; NCAM, Neural cell adhesion molecule.
Company Locations: BD Bioscience, San José, CA, USA; R&D Systems, Minneapolis, MN, USA; Dako, Glostrup, Denmark; eBioscience, San Diego, CA, USA; Miltenyi Biotec, Bergisch Gladbach, Germany; BioLegend, San Diego, CA, USA; Santa Cruz Biotechnology, Dallas, TX, USA; Abcam, Cambridge, UK.

Table S2

Patients´ characteristics – chronic myeloid leukemia (CML)

No. #	age (yrs)	gender (m/f)	diagnosis - CML phase	karyotype	WBC (G/L)	Hb (g/dL)	PLT (G/L)	basophils (%) PB	blasts (%) PB	blasts (%) BM	*BCR-ABL1* mutations
1	52	m	CP	46,XY,t(9;22)	45.7	14.7	577	10	0	1	n.t.
2	57	f	CP	46,XX,t(9;22)	68.7	12.6	818	7	0	1	n.t.
3	41	f	CP	46,XX,t(9;22)	30.4	9.6	1172	19	1	1	n.t.
4	65	m	AP	46,XY,t(9;22)	273.6	7.7	470	8	10	6	n.t.
5	49	m	CP	46,XY,t(9;22)	232.8	9.3	1062	6	3	1	n.t.
6	42	f	CP	46,XX,t(9;22)	181.9	14.1	148	4	1	2	n.t.
7	37	m	CP	46,XY,t(9;22)	396.0	9.3	225	4	5	3	n.t.
8	24	f	CP	46,XX,t(9;22)	77.0	12.3	363	5	2	1	n.t.
9*	47	m	BP	complex	3.2	7.9	30	0	35	97	G250E, E355A
10	60	m	CP	46,XY,t(9;22)	47.9	14.3	261	3	0	1	n.t.
11	57	f	CP	46,XX,t(9;22)	398.9	8.6	165	7	1	1	n.t.
12*	38	f	BP	complex	3.5	9.6	18	0	52	30	n.t.
13	68	f	CP	46,XX,t(9;22)	97.8	10.9	450	6	1	1	n.t.
14	63	m	CP	45,X,-Y,t(9;22)	93.5	13.1	305	1	1	1	n.t.
15	80	f	CP	46,XX,t(9;22)	96.3	10.5	477	1	0	1	n.t.
16	67	m	CP	46,XY,t(9;22)	35.9	9.1	186	2	0	2	n.t.
17	39	m	AP	46,XY,t(9;22),t(8;17)	190.9	11.0	347	15	3	2	n.t.
18	82	f	CP	46,XX,t(9;22)	32.6	13.6	326	5	0	1	n.t.
19	19	m	CP	46,XY,t(9;22)	175.8	8.7	282	3	2	1	n.t.
20	59	m	CP	46,XY,t(9;22)	83.7	13.5	297	4	2	3	n.t.
21	46	f	CP	46,XX,t(9;22)	570.5	7.5	342	3	1	2	n.t.
22	22	f	CP	46,XX,t(9;22)	26.6	13.4	354	5	0	1	n.t.
23*	49	m	CP	46,XY,t(9;11;22)	34.5	14.0	145	1	0	1	n.t.
24	53	m	CP	46,XY,t(9;22)	54.2	12.9	302	4	0	1	n.t.
25	73	f	CP	46,XX,t(9;22)	46.8	8.8	1060	7	1	1	n.t.

No. #	age (yrs)	gender (m/f)	diagnosis - CML phase	karyotype	WBC (G/L)	Hb (g/dL)	PLT (G/L)	basophils (%) PB	blasts (%) PB	blasts (%) BM	*BCR-ABL1* mutations
26	57	m	CP	46,XY,t(9;22)	271.5	9.2	607	8	2	2	n.t.
27	33	m	CP	46,XY,t(9;22)	142.1	10.5	822	6	6	3	n.t.
28	72	m	CP	46,XY,t(9;22)	179.1	11.1	83	4	1	2	n.t.
29	84	f	BP	complex	149.8	5.5	62	1	63	83	n.t.
30	36	f	CP	46,XX,t(9;10;22)	193.8	11.5	222	4	1	2	n.t.
31	71	m	CP	46,XY,t(9;22)	52.5	15.0	340	4	2	2	n.t.
32	57	f	CP	46,XX,t(9;22)	39.1	12.1	150	1	0	1	n.t.
33	55	m	CP	46,XY; (FISH: BCR-ABL)	33.7	12.9	417	5	0	4	n.t.
34	29	m	CP	46,XY,t(9;22)	96.8	12.9	174	2	1	1	n.t.
35	40	m	CP	46,XY,t(9;22)	13.0	13.9	601	1	0	1	n.t.
36	38	f	CP	46,XX,t(9;22)	27.3	12.7	412	5	0	1	n.t.
37	85	m	CP	46,XY,t(9;22)	45.7	12.4	330	2	1	2	n.t.
38*	44	m	BP	n.t.	74.0	8.0	33	33	44	n.t.	E255K
39	63	f	CP	46,XX,t(9;22)	43.5	11.7	265	19	1	4	n.t.
40*	74	f	BP	46,XX,t(9;22)	3.0	10.2	156	n.t.	6	43	none
41	46	f	CP	46,XX,t(9;22)	47.0	11.1	591	2	0	1	n.t.
42	33	m	CP	46,XY,t(9;22)	190.7	9.7	750	4	3	8	n.t.
43	35	m	AP	46,XY,t(9;22)	263	11.7	124	11	10	2	none
44*	63	f	CP	46,XX,t(9;22)	36.8	11.5	105	2	0	n.t.	V379I
45	28	f	CP	46,XX,t(9;22)	108.6	8.5	432	3	0	1	n.t.
46	68	f	CP	46,XX,t(9;22)	40.2	12.1	413	5	0	2	n.t.
47	27	f	AP	46,XX,t(9;22)	178.3	10.2	626	15	7	5	n.t.
48*	75	f	CP	47,XX,t(9;22),+der(22)t(9;22)	6.6	11.4	286	1	0	1	L248V
49	67	f	CP	46,XX,t(9;22)	37.6	10.5	1375	3	1	4	n.t.
50	57	f	CP	n.t.	143.7	11.5	204	4	3	4	n.t.
51	60	f	CP	46,XX,t(9;22)	39.8	13.1	793	9	1	3	n.t.
52	63	m	CP	46,XY,t(9;22)	46.5	11.8	419	4	1	2	n.t.
53	84	f	CP	46,XX,t(9;22)	67.6	12.8	305	2	0	1	n.t.

No. #	age (yrs)	gender (m/f)	diagnosis - CML phase	karyotype	WBC (G/L)	Hb (g/dL)	PLT (G/L)	basophils (%) PB	blasts (%) PB	blasts (%) BM	*BCR-ABL1* mutations
54	43	m	BP	46,XY,t(9;22)	44.6	9	769	31	20	23	n.t.
55	55	f	CP	46,XX,t(9;22)	541.2	7.6	782	3	7	2	n.t.
56*	48	f	BP	complex	71.0	11.9	49	0	34	11	none
57	57	m	CP	46,XY,t(9;22)	48.1	13.9	217	1	0	1	n.t.
58	45	f	CP	n.t.	31.9	12.9	411	3	0	1	n.t.
59	67	f	CP	46,XX,t(9;22)	332.2	10.3	568	4	4	4	n.t.
60	47	m	CP	46,XY,t(9;22)	55.7	13.9	250	1	1	3	n.t.
61	59	m	CP	46,XY,t(9;22)	222.1	10.5	789	8	1	2	n.t.
62	75	m	CP	n.t.	65.0	9.2	185	0	0	1	n.t.
63	33	m	CP	46,XY,t(9;22)	173.3	10.8	787	5	3	2	n.t.
64	46	m	CP	46,XY,t(9;22)	53.8	14	480	8	2	1	n.t.
65	66	m	CP	46,XY,t(9;22)	137.2	13.8	173	1	2	1	n.t.
66	52	m	CP	46,XY,t(9;22)	174.2	11.6	275	1	1	1	n.t.
67*	45	m	AP	46,XY,t(9;22)	31.1	9	306	0	6	4	n.t.
68	28	m	CP	46,XY,t(9;22)	23.7	13	209	1	0	1	n.t.
69	43	m	CP	46,XY; (FISH: BCR-ABL)	76.1	14	151	2	2	4	n.t.
70	34	f	CP	46,XX,t(9;22)	396.9	7.2	425	6	2	1	n.t.
71	72	m	CP	46,XY,t(2;10),t(9;22)	112.5	11.9	394	13	6	4	n.t.
72	60	f	BP	46,XX,t(9;22)	94.4	10.9	45	1	35	75	none
73	46	f	CP	46,XX,t(9;22)	184.0	10.1	700	1	1	1	n.t.
74	75	f	CP	46,XX,t(9;22)	34.2	12.8	911	6	0	1	n.t.
75	40	f	CP	46,XX,t(9;22)	24.4	13	643	8	0	1	n.t.
76	60	f	CP	46,XX,t(9;22)	22.4	13.2	319	5	0	1	n.t.
77	22	f	CP	46,XX,t(9;22)	230.6	10.4	720	5	1	1	n.t.
78*	69	m	AP	complex	1.7	9.5	112	7	4	8	Q252H
79	86	f	AP	46,XX,t(9;22)	230.0	8.9	783	4	11	3	n.t.
80	64	m	CP	46,XY,t(9;22)	23.9	13.3	164	1	0	1	n.t.
81*	73	m	CP	n.t.	169.5	7.8	205	3	2	n.t.	n.t.

No. #	age (yrs)	gender (m/f)	diagnosis - CML phase	karyotype	WBC (G/L)	Hb (g/dL)	PLT (G/L)	basophils (%) PB	blasts (%) PB	blasts (%) BM	*BCR-ABL1* mutations
82	44	f	CP	46,XX,t(9;22)	113.7	12.3	514	5	3	2	n.t.
83	54	m	CP	complex	290.4	12.4	441	3	2	2	n.t.
84*	23	m	CP	47,XY,+8,t(9;22)	127.7	11.7	93	1	1	1	none
85	65	m	CP	46,XY,t(9;22)	38.4	11.8	311	5	0	1	n.t.
86	33	f	CP	46,XX,t(9;22)	38.7	13.2	732	8	1	1	n.t.
87	67	m	CP	46,XY,t(9;22)	37.8	14.5	114	7	1	1	n.t.
88*	73	f	AP	n.t.	23.4	8.3	69	1	5	n.t.	none
89	48	m	CP	46,XY,t(9;22)	315.9	10.8	190	5	2	n.t.	n.t.
90	65	m	CP	46,XY,t(9;22)	30.6	15.2	216	3	0	1	n.t.
91	42	f	CP	46,XX,t(9;22)	61.2	13.5	319	3	0	1	n.t.
92	74	m	CP	46,XY,t(9;22)	48.4	13.1	321	1	1	1	n.t.
93*	76	m	CP	46,XY,t(9;22)	17.5	12.7	595	4	0	n.t.	F359V
94*	56	f	CP	46,XX,t(9;22),i(17q)	9.9	7.5	171	2	2	2	E279K
95*	74	f	AP	46,XX,t(9;22)	32.0	8.7	1150	10	1	1	F317L
96	51	m	CP	46,XY,t(9;22)	170.0	17	176	7	3	1	n.t.
97	29	f	CP	46,XX,t(9;22)	34.1	12.7	606	2	0	1	n.t.

Abbreviations: No. #, patient number; WBC, white blood count; Hb, hemoglobin; Plt, platelet count; yrs, years; m, male; f, female; PB, peripheral blood; BM, bone marrow; G/L, 10^9 cells per liter; g/dL, gram per deciliter; CP, chronic phase; AP, accelerated phase; BP, blast phase; complex karyotype: t(9;22) and at least two additional cytogenetic abberations; FISH: a cryptic BCR-ABL fusion gene was detected by fluorescence in situ hybridization; n.t., not tested.

* These patients were analyzed at relapsed/refractory disease only and the patients' characteristics refer to the time of sampling.

Table S3

Patients' characteristics – acute myeloid leukemia (AML)

	age	gender	sample	AML diagnosis		Mutations or		WBC	Hb	Plt	Blast (%)	
No. #	(yrs)	(m/f)	used at	FAB	WHO	molecular translocations	karyotype	(G/L)	(g/dL)	(G/L)	PB	BM
1	53	m	D,FU	M1	AML without maturation	none found	47,XY,+13	2.4	10.4	20	30	80
2	37	f	D	M5a	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46, XX	25.1	8.8	71	8	42
3	43	f	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	65.1	7.7	46	72	72
4	51	f	D,FU	M2	AML with mutated *NPM1*	*NPM1*	46,XX	44.0	9.1	32	59	66
5	34	m	D,FU	M4eo	AML with inv(16)	*CBFB-MYH11*	46,XY,inv(16)	57.1	9.3	11	60	34
6	71	f	D	M1	AML with mutated *NPM1*	*NPM1*	46,XX	52.4	11.0	15	87	85
7	74	f	D,FU	M0	AML with myelodysplasia	none found	46,XX,t(1;17;X),del(5q)	2.5	9.3	65	10	50
8	80	f	D	M5a	AML with mutated *NPM1*	*NPM1*	47,XX,+8,add(21)/47,XX,+8	10.2	10.1	44	5	42
9	66	f	D,FU	M2	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	97.4	10.3	56	94	83
10	69	m	D	M4	AML with myelodysplasia	none found	complex, del(5q)	1.0	10.3	88	0	37
11	66	f	D	M4	AML with myelodysplasia	none found	complex, del(5q)	62.2	10.3	50	79	70
12	55	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	76.1	9.4	60	70	85
13	61	f	D,FU	M2	AML with mutated *NPM1*	*NPM1*	46,XX	1.3	10.2	73	3	41
14	71	m	D	M2	AML with maturation	*FLT3* ITD	46,XY	6.1	9.2	73	45	26
15	70	m	D	M4	AML with myelodysplasia	none found	45,XY,-7	13.7	6.6	39	18	24
16	54	m	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XY	361.5	6.9	74	95	92
17	56	m	D	M2	AML with myelodysplasia	none found	complex	18.7	9.2	142	13	33
18	69	m	D	n.a.	AML with myelodysplasia	none found	46,XY	3.2	8.0	1	0	65
19	49	f	D	M4eo	AML with inv(16)	*CBFB-MYH11*, *FLT3* D835	46,XX,inv(16)	94.2	8.1	15	47	57
20	72	f	D	M2	AML with mutated *NPM1* and *CEBPA*	*NPM1*, *CEBPA*	46,XX	2.1	10.5	166	27	50
21	62	f	D	M1	AML without maturation	none found	47,XX,+11	2.8	12.1	158	77	60
22	64	f	D,FU	M0	AML with inv(3) or t(3;3)	none found	46,XX,t(3;3)	60.2	9.2	13	77	90
23	77	f	D	M4	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	200.0	8.3	26	41	65

No. #	age (yrs)	gender (m/f)	sample used at	AML diagnosis FAB	AML diagnosis WHO	Mutations or molecular translocations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
24	59	m	D	M2	Therapy-related AML with t(8;21)	*AML1-ETO*	46,XY,t(8;21)	100.0	9.5	29	60	58
25	63	m	D	n.a.	AML with myelodysplasia	*NPM-MLF1*; t(3;5)(q25.1;q34)	complex	26.5	9.8	11	79	70
26	69	m	D	M4	AML with myelodysplasia	none found	46,XY	3.5	9.0	118	0	20
27	59	m	FU	M5a	AML with myelodysplasia	none found	complex, del(5q)	1.0	7.9	43	0	9
28	52	m	D,FU	M2	AML with t(8;21)	*AML1-ETO*	45,X,-Y,del7q,t(8;21)	51.8	7.3	17	88	70
29	26	m	D	M4	Acute myelomonocytic leukemia	none found	46,XY	2.3	11.4	159	1	37
30	64	m	D	n.a.	AML with myelodysplasia	none found	46,XY	1.8	8.2	12	0	26
31	78	f	FU	M4	Acute myelomonocytic leukemia	none found	46,XX	26.1	10.9	7	4	23
32	53	f	D	M0	AML with minimal differentiation	none found	46,XX	0.4	8.2	104	0	75
33	24	m	D	M0	AML with myelodysplasia	none found	complex	2.4	11.1	332	7	25
34	65	m	D	M4eo	AML with inv(16)	*CBFB-MYH11A*, *KIT* D816V	46,XY,inv(16)	51.8	8.6	16	33	22
35	56	m	D	M2	AML with t(8;21)	*AML1-ETO*, t(8;21)(q22;q22)	46,XY,t(8;21)	6.8	11.1	53	27	58
36	63	f	D	M4	AML with myelodysplasia	none found	complex, del(5q)	1.0	8.9	71	21	57
37	57	f	D	M4eo	AML with inv(16)	*CBFB-MYH11*	46,XX,inv(16)	27.6	10.0	20	56	65
38	69	f	D	M2	AML with myelodysplasia	none found	complex	1.5	8.6	49	11	22
39	36	f	FU	M6	AML with myelodysplasia	*FLT3* ITD, *NPM1*	46,XX	5.0	10.0	128	39	65
40	53	m	FU	M6	AML with myelodysplasia	none found	complex	2.6	7.5	97	11	22
41	67	m	D	M4	AML with myelodysplasia	none found	45,XY,-7	84.5	11.4	121	4	28
42	51	m	D	M1	AML without maturation	none found	46,XY	0.7	8.8	30	8	73
43	27	m	FU	M4	Acute myelomonocytic leukemia	none found	46,XY	0.9	11.6	14	19	77
44	42	m	D,FU	M3	Acute promyelocytic	*PML-RARA*	46,XY,t(15;17)	6.3	14.9	18	22	88

No. #	age (yrs)	gender (m/f)	sample used at	AML diagnosis FAB	AML diagnosis WHO	Mutations or molecular translocations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
					leukemia							
45	62	f	D,FU	n.a.	AML with myelodysplasia	*CEBPA*	46,XX,del(13q)/45,XX,-13	2.2	9.2	40	0	24
46	65	f	D,FU	M1	AML with mutated *CEBPA*	*CEBPA*	46,XX	25.8	9.7	14	89	80
47	36	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	93.9	8.9	50	37	64
48	38	m	D	M5a	AML with myelodysplasia	none found	complex	13.4	7.9	108	96	75
49	65	m	D,FU	M1	AML without maturation	none found	46,XY	2.9	9.3	56	40	88
50	65	f	D	M1	AML without maturation	none found	46,XX	1.3	10.8	108	28	66
51	78	m	D	n.a.	AML with myelodysplasia	none found	45,XY,-7	53.1	10.3	20	51	40
52	79	m	D	M2	AML with maturation	none found	46,XY	52.4	11.6	65	72	60
53	65	f	D	M0	AML with myelodysplasia	none found	46,XX	2.0	10.5	31	21	78
54	74	m	D,FU	M1	AML with myelodysplasia	none found	complex, 5q-,7q-	1.6	9.0	17	16	25
55	56	m	D	M1	AML with mutated *NPM1*	*NPM1*	46,XY	127.4	8.5	307	95	96
56	70	f	D	M2	AML with myelodysplasia	none found	46,XX,t(1;6)	2.4	2.6	2	0	23
57	71	f	D	M0	AML with minimal differentiation	none found	46,XX	2.4	10.8	208	48	84
58	68	m	FU	M6	Acute erythroid leukemia	none found	46,XY	4.4	12.1	81	0	22
59	65	m	D	n.a.	AML with myelodysplasia	*KIT* D816V	47,XY,+8	6.6	8.2	20	13	85
60	66	f	D	n.a.	AML with myelodysplasia	none found	46,XX	1.3	9.0	85	0	20
61	58	m	D	n.a.	Therapy-related AML	none found	46,XY	1.7	8.8	34	0	20
62	40	f	D	M4	AML with mutated *NPM1*	*NPM1*	46,XX	18.6	10.3	91	16	28
63	83	f	D	M6	Acute erythroid leukemia	none found	n.t.	1.8	9.2	912	3	27
64	53	f	D	M4	Therapy related AML	none found	complex	2.0	11.4	10	2	25
65	82	m	D	M0	AML with myelodysplasia	*FLT3* ITD	46,XY,del(5q)	6.7	8.2	74	23	60
66	55	f	D	M2	AML with myelodysplasia	*NPM1*	46,XX	10.3	7.7	39	28	24
67	72	m	D	M2	AML with myelodysplasia	none found	47,XY,+8	1.9	10.2	62	1	47
68	75	m	D	n.a.	AML with myelodysplasia	*JAK2* V617F	47,XY,+mar	20.7	8.2	480	28	21
69	67	m	D	M2	AML with mutated *CEBPA*	*CEBPA*	46,XY	77.9	13.9	193	81	71
70	59	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	6.1	8.6	212	21	40

No. #	age (yrs)	gender (m/f)	sample used at	AML diagnosis FAB	AML diagnosis WHO	Mutations or molecular translocations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
71	21	m	D	M1	AML without maturation	none found	46,XY,add(17p)	133.2	9.5	28	95	88
72	54	f	D	M1	AML without maturation	none found	n.t.	1.2	11.1	271	0	71
73	56	f	D	M4	Acute myelomonocytic leukemia	none found	47,XX,+der(11)	55.7	9.5	61	28	55
74	49	f	D	M5a	AML with mutated *NPM1*	*NPM1*	47,XX,+8	12.1	11.8	97	42	75
75	79	f	D	M1	AML without maturation	none found	48,XX,+8,+21	4.3	8.3	191	77	n.t.
76	71	f	D	M2	AML with myelodysplasia	none found	complex, del(5q)	5.3	9.5	159	20	62
77	60	m	D	M1	AML with myelodysplasia	none found	46,XY,del(13q)	1.7	12.4	112	0	60
78	65	m	D	M4	AML with myelodysplasia	none found	47,XY,+8	72.4	12.5	129	2	55
79	54	m	D	M2	AML with t(8;21)	*AML1-ETO*	45,X,-Y,t(8;21)	14.0	7.5	13	22	23
80	73	m	D,FU	M5a	AML with mutated *NPM1*	*NPM1*	46,XY	50.0	7.8	143	29	78
81	59	m	FU	n.a.	AML with myelodysplasia	none found	47,XY,add(3),+8	34.3	10.4	7	26	n.t.
82	51	f	D	M4	AML with myelodysplasia	none found	complex	90.0	8.7	79	60	70
83	61	f	D	M3	Acute promyelocytic leukemia	*PML-RARA*, *FLT3* ITD	45,XX,der(15)t(15;17),-17	1.0	11.8	122	3	81
84	63	f	FU	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	33.8	10.0	25	95	94
85	74	m	D	M0	AML with minimal differentiation	none found	46,XY	13.9	11.2	206	51	80
86	68	m	D,FU	M1	AML without maturation	none found	46,XY	24.4	7.3	41	17	20
87	54	f	D	M1	AML without maturation	none found	47,XX,+8	0.5	8.4	28	20	72
88	39	f	D,FU	M2	AML with t(8;21)	*KIT* D816V, *AML1-ETO*	47,XX,+4,t(8;21)	9.2	9.6	17	30	65
89	73	m	D	M2	AML with mutated *CEBPA*	*CEBPA*	46,XY	2.0	14.9	17	10	33
90	38	f	D	M4	Acute myelomonocytic leukemia	*FLT3* D835	46,XX	45.2	9.0	69	11	45
91	71	m	D,FU	M4	Acute myelomonocytic leukemia	none found	46,XY	33.2	9.8	24	26	43
92	75	f	D	M5b	Acute monocytic leukemia	*JAK2* V617F	46,XX	77.9	9.5	22	2	66
93	55	m	D	n.a.	AML with myelodysplasia	none found	46,XY,t(10;11)	44.4	10.0	76	16	30

	age	gender	sample	AML diagnosis		Mutations or		WBC	Hb	Plt	Blast (%)	
No. #	(yrs)	(m/f)	used at	FAB	WHO	molecular translocations	karyotype	(G/L)	(g/dL)	(G/L)	PB	BM
94	25	f	D	M3	Acute promyelocytic leukemia	*PML-RARA*	46,XX,t(15;17)	7.5	9.7	26	47	90
95	85	f	D	M4	Acute myelomonocytic leukemia	none found	46,XX	132.0	10.0	57	8	29
96	67	m	D	M2	AML with t(8;21)	*AML1-ETO*	46,XY,t(8;21)	1.4	8.5	54	35	53
97	67	m	D	n.a.	AML with myelodysplasia	none found	46,XY	1.8	11.6	144	0	40
98	76	m	D	M2	AML with myelodysplasia	*FLT3* ITD	48,XY,+8,+14	3.3	10.4	35	18	28
99	70	f	D	M1	AML with myelodysplasia	none found	46,XX	0.3	10.0	176	n.t.	22
100	62	m	FU	n.a.	AML with myelodysplasia	none found	46,XY	1.2	11.2	6	75	70
101	62	m	D	M4	AML with myelodysplasia	none found	complex	7.3	9.8	44	10	65
102	67	m	D	n.a.	AML with myelodysplasia	*FLT3* ITD	46,XY	13.1	5.9	29	63	50
103	62	f	D,FU	M1	AML with myelodysplasia	*FLT3* ITD, *NPM1*	46,XX	4.7	11.3	116	19	62
104	56	m	D,FU	M4eo	AML with inv(16)	*CBFB-MYH11*	46,XY	14.9	10.5	48	43	57
105	78	m	D	n.a.	AML with myelodysplasia	none found	complex	40.6	8.8	21	50	45
106	50	m	D,FU	M2	AML with t(8;21)	*AML1-ETO*	45,X,-Y,t(8;21)	4.6	5.9	18	35	63
107	57	m	FU	n.a.	AML, NOS	none found	46,XY,dup(1q)	2.0	8.9	18	0	12
108	83	m	D	M0	AML with minimal differentiation	none found	46,XY	116.9	6.1	12	81	85
109	51	f	D	M1	AML without maturation	none found	47,XX,+8	2.4	7.7	11	19	82
110	45	m	FU	M2	AML with myelodysplasia	*FLT3* ITD, *NPM1*	46,XY	1.6	13.1	86	0	60
111	38	m	D,FU	M4	AML with mutated *NPM1*	*NPM1*	46,XY	14.0	11.1	87	11	24
112	77	f	D	n.a.	AML with myelodysplasia	none found	complex	1.3	7.4	53	0	23
113	73	m	D	M4	Acute myelomonocytic leukemia	*FLT3* D835	46,XY,+11,-16	69.0	10.6	44	59	60
114	28	m	D	M2	AML with t(8;21)	*AML1-ETO*	45,X,-Y	11.5	9.9	68	24	20
115	48	m	D	M2	AML with maturation	none found	46,XY	11.8	8.4	576	42	27
116	71	m	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	n.t.	152.3	9.1	22	96	n.t.
117	66	f	FU	M4eo	AML with inv(16)	*CBFB-MYH11*	46,XX,inv(16)	3.3	12.2	72	5	19

	age	gender	sample	AML diagnosis		Mutations or		WBC	Hb	Plt	Blast (%)	
No. #	(yrs)	(m/f)	used at	FAB	WHO	molecular translocations	karyotype	(G/L)	(g/dL)	(G/L)	PB	BM
118	83	m	D	M2	AML with myelodysplasia	none found	47,XY,+8	8.1	9.1	28	21	24
119	68	f	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	n.t.	198.1	9.2	14	90	91
120	74	f	D	M4	Therapy-related AML	*FLT3* ITD	46,XX	109.5	9.2	67	37	70
121	65	f	FU	M0	AML with minimal differentiation	n.t.	47,XX,t(3;12),+8	0.6	10.9	10	n.t.	88
122	68	f	D	M2	AML with myelodysplasia	none found	49,XX,+8,+12,+13	7.4	9.8	5	72	30
123	77	m	D	M2	Therapy-related AML	none found	46,XY	4.9	11.1	61	32	37
124	43	m	D	M2	AML with mutated *NPM1*	*NPM1*	46,XY	135.7	12.5	54	50	49
125	49	f	FU	M0	AML with minimal differentiation	*TLS-ERG*	46,XX,t(16;21;13)	7.4	13.3	242	1	5
126	73	f	D	M4	Therapy-related AML	*FLT3* ITD, *NPM1*	46,XX	93.2	8.1	90	62	62
127	54	f	D	M5a	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	144.2	10.3	62	96	95
128	62	m	D	M4	AML with t(11;17), *MLL-MSF*	*MLL1-MSF*	47,XY,t(11;17)	35.1	11.3	32	33	72
129	41	m	D,FU	M3	Acute promyelocytic leukemia	*PML-RARA*	46,XY,t(15;17)	54.0	11.1	23	88	90
130	55	m	D,FU	M2	AML with maturation	*FLT3* ITD	47,XY,+8	6.8	12.0	112	56	66
131	54	f	D,FU	M2	AML with myelodysplasia	none found	complex, del(5q), del(7q)	2.4	9.4	55	1	21
132	44	f	D	n.a.	AML with myelodysplasia	none found	45,XX,inv(3),-7	1.5	9.3	13	16	25
133	78	m	FU	M4	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XY	91.6	8.8	33	93	81
134	34	f	D	M5a	AML with mutated *NPM1*	*NPM1*	46,XX	30.8	9.6	41	5	70
135	83	f	D	M1	AML with myelodysplasia	n.t.	47,XX,del(5q),+8	1.2	8.0	151	5	60
136	39	f	D	M2	AML with maturation	none found	46,XX	19.9	11.7	87	82	81
137	68	m	D	n.a.	AML with myelodysplasia	none found	complex	2.2	8.2	34	0	34
138	65	f	D	M6	Acute erythroid leukemia	*FLT3* ITD	46,XX	2.5	9.6	189	1	30
139	64	f	D	n.a.	AML with myelodysplasia	*JAK2* V617F	n.t.	10.3	10.7	352	24	n.t.
140	43	f	FU	M1	AML without maturation	none found	46,XX	2.4	10.9	168	0	14
141	21	f	D	M4eo	AML with inv(16)	*CBFB-MYH11*	46,XX,inv(16)	192.9	8.4	18	78	86
142	76	m	D	n.a.	AML with myelodysplasia	none found	46,XY	1.9	12.4	111	0	21

	age	gender	sample	AML diagnosis		Mutations or		WBC	Hb	Plt	Blast (%)	
No. #	(yrs)	(m/f)	used at	FAB	WHO	molecular translocations	karyotype	(G/L)	(g/dL)	(G/L)	PB	BM
143	71	m	D	M6	Acute erythroid leukemia	none found	48,XY,+8,+15	3.9	6.8	145	28	48
144	49	m	FU	n.a.	AML with myelodysplasia	none found	46,XY	2.0	14.8	41	1	22
145	85	f	D	M1	AML with myelodysplasia	none found	46,XX,del(5q)	1.7	11.1	101	0	33
146	39	f	FU	M2	AML with maturation	n.t.	46,XX	1.7	10.5	29	6	27
147	68	m	D	M0	AML with minimal differentiation	none found	46,XY	1.8	6	38	1	46
148	64	f	D	M4	AML with myelodysplasia	*FLT3* ITD	46,XX,del(9q)	63.9	8.2	36	28	79
149	72	m	D	M6	AML with myelodysplasia	none found	complex with (del5q)	2.7	7.2	86	1	30
150	62	m	D	n.a.	AML with myelodysplasia	none found	complex	1.6	9.6	31	2	30
151	67	m	D	M1	AML with myelodysplasia	*JAK2* V617F, *FLT3* ITD	46,XY	38.4	9.1	12	78	69
152	28	f	FU	M1	AML without maturation	none found	46,XX	4.0	12.0	114	10	37
153	70	m	D	M2	AML with myelodysplasia	none found	46,XY,+mar	1.3	7.0	95	0	1
154	36	f	D	M4	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	26.2	8.2	82	5	40
155	80	m	D	n.a.	AML, NOS	none found	46,XY	2.5	9.4	47	17	38
156	83	m	D	M2	AML with myelodysplasia	none found	complex	1.4	8.0	43	5	23
157	50	f	D,FU	M4	AML with mutated *NPM1* and *CEBPA*	*FLT3* ITD, *NPM1*, *CEBPA*	46,XX	15.4	7.3	68	16	63
158	73	f	D	M2	AML with myelodysplasia	none found	46,XX,7,+mar,del(20q)/ 46,XX,del(20q)	70.6	9.8	19	77	50
159	57	m	D	M1	AML without maturation	*FLT3* ITD	46,XY	72.6	8.5	91	80	73
160	46	f	D	M1	AML with t(10;11), *MLL-AF10*	*MLL1-AF10*	46,XX,t(8;12)	25.0	8.1	35	81	93
161	92	m	D	M0	AML with minimal differentiation	*FLT3* ITD	n.t.	107.0	10.3	63	70	80
162	50	m	D	M3	Acute promyelocytic leukemia	*PML-RARA*	n.t.	0.9	11.9	61	2	n.t.
163	78	m	D	n.a.	AML with myelodysplasia	none found	46,XY,t(X;2)/47,XY,t(2;X),+9	9.0	9.5	24	89	63
164	71	f	D	M2	AML with myelodysplasia	none found	46,XX	10.5	10.0	24	6	33
165	63	m	D	M1	AML with inv(16)	*CBFB-MYH11*	46,XY,inv(16)	27.5	9.0	22	65	64

No. #	age (yrs)	gender (m/f)	sample used at	AML diagnosis FAB	AML diagnosis WHO	Mutations or molecular translocations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
166	71	m	FU	M4	AML with myelodysplasia	none found	46,XY	17.8	11.4	56	2	22
167	62	m	D	M2	AML with myelodysplasia	*JAK2* V617F, *CEBPA*	46,XY,-18,+mar	17.4	10.8	560	41	32
168	64	m	D,FU	M1	AML without maturation	none found	46,XY	53.5	9.0	52	94	84
169	70	m	D	M2	AML with maturation	none found	46,XY	1.9	9.1	36	0	37
170	56	m	D,FU	M5b	Acute monocytic leukemia	none found	46,XY	1.7	9.3	90	6	35
171	76	m	D,FU	M2	AML with maturation	none found	47,XY,+8	21.7	7.3	15	79	40
172	33	f	FU	M1	AML with t(9;11), *MLL1-AF9*	*MLL1-AF9*	46,XX,t(9;11)	0.4	7.5	16	n.t.	73
173	60	m	D	M2	AML with myelodysplasia	none found	46,XY	3.4	11.4	50	38	55
174	49	f	FU	M2	AML with maturation	n.t.	46,XX,add(17q)	37.4	9.3	23	95	90
175	66	m	D	M5a	Acute monoblastic leukemia	none found	46,XY	30.8	10.5	26	1	n.t.
176	88	m	D	M6	Acute erythroid leukemia	none found	complex	3.6	8.4	124	1	21
177	82	f	D	M2	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	112.9	9.5	54	73	75
178	50	m	D	n.a.	n.t.	n.t.	n.t.	330.0	7.3	100	67	n.t.
179	71	m	D	n.a.	AML with t(6;11), *MLL1-AF6*	*MLL1-AF6*	46,XY,t(6;11)	312.0	11.4	94	77	nA
180	66	f	D	M4	AML with myelodysplasia	*FLT3* ITD	46,XX	4.2	9.4	14	0	19
181	76	m	D	M4	AML with myelodysplasia	*TP53*, *BCR-ABL*	complex with del(5q)	3.6	6.5	151	33	50
182	71	f	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	33.1	10.0	9	44	75
183	69	m	D	M2	AML with myelodysplasia	none found	complex (5q-,-7)	8.2	9.6	126	28	17
184	50	f	FU	M4	AML with mutated *NPM1*	*NPM1*, *FLT3* ITD	46,XX	75.6	12.7	158	81	79
185	38	f	D,FU	M3	Acute promyelocytic leukemia	*PML-RARA*	46,XX,t(15;17)	5.1	9.8	22	37	73
186	71	m	FU	M1	AML with myelodysplasia	none found	45,XY,-7	1.4	11.1	43	7	56
187	53	f	D,FU	M1	AML with mutated *NPM1*	*NPM1*	46,XX	64.1	8.5	39	88	80
188	51	f	D	M1	AML without maturation	*FLT3* ITD	46,XX	55.9	9.4	33	94	94
189	36	f	D	M2	AML with myelodysplasia	*FLT3* ITD	46,XX,del(3)	5.2	8.7	8	41	56
190	44	f	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	219.8	5.1	38	94	83
191	76	f	D	n.a.	AML with myelodysplasia	*FLT3* ITD	46,XX,del(5q)	20.1	12.1	206	36	49

No. #	age (yrs)	gender (m/f)	sample used at	AML diagnosis FAB	AML diagnosis WHO	Mutations or molecular translocations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
192	66	f	D,FU	M5a	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	56.0	8.9	88	11	62
193	78	m	D	M3	Acute promyelocytic leukemia	*PML-RARA*	46,XY,t(15;17)	2.6	10.1	29	33	81
194	29	f	D,FU	M2	AML with maturation	none found	46,XX	31.0	11.5	20	79	56
195	26	f	D	M2	AML with maturation	none found	46,XX	20.8	11.4	124	39	65
196	19	m	D,FU	M1	AML without maturation	none found	46,XY	34.4	8.2	11	81	79
197	62	f	FU	M2	AML with maturation	none found	46,XX	1.7	14.0	52	0	16
198	70	f	D	M2	AML with mutated *NPM1*	*NPM1*	46,XX	6.7	9.4	92	38	68
199	64	f	FU	M3	Acute promyelocytic leukemia	*PML-RARA*	47,XX,+8,t(15;17)	2.5	12.2	143	0	8
200	68	f	D	M2	AML with myelodysplasia	none found	46,XX	1.2	11.9	81	3	30
201	50	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	27.2	8.2	99	0	40
202	61	m	D	M4	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XY	34.7	9.3	180	32	60
203	75	f	D	n.a.	Therapy related AML	none found	n.t.	1.07	8.4	8	3	47
204	53	f	D	M2	AML with myelodysplasia	none found	45,XX,-7	2.4	9.3	84	13	30
205	30	m	D	M4eo	AML with inv(16)	*FLT3* ITD, *CBFB-MYH11*	47,XY,+8,inv(16)	110.9	10.7	37	80	68
206	73	m	D	M0	AML with myelodysplasia	none found	45,XY,-7	25.6	11.4	121	88	66
207	66	m	FU	M2	AML with myelodysplasia	none found	46,XY	0.9	8.6	41	2	22
208	70	f	D	M2	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XX	40.5	9.8	447	78	71
209	44	m	D	M0	AML with minimal differentiation	none found	46,XY	1.7	9.7	186	4	65
210	68	m	D	M1	AML with myelodysplasia	none found	complex	74.6	12.0	146	95	84
211	39	m	D,FU	M4	Acute myelomonocytic leukemia	none found	46,XY,t(11;17)	28.5	6.7	42	40	47
212	42	m	D	M1	AML without maturation	none found	46,XY	15.2	10.8	39	57	53
213	41	m	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XY	202.3	8.3	24	61	65
214	62	f	FU	M5b	AML with myelodysplasia	*NPM1*	46,XX,del(12p),del(20q)	12.3	9.4	12	47	74

	age	gender	sample	AML diagnosis		Mutations or		WBC	Hb	Plt	Blast (%)	
No. #	(yrs)	(m/f)	used at	FAB	WHO	molecular translocations	karyotype	(G/L)	(g/dL)	(G/L)	PB	BM
215	53	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	31.7	8.2	18	1	25
216	43	m	D	M2	AML with mutated *NPM1*	*NPM1*	46,XY	1.0	10.2	39	11	56
217	54	f	D,FU	M4	AML with myelodysplasia	none found	complex, del(5q)	5.4	8.6	13	5	64
218	56	m	D,FU	M4	Acute myelomonocytic leukemia	*FLT3* ITD	46,XY	47.0	6.7	145	12	43
219	73	f	FU	M1	AML without maturation	*FLT3* ITD	46,XX	136.4	10.5	79	57	
220	69	m	D	M0	AML with myelodysplasia	none found	46,XY,inv(3),del(7q)	2.4	8.2	19	2	37
221	44	m	D,FU	M4	Acute myelomonocytic leukemia	none found	46,XY,t(5;9)	25.0	9.1	24	11	31
222	64	m	D	M4	AML with t(11;19), *MLL1-ELL*	*MLL1-ELL*, t(11;19)	46,XY	219.0	7.1	34	68	61
223	54	m	D	M2	AML with myelodysplasia	none found	45,XY,-7	2.8	11.5	24	16	37
224	20	f	D,FU	M2	Therapy related AML	*CEBPA*	46,XX	2.4	8.1	40	32	25
225	71	m	D	M4	Acute myelomonocytic leukemia	none found	47,XY,+mar	46.7	10.8	116	90	72
226	46	m	D	M4	AML with mutated NPM1	*NPM1*	46,XY	19.0	5.0	24	0	50
227	58	f	D	M4	AML with t(11;17), *MLL1-AF17*	*MLL1-AF-17*, t(11;17)	46,XX,t(11;17)	13.3	6.3	66	71	81
228	55	f	D	M3	Acute promyelocytic leukemia	*PML-RARA*	47,XX,t(15;17)	3.3	9.1	67	27	63
229	70	f	D	M2	AML with maturation	none found	46,XX	2.2	11.6	45	0	21
230	79	m	D	M1	AML without maturation	*FLT3* ITD	46,XY	1.7	8.5	113	49	77
231	55	m	D	M2	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	46,XY	42.0	8.5	242	82	62
232	23	m	D	M4eo	AML with inv(16)	*CBFB-MYH11*	complex, inv(16)	51.0	8.1	21	17	32
233	62	m	D	M1	AML with mutated *NPM1*	*NPM1*, *FLT3* ITD	n.t.	116.7	13.2	50	95	95
234	41	f	D	M4eo	AML with inv(16)	*CBFB-MYH11*	46,XX,inv(16)	86.4	6.7	30	7	28
235	43	m	FU	M2	AML with myelodysplasia	none found	45,XY,der(6),-7	6.8	11.5	58	86	63
236	70	m	D,FU	M2	AML with maturation	none found	47,XY,+8	2.3	9.1	77	0	33
237	56	m	D,FU	M1	AML without maturation	none found	46,XY	2.0	8.9	45	0	50

	age	gender	sample	AML diagnosis		Mutations or		WBC	Hb	Plt	Blast (%)	
No. #	(yrs)	(m/f)	used at	FAB	WHO	molecular translocations	karyotype	(G/L)	(g/dL)	(G/L)	PB	BM
238	65	f	D	M1	AML with myelodysplasia	none found	46,XX	1.1	7.3	84	7	63
239	36	m	D	M1	AML with myelodysplasia	none found	47,XY,del(9q),+21	18.5	9.2	28	77	77
240	72	m	D	M2	AML with myelodysplasia	*NPM1*, *KIT* D816V	46,XY	13.0	10.3	163	77	46
241	41	f	FU	n.a.	n.t.	*DEK-CAN*	46,XX	1.4	8.5	7	1	75
242	54	f	FU	M2	AML with maturation	none found	46,XX	1.8	11.8	45	5	30
243	36	m	D,FU	M0	AML with t(10;11), *MLL-AF10*	*MLL1-AF10*	complex	1.5	12.0	192	6	66
244	74	f	D	M4	AML with t(6;11), *MLL1-AF6*	MLL1-AF6.t(6;11)	46,XX	3.4	8.0	3	0	40
245	76	m	D,FU	M3	Acute promyelocytic leukemia	*PML-RARA*	46,XY,t(15;17)	22.8	11.9	36	88	90
246	81	m	D	M5a	AML with t(9;11), *MLL1-AF9*	*MLL1-AF9*	46,XY	66.2	8.7	36	90	93
247	73	m	FU	M1	AML without maturation	none found	47,XY,+8	2.0	10.0	78	0	2
248	66	m	D	M1	AML without maturation	none found	46,XY	3.3	8.6	15	79	20
249	64	m	D	M1	AML with mutated *NPM1*	*FLT3* ITD, *NPM1*	45,XY	130.3	11.8	22	86	85
250	81	f	D	M1	AML with mutated *NPM1*	*NPM1*	46,XX	36.4	8.9	94	86	80
251	36	f	FU	M1	AML without maturation	*FLT3* ITD	46,XX	1.9	10.2	92	0	15
252	66	m	D	M2	AML with myelodysplasia	none found	47,XY,+11,der(11)	2.9	10.1	21	0	31
253	41	f	D	M4	Acute myelomonocytic leukemia	none found	46,XX	2.8	8.2	61	51	72
254	45	f	D	M4	AML with myelodysplasia	*FLT3* ITD	46,XX	66.8	10.2	46	53	66
255	20	m	D	M3	Acute promyelocytic leukemia	*PML-RARA*	46,XY,t(15;17)	51.2	10.6	25	90	95
256	27	m	FU	M4	Acute myelomonocytic leukemia	*FLT3* ITD	46,XY	93.9	10.3	88	76	76
257	32	f	D	M4	AML with mutated *NPM1*	*NPM1*, *FLT3* ITD	46,XX	126.2	9.1	35	42	32
258	41	m	D	M3v	Acute promyelocytic leukemia	*PML-RARA*, *FLT3* ITD	46,XY,t(15;17)	57.5	9.9	15	63	90
259	63	f	D	n.a.	Therapy-related AML	none found	46,XX	0.8	6.5	24	0	48
260	83	m	D	M2	AML with myelodysplasia	none found	complex, del(5q)	2.4	7.9	25	0	22

No. #	age (yrs)	gender (m/f)	sample used at	AML diagnosis FAB	AML diagnosis WHO	Mutations or molecular translocations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
261	62	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	233.7	7.9	53	9	28
262	81	m	D	M4	AML with mutated *NPM1*	*NPM1*, *FLT3* ITD, *KIT* D816V	46,XY	198.5	10.2	27	25	66
263	69	m	D	M0	AML with minimal differentiation	none found	46,XY	1.1	9.8	106	0	78
264	58	m	D	M1	AML with t(8;21)	*AML1-ETO*	46,XY,t(8;21)	9.6	9.5	12	85	77
265	53	m	D	M2	AML with mutated *NPM1*	*NPM1*	46,XY	5.0	8.2	47	46	50
266	81	f	D	M0	AML with myelodysplasia	none found	94,XXXX, complex	1.4	11.4	122	16	46
267	68	m	D	M4	AML with mutated *NPM1*	*NPM1*	46,XY	22.7	10.4	28	53	72
268	73	f	D	M6	Acute erythroid leukemia	none found	46,XX	4.4	8.2	173	1	43
269	64	m	D	M5a	Acute monoblastic leukemia	none found	46,XY,dup(11q)	56.0	9.1	12	70	70
270	22	f	D	M1	AML without maturation	none found	47,XX,+11	2.8	9.5	78	57	65
271	81	f	D	M1	AML with myelodysplasia	none found	complex	2.6	8.4	25	53	77
272	55	f	D	M2	AML with myelodysplasia	none found	45,XX,-7,-17	10.5	9.8	94	22	40
273	76	m	D	M2	Therapy-related AML	none found	complex	51.3	7.7	63	69	61
274	88	f	D	n.a.	AML with mutated *NPM1*	*NPM1*, *FLT3* ITD	n.t.	62.4	7.0	21	89	95

Abbreviations: No. #, patient number; WBC, white blood count; Hb, hemoglobin; Plt, platelet count; yrs, years; m, male; f, female; FAB, French-American-British cooperative study group; WHO, world health organization classification, 4th Edition; PB, peripheral blood; BM, bone marrow; G/L, 10^9 cells per liter; g/dL, gram per deciliter; m, male; f, female; D, used at time of diagnosis or persistence of blasts after induction chemotherapy; FU, used during follow up; n.a., not available; n.t., not tested; AML, acute myeloid leukemia; *FLT3* ITD, fms like tyrosine kinase 3 internal tandem duplication; NPM1, nucleophosmin mutation; complex karyotype: at least three cytogenetic aberrations.

Table S4

Patients' characteristics - other hematologic neoplasms

No. #	age (yrs)	gender (m/f)	diagnosis	FAB	WHO	mutations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
1	83	m	MDS	RA	RCMD	n.t.	n.t.	5.5	11.0	78	0	2
2	86	f	MDS	RARS	RARS	n.t.	46,XX	2.0	10.3	151	0	2
3	84	m	MDS	RAEB-2	RAEB-2	n.t.	n.t.	5.5	10.5	25	3	15
4	66	f	MDS	RAEB-2	RAEB-2	n.t.	47,XX,+21	2.9	9.5	231	2	10
5	70	m	MDS	RAEB-2	RAEB-2	n.t.	n.t.	1.2	7.9	76	0	14
6	78	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)	8.4	8.2	152	0	3
7	81	m	MDS	RA	RCUD	n.t.	n.t.	13.3	11.6	19	1	8
8	69	m	MDS	RAEB-2	RAEB-2	n.t.	47,XY,+8	1.1	14.8	44	0	19
9	82	m	MDS	RAEB-1	RAEB-1	n.t.	n.t.	1.7	10.3	105	0	7
10	72	f	MDS	RAEB-1	RAEB-1	n.t.	46,XX,del(20q)/47,XX,del(20q),+del(20q)	4.2	5.6	25	0	9
11	76	m	MDS	RAEB-1	RAEB-1	n.t.	45,XY,-7	2.6	10.6	39	0	5
12	52	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)	4.9	8.0	409	0	1
13	51	f	MDS	RAEB-1	RAEB-1	n.t.	46,XX,del(7q)	1.8	10.0	82	0	5
14	59	m	MDS	RA	RCMD	n.t.	46,XY	3.0	6.7	71	0	1
15	57	m	MDS	RAEB-2	RAEB-2	n.t.	46,XY	4.1	10.3	47	0	11
16	72	f	MDS	RARS	RARS	n.t.	46,XX	2.6	8.9	349	0	1
17	70	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)/47,XX,del(5q),+8	2.7	8.9	267	0	2
18	72	m	MDS	RA	RCMD	n.t.	45,X,-Y/45,XY,-21/45,XY,-22	11.7	8.1	342	0	2
19	80	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)	8.7	8.6	354	0	1
20	54	m	MDS	RA	RCMD	n.t.	46,XY	1.3	13.2	16	0	1
21	63	m	MDS	RA	del(5q)	n.t.	46,XY,del(5q)	1.9	10.5	317	0	3

No. #	age (yrs)	gender (m/f)	diagnosis	FAB	WHO	mutations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
22	76	m	MDS	RA	RCUD	n.t.	45,X,-Y	2.4	11.8	111	0	1
23	74	m	MDS	RA	del(5q)	n.t.	46,XY,del(5q)	1.8	8.4	89	0	2
24	64	m	MDS	RAEB	RAEB-2	n.t.	46,XY,del(7p)	1.8	7.8	118	0	14
25	63	m	MDS	RA	RCMD	n.t.	complex	3.3	10.0	134	0	5
26	73	f	MDS	RAEB-2	RAEB-2	n.t.	n.t.	3.0	11.4	93	0	15
27	58	m	MDS	RAEB	RAEB-1	n.t.	46,XY	4.2	11.4	122	0	6
28	88	m	MDS	RA	RCMD	n.t.	45,XY,-7	2.0	9.1	90	0	3
29	69	f	MDS	RA	RCMD	n.t.	46,XX	4.0	8.9	114	0	1
30	76	m	MDS	RAEB-2	RAEB-2	n.t.	46,XY	4.5	8.6	41	0	11
31	39	m	MDS	RAEB-2	RAEB-2	n.t.	48,XY,+8,+12	2.8	12.6	52	4	15
32	72	m	MDS	RA	RCUD	TCR Rearrangement	46,XY	7.1	11.0	58	0	1
33	76	m	MDS	RAEB-2	RAEB-2	n.t.	46,XY	1.4	10.8	22	0	12
34	84	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)	6.2	9.3	332	0	2
35	57	f	MDS	RAEB-1	RAEB-1	n.t.	46,XX,del(11q)	4.2	12.6	295		5
36	78	m	MDS	RARS	del(5q)	n.t.	46,XY,del(5q)	4.2	9.6	122	0	1
37	63	f	MDS	RARS	RARS	n.t.		5.7	8.0	85	0	3
38	80	f	MDS	RAEB-1	RAEB-1	n.t.	46,XX	1.8	10.8	46	0	4
39	73	f	MDS	RAEB-1	RAEB-1	n.t.		10.0	11.3	34	0	1
40	78	m	MDS	RAEB-2	RAEB-2	*FLT3* ITD	46,XY,del(20q)	2.1	9.6	43	0	17
41	72	m	MDS	RARS	RARS	n.t.	46,XY	7.3	9.5	266	0	1
42	53	f	MDS	RAEB-2	RAEB-2	n.t.	45,XX,-7	2.0	7.3	203	2	13
43	64	m	MDS	RAEB-1	RAEB-1	n.t.		1.4	8.6	117	0	9
44	63	m	MDS	RARS	RARS	n.t.		8.5	8.6	286	0	1
45	70	m	MDS	RA	RCMD	n.t.	46,XY	8.0	9.5	157	0	2
46	66	m	MDS	RAEB-2	RAEB-2	n.t.	complex	1.9	8.6	24	2	11
47	65	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)	5.7	10.5	284	0	2
48	71	m	MDS	RAEB	RAEB-2	n.t.	46,XY	1.5	10.1	10	8	16

No. #	age (yrs)	gender (m/f)	diagnosis	FAB	WHO	mutations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
49	77	f	MDS	RA	del(5q)	n.t.	46,XX,del(5q)	2.5	8.8	13	0	n.t.
50	67	m	MDS	RAEB	RAEB-2	n.t.	46,XY,del(5q)	3.8	9.6	109	1	11
51	85	m	MDS	RAEB	RAEB-2	n.t.	46,XY,del(5q)	2.6	6.7	14	9	12
52	66	m	MDS	RAEB-2	RAEB-2	n.t.	46,XY,del(10q),t(10;21)	6.2	8.9	16	2	14
53	64	m	MDS	RAEB	RAEB-2	n.t.	46,XY	3.7	12.3	58	0	11
54	75	f	MDS	RA	del(5q)	n.t.	47,XX,del(5q),+8	3.6	9.7	413	0	3
55	65	f	MDS	RA	RCMD	n.t.	n.t.	3.6	8.2	110	0	3
56	69	m	MDS	RA	RCUD	n.t.	46,XY	5.8	12.9	79	0	1
57	71	f	MDS	RA	RCMD	n.t.	46,XX	1.6	11.4	27	0	2
58	77	m	MDS	RAEB-2	RAEB-2	n.t.	complex, del(5q)	2.8	9.9	33	3	18
59	56	m	MDS/MPN	CMML	CMML-1	n.t.	46,XY	16.0	8.7	49	1	2
60	72	m	MDS/MPN	n.a.	MDS/MPN-U	n.t.	n.t.	45.3	8.7	40	0	1
61	75	m	MDS/MPN	CMML	CMML-2	n.t.	n.t.	17.7	11.3	132	2	15
62	62	m	MDS/MPN	CMML	CMML-2	JAK2 V617F	n.t.	24.6	8.3	49	15	n.t.
63	46	m	MDS/MPN	CMML	CMML-2	n.t.	complex	39.1	8.5	22	1	6
64	81	m	MDS/MPN	CMML	CMML-2	n.t.	46,XY	8.9	11.0	8	2	10
65	73	m	MDS/MPN	CMML	CMML-1	n.t.	46,XY	4.7	8.1	36	0	6
66	62	m	MDS/MPN	CMML	CMML-2	n.t.	46,XY	77.1	10.3	26	7	11
67	71	m	MDS/MPN	RAEB-2	MDS/MPN-U	n.t.	47,XY,+8	25.4	7.6	137	11	8
68	66	m	MDS/MPN	RAEB-2	MDS/MPN-U	n.t.	n.t.	26.2	10.5	39	13	15
69	61	m	MDS/MPN	n.a.	MDS/MPN-U	n.t.	46,XY	9.5	9.9	512	0	1
70	72	m	MDS/MPN	CMML	CMML-1	n.t.	46,XY	21.6	11.8	64	0	4
71	76	f	MDS/MPN	n.a.	MDS/MPN-U	n.t.	complex	21.0	9.1	19	4	6
72	83	f	MDS/MPN	CMML	CMML-1	n.t.	46,XX	16.1	10.3	160	1	3
73	78	f	MDS/MPN	CMML	CMML-1	n.t.	46,XX	6.0	10.1	137	0	4
74	71	f	MDS/MPN	CMML	CMML-1	n.t.	46,XX,del(20q)	9.4	11.5	54	0	4
75	77	m	MDS/MPN	CMML	CMML-1	n.t.	n.t.	17.6	9.8	11	0	2

No. #	age (yrs)	gender (m/f)	diagnosis	FAB	WHO	mutations	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
76	56	m	MDS/MPN	n.a.	MDS/MPN-U	n.t.	n.t.	15.7	10.5	256	0	1
77	62	m	MDS/MPN	CMML	CMML-1	n.t.	46,XY	70.3	10.2	148	2	8
78	73	f	MDS/MPN	CMML	CMML-2	n.t.	46,XX	18.1	10.6	28	9	11
79	53	f	MDS/MPN	CMML	CMML-2	n.t.	47,XX,+6	3.3	9.5	33	18	17
80	47	f	MDS/MPN	CMML	CMML-2	n.t.	46,XX	39.9	10.1	26	3	14
81	71	m	MDS/MPN	CMML	CMML-2	n.t.	47,XY,+8	19.5	8.4	259	0	20

Abbreviations: No. #, patient number; WBC, white blood count; Hb, hemoglobin; Plt, platelet count; yrs, years; m, male; f, female; FAB, French-American-British classification; WHO, World Health Organisation classification, 4th edition; PB, peripheral blood; BM, bone marrow; MDS/MPN, myelodysplastic/myeloproliferative overlap neoplasm; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; SM, systemic mastocytosis; CMML, chronic myelomonocytic leukemia; RARS, refractory anemia with ring sideroblasts; RAEB, RA with excess of blasts; del(5q), deletion of chromosome 5q; RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; n.a., not available; n.t., not tested.

Table S5

Patients' characteristics of control patients: lymphoma, normal bone marrow, premalignant conditions and cord blood samples

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	Blast (%) BM
1	77	m	ICUS	46,XY	3.2	12.5	93	0	2
2	67	f	ICUS	n.t.	2.9	12.9	33	0	3
3	82	f	ICUS	n.t.	7.4	11.8	82	0	1
4	56	f	ICUS	n.t.	1.6	11.6	25	0	1
5	59	f	ICUS	n.t.	2.9	13.5	58	0	3
6	78	m	ICUS	n.t.	2.5	16.0	118	0	1
7	50	f	ICUS	46,XX	3.9	11.1	205	0	2
8	60	m	ICUS	46,XY	1.5	10.9	45	0	2
9	44	f	ICUS	46,XX	4.0	10.8	189	0	1
10	75	m	ICUS	n.t.	7.9	14.8	12	n.t.	1
11	36	f	ICUS	46,XX	2.2	11.1	310	0	1
12	82	m	ICUS	n.t.	3.0	14.4	211	0	1
13	23	f	ICUS	n.t.	6.0	13.8	37	0	1
14	70	f	ICUS	46,XX	2.8	10.5	97	0	1
15	20	m	ICUS	46,XY	3.8	12.3	47	0	1
16	69	f	ICUS	n.t.	12.8	11.4	45	0	1
17	53	f	ICUS	46,XX	3.2	11.3	173	0	1
18	34	f	ICUS	n.t.	2.5	13.7	159	0	1
19	64	m	ICUS	46,XY	5.3	8.2	96	0	1
20	79	m	ICUS	n.t.	3.4	13.2	89	0	1
21	69	m	ICUS	46,XY	4.6	9.9	136	0	1
22	53	f	ICUS	n.t.	11.0	11.2	11	0	1
23	56	f	ICUS	n.t.	7.9	14.2	261	0	n.t.
24	34	f	ICUS	46,XX	4.8	11.6	201	0	1

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	BM
25	81	m	ICUS	45,X,-Y	9.1	10.3	258	0	1
26	69	m	ICUS	n.t.	2.4	10.6	1	0	1
27	39	m	ICUS	n.t.	9.5	14.8	27	0	n.t.
28	80	m	ICUS	46,XY	3.6	15.1	107	0	4
29	68	f	ICUS	46,XX	2.5	13.8	189	0	2
30	72	m	ICUS	46,XY	3.6	13.3	120	0	1
31	38	f	ICUS	46,XX	2.7	12.9	289	0	1
32	58	m	ICUS	n.t.	1.5	12.8	216	0	2
33	62	m	ICUS	n.t.	5.0	8.4	94	0	2
34	33	f	ICUS	46,XX	12.0	11.7	133	0	2
35	73	f	ICUS	n.t.	2.6	9.3	256	0	2
36	37	f	ICUS	n.t.	2.7	11.6	196	0	0
37	64	m	ICUS	n.t.	3.7	9.8	67	0	1
38	70	m	ICUS	n.t.	7.9	15.6	59	0	1
39	82	f	ICUS	n.t.	3.6	11.5	134	0	2
40	79	m	ICUS	n.t.	6.9	12.8	102	0	1
41	41	m	Hodgkin’s lymphoma	n.t.	10.0	15.5	279	0	1
42	57	m	suspected NHL	46,XY	6.0	17.6	95	0	1
43	38	f	suspected CML	n.t.	85.1	10.8	285	0	1
44	28	f	NHL	n.t.	6.0	10.8	297	0	1
45	43	m	Hodgkin’s lymphoma	n.t.	8.5	14.3	288	0	1
46	47	f	Hodgkin’s lymphoma	n.t.	8.2	13.2	345	0	1
47	78	m	NHL	n.t.	12.6	15.0	266	0	1
48	70	m	suspected NHL	n.t.	3.6	10.2	189	0	2
49	88	f	NHL	n.t.	9.8	12.9	297	0	1
50	74	m	NHL	n.t.	2.5	11.6	56	0	1
51	55	m	NHL	n.t.	6.6	13.4	86.3	0	1
52	23	m	NHL	n.t.	11.7	14.1	210	0	2

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	BM
53	52	m	NHL	n.t.	10.2	12.0	192	0	1
54	63	f	NHL	n.t.	6.8	14.6	208	0	1
55	66	f	NHL	n.t.	6.4	13.4	268	0	1
56	53	m	Hodgkin's lymphoma	n.t.	7.4	9.5	316	0	1
57	69	f	NHL	46,XX	6.2	12.9	276	n.t.	2
58	52	f	NHL	n.t.	7.4	11.6	587	0	1
59	48	f	NHL	n.t.	17.0	11.4	387	0	1
60	46	f	Hodgkin's lymphoma	n.t.	6.5	14.4	165	0	1
61	58	f	NHL	complex	5.1	12.0	230	0	2
62	50	m	suspected mastocytosis	n.t.	7.4	15.3	242	n.t.	1
63	65	f	normal bone marrow	46,XX	5.4	13.3	289	0	1
64	26	f	NHL	n.t.	6.3	12.8	345	0	1
65	37	m	Hodgkin's lymphoma	n.t.	7.0	15.1	245	0	1
66	87	m	normal bone marrow	46,XY	9.7	12.5	254	0	1
67	79	m	NHL	46,XY	12.7	12.5	627	0	1
68	49	f	NHL	n.t.	5.1	11.9	214	0	n.t.
69	47	f	MGUS	n.t.	5.6	12.1	222	0	2
70	69	f	Hodgkin's lymphoma	n.t.	8.7	14.2	292	0	1
71	82	m	NHL	n.t.	5.4	13.2	226	0	1
72	73	m	NHL	46,XY	4.5	8.5	n.t.	n.t.	1
73	76	m	Hodgkin's lymphoma	n.t.	4.6	9.0	142	0	1
74	40	m	NHL	n.t.	5.5	14.6	247	0	1
75	36	f	Ewing´s Sarcoma	n.t.	7.7	11.6	357	0	1
76	28	m	NHL	n.t.	13.2	14.6	303	0	1
77	38	f	Hodgkin's lymphoma	n.t.	7.7	14.0	280	0	1
78	28	m	NHL	n.t.	5.2	16.9	198	0	2
79	66	m	NHL	n.t.	19.3	15.6	223	0	1
80	30	m	Hodgkin's lymphoma	46,XY	6.0	13.0	258	0	2

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	BM
81	62	m	NHL	n.t.	4.5	13.1	60	0	1
82	19	m	suspected mastocytosis	n.t.	5.4	15.5	177	0	1
83	19	m	Hodgkin's lymphoma	n.t.	9.4	9.3	557	0	1
84	56	m	NHL	n.t.	6.6	16.6	166	0	1
85	61	m	NHL	46,XY	8.1	7.6	530	0	1
86	54	f	NHL	n.t.	6.4	13.4	202	0	1
87	23	m	suspected mastocytosis	46,XY	6.0	14.4	165	0	1
88	28	m	NHL	n.t.	6.8	14.0	326	n.t.	1
89	54	m	suspected mastocytosis	46,XY	9.5	16.0	356	0	1
90	27	f	Dysgerminoma	n.t.	9.1	13.8	354	0	1
91	71	f	NHL	n.t.	7.3	10.8	177	0	1
92	35	m	suspected mastocytosis	n.t.	7.3	14.2	241	0	1
93	81	f	NHL	n.t.	5.2	8.8	139	0	2
94	66	f	NHL	n.t.	5.9	14.9	307	0	1
95	24	f	NHL	n.t.	8.4	11.1	317	0	1
96	77	f	NHL	n.t.	3.9	10.2	110	0	1
97	32	m	suspected mastocytosis	46,XY	4.3	15.2	278	0	1
98	59	f	NHL	n.t.	4.9	12.3	238	0	1
99	42	m	suspected NHL	46,XY	15.7	7.8	259	1	1
100	73	m	NHL	n.t.	5.2	11.9	212	0	1
101	71	f	NHL	n.t.	17.8	9.7	530	0	1
102	24	m	Hodgkin's lymphoma	n.t.	6.3	16.4	191	0	1
103	57	m	NHL	n.t.	4.2	14.4	196	0	1
104	54	f	NHL	n.t.	15.5	9.1	331	0	1
105	47	f	cutaneous mastocytosis	n.t.	7.7	14.9	215	0	1
106	78	f	Hodgkin's lymphoma	n.t.	13.2	10.2	516	0	1
107	29	m	NHL	n.t.	12.1	13.7	375	0	1
108	39	f	Hodgkin's lymphoma	n.t.	11.0	9.6	614	0	1

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	BM
109	52	f	NHL	n.t.	1.9	13.2	67	0	1
110	64	f	NHL	46,XX	5.6	8.7	444	0	1
111	59	m	NHL	n.t.	31.0	14.9	276	0	1
112	44	f	suspected mastocytosis	n.t.	7.1	14.1	267	0	1
113	74	m	NHL	n.t.	8.1	9.7	345	0	1
114	46	m	NHL	n.t.	17.8	13.8	332	n.t.	1
115	75	m	NHL	n.t.	102.0	14.3	123	2	n.t.
116	38	m	Hodgkin's lymphoma	n.t.	14.8	13.1	234	n.t.	2
117	55	m	NHL	n.t.	1.7	9.2	49	0	1
118	27	m	Hodgkin's lymphoma	n.t.	7.8	13.1	336	0	n.t.
119	86	f	NHL	46,XX	8.8	11.9	184	0	1
120	68	f	NHL	46,XX	6.1	11.8	350	0	1
121	28	m	NHL	n.t.	6.7	13.7	305	0	1
122	71	m	NHL	n.t.	13.0	14.7	108	0	1
123	24	m	Hodgkin's lymphoma	n.t.	6.1	13.1	243	0	1
124	80	m	MGUS	n.t.	10.7	16.0	172	0	1
125	48	f	Hodgkin's lymphoma	n.t.	6.6	11.8	282	0	1
126	46	m	NHL	n.t.	2.9	11.4	28	0	1
127	60	m	NHL	n.t.	7.8	10.5	723	0	1
128	36	f	Hodgkin's lymphoma	n.t.	11.5	10.1	502	0	2
129	37	m	NHL	n.t.	8.8	12.9	391	0	1
130	64	m	NHL	n.t.	3.5	13.6	64	0	1
131	71	m	NHL	n.t.	8.5	13.6	228	0	1
132	23	m	Hodgkin's lymphoma	n.t.	11.9	12.1	427	0	2
133	70	m	NHL	n.t.	8.2	16.0	289	0	1
134	27	f	Hodgkin's lymphoma	n.t.	13.6	8.3	376	0	1
135	62	m	NHL	46,XY	2.9	14.8	131	0	1
136	31	f	Suspected mastocytosis	n.t.	4.7	13.7	229	0	1

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	BM
137	72	f	Breast Cancer	n.t.	23.5	8.6	91	0	1
138	51	m	NHL	n.t.	6.7	14.0	84	0	1
139	19	m	NHL	n.t.	9.5	12.2	362	n.t.	2
140	68	f	NHL	n.t.	9.3	10.6	350	0	1
141	52	m	Ewing´s sarcoma	n.t.	8.0	15.0	203	0	1
142	64	m	NHL	n.t.	6.3	15.1	211	n.t.	1
143	70	m	NHL	n.t.	13.7	10.4	91	0	2
144	70	m	NHL	n.t.	8.5	12.8	291	n.t.	1
145	60	m	NHL	n.t.	5.9	10.9	334	1	1
146	72	m	NHL	n.t.	6.9	13.7	205	n.t.	1
147	72	m	NHL	n.t.	13.8	14.3	286	0	1
148	20	m	NHL	n.t.	12.7	13.6	520	1	2
149	49	f	NHL	46,XX	2.3	8.4	541	0	1
150	52	m	MGUS	n.t.	7.2	15.3	219	0	1
151	34	m	NHL	n.t.	10.7	12.6	196	0	1
152	48	m	NHL	46,XY	12.4	15.7	196	0	1
153	47	m	Agranulocytosis	n.t.	1.7	10.6	399	0	2
154	19	m	NHL	n.t.	11.5	13.4	287	0	1
155	34	m	NHL	n.t.	18.1	12.8	312	0	n.t.
156	61	m	NHL	n.t.	20.9	12.7	263	0	1
157	45	m	NHL	n.t.	5.1	14.5	245	0	1
158	57	f	NHL	n.t.	6.1	9.3	206	0	2
159	58	m	NHL	n.t.	12.1	14.1	222	n.t.	1
160	71	m	NHL	complex	44.8	11.9	19	0	2
161	66	m	NHL	n.t.	5.2	11.9	76	0	2
162	34	m	Suspected NHL	n.t.	4.8	11.2	189	n.t.	3
163-179	n.a.	n.a.	Normal bone marrow (purchased from Lonza)	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

No. #	age (yrs)	gender (m/f)	diagnosis	karyotype	WBC (G/L)	Hb (g/dL)	Plt (G/L)	Blast (%) PB	BM
180-205	n.a.	n.a.	Cord blood samples	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

Abbreviations: No. #, patient number; WBC, white blood count; Hb, hemoglobin; Plt, platelet count; yrs, years; m, male; f, female; PB, peripheral blood; BM, bone marrow; ICUS, idiopathic cytopenia of undetermined significance; NHL, Non-Hodgkin's lymphoma; CTLC, cutaneous T-cell lymphoma; MGUS, monoclonal gammopathy of undetermined significance; n.t., not tested.

Table S6

Sequences of oligonucleotide primers used for qPCR analyses

Gene	Sequence
huABCB10-fwd	GTGGCCTTCATCCGGAATTT
huABCD10-rev	ATCGCAATCCGCTGTTTCTG
huIL2Ra-fwd	GGACTGCTCACGTTCATCATGG
huIL2Ra-rev	GCTTTGAATGTGGCGTGTGG
huCD9-fwd	ACATCATCGGCGCAGTGG
huCD9-rev	GGTTCCTGCGGATAGCACAG
huCD26-fwd	TCAATATCTCCTGATGGGCAGTTT
huCD26-rev	TGTCATATGAAGCTGTGTAGGAATGC
huCD33-fwd	CCCAGCTCTCTGTGCATGTGA
huCD33-rev	GAGTGCCAGGGATGAGGATTT
huCD36-fwd	TGATGAACAGCAGCAACATTCA
huCD36-rev	TCCTCAGCGTCCTGGGTTAC
huCD44-fwd	ATGGCCCAGATGGAGAAAGC
huCD44-rev	GGAATCACCACGTGCCCTTC
huCD46-fwd	CTTTCCTTCCTGGCGCTTTC
huCD46-rev	CAAATGTTGGTGGCTCCTCA
huCD52-fwd	TCCTCCTACTCACCATCAGCCTCCTG
huCD52-rev	GGGCTGCTGGTTGGCTGGT
huCD90-fwd	CTGCAGCAGCGGAAGACC
huCD90-rev	GCAGGAGAGCGATGCTGATG
huCD96-fwd	GCTCTGTCTCCAGTCCCAGGAA
huCD96-rev	TCAGTGGAGGGTCTGTTGAGGA
huCD114-fwd	CTGACTTGGGCTGCCCTGAT
huCD114-rev	ACTGATGTGCCCGCACTCCT
huCD115-fwd	GCCGCCATCCACCTCTATGT
huCD115-rev	GAACACGACCACCTCCTGTGC
huCD116-fwd	CCACACCCAGCATTCCTCCT
huCD116-rev	AGGCTGGTGCCACTGTTCG
huCD117-fwd	GCGTTCTGCTCCTACTGCTTCG
huCD117-rev	CCCTGGACTCACAGATGGTTGA
huCD122-fwd	GGGCTCTGCAGGACACTTCC
huCD122-rev	GGAGCAGCTCACAGGTTTGG
huCD123-fwd	CCACGAAGGCTGTTTCTTCCA
huCD123-rev	CTCCGGAACGCAGGACAGAG
huCD131-fwd	CCGTCACTCTGACCCAGCAT
huCD131-rev	AAGTGGTCCTGGTCGGTGCT

Gene	Sequence
huCD133-fwd	GCGGAATTCTTGACCGACTGA
huCD133-rev	CCAACGCCTCTTTGGTCTCC
huCD135-fwd	TCAGCTGGCAGATGCAGAAG
huCD135-rev	CATTCCGAAACACGGCCATC
huCD151-fwd	CGAGACCATGCCTCCAACAT
huCD151-rev	CCAATGACCCTCAGGTGCTC
huCD164-fwd	TAGCGCCCATCTCCAACGTA
huCD164-rev	ACGCAGCTGTTTCGACCTTC
huCD184-fwd	GCTACCTGGCCATCGTCCAC
huCD184-rev	AGGAGGGCAGGGATCCAGAC
huCD200-fwd	GTTTGGGTCATGGCAGCAGT
huCD200-rev	CATCCTGGGTCACCACTTGC
huCD203c-fwd	TGAGGATGCCCATGTGGAGTTCA
huCD203c-rev	TGGGAGGCAGAGGCGATGTG
huCD221-fwd	TGGCCGACGAGTGGAGAAAT
huCD221-rev	CTGCTGATAGTCGTTGCGGATG
huCD243-fwd	GCAGCAAAGGAGGCCAACAT
huCD243-rev	TCTGGCCACCAGAGAGCTGA
huCD271-fwd	CTGCACCGTGTGCGAGGAC
huCD271-rev	AATCCAACGGCCAGGGATCT
huCD309-fwd	GCGTGTGGCACCCACGATCA
huCD309-rev	GTTCCCCTCCATTGGCCCGC
huCLL1-fwd	TGCTGCTAGCTGGGATGTGG
huCLL1-rev	GCTCCGCAAATGAGAAGCAA
hu-MET-fwd	CGG ACC CAA TCA TGA GCA CTG
hu-MET-rev	ATC ACG GCG CGC TTC ACA G
huEPO-R-fwd	CCCGGGCAACTACAGCTTCT
huEPO-R-rev	GCAGGCGACACAGCT TCCAT
huITGA6-fwd	CCCGCTGGTTATAATCCTTCAA
huITGA6-rev	TGAACTCTTGAGGATAGCCCAGAT
huITGA2B-fwd	ATGGAGACACCCATGTGCAG
huITGA2B-rev	ACGCTGGCAGTGAGCTGAAG
huITGB3-fwd	TGTGTGCCTGGTGCTCTGAT
huITG3B-rev	CCTTCAGGTCACAGCGAGGT
huITGB3BP-fwd	AACTGAAAGCAAAGAATCTACAACAAA
huITGB3BP-rev	TTGCATTATCTCCATGATTTCTTCTG
huGP1BA-fwd	TGCCTTCGGAGGTCTTTCTG

Gene	Sequence
huGP1BA-rev	CAGGAGCAGCAAGAGGAGGA
huIL-1RAP-fwd	CCGGGCTCATTTTGGAACAG
huIL-1RAP-rev	TTCTTCCGCATTCCTTGCAT
huNPDC1-fwd	GCACAGAGCGCGGAGATGTA
huNPDC1-rev	CAGCTCCTTGGGTGGCTCTT
huOSM-R-fwd	TGGGTGCTTCTCCTGCTTCTG
huOSM-R-rev	CCACCCTCTGTGCCTGCAAT
huROBO4-fwd	CCTGGCAGTGGCTGTGGATA
huROBO4-rev	GGAGGGAGGTGATGAGGCATCT
huBCR/ABL1-fwd	TCCGCTGACCATCAATAAGGA
huBCR/ABL1-rev	CACTCAGACCCTGAGGCTCAA
huABL1-fwd	TGTATGATTTTGTGGCCAGTGGAG
huABL1-rev	GCCTAAGACCCGGAGCTTTTCA
huPDCD10-fwd	TGAAGCTGAGACCACATCCA
huPDCD10-rev	TCTGGGCTGCAGACAGATTT
huSELP-fwd	CAGTCTGGGTTGGGCAGAAG
huSELP-rev	AAGATGGCTATTTGGCAGTTGG

Abbreviations: IL-2R, interleukin-2 receptor; CLL1, C-type lectin-like molecule-1; EPO-R, erythropoietin receptor; NPDC1, neural proliferation differentiation and control protein 1; IL-1RAP, interleukin-1 receptor accessory protein; OSMR, oncostatin M receptor; ROBO4, roundabout 4; fwd, forward; rev, reverse.

Table S7

FISH probes used for detection of cytogenetic abnormalities in purified AML cells

Chromosomal aberration	FISH probes*
inv(16) or -16	CBFB t(16;16); inv(16)
t(3;3)	MECOM t(3;3); inv(3)(3q26)
del(5q)	5q- (5q31; 5q33)
t(8;21)	RUNX1/RUNX1T t(8;21)

Abbreviations: FISH, fluorescence in situ hybridization; AML, acute myeloid leukemia. *FISH probes were purchased from Kreatech (Amsterdam, The Netherlands).

Table S8

FISH analysis of purified $CD34^+/CD38^-$ and $CD34^+/CD38^+$ AML cells

No. #	AML variant	Chromosomal aberration	% of cells with abnormal karyotype by FISH		
			MNC	$CD34^+/CD38^-$	$CD34^+/CD38^+$
#5	AML M4eo	inv(16)	88%	100%	100%
#19	AML M4eo	inv(16)	70%	100%	100%
#22	AML M0	t(3;3)	80%	100%	100%
#113	AML M4	-16	81%	100%	100%
#191	sAML	del(5q)	92%	100%	100%
#210	AML M1	del(5q)	81%	100%	100%
#264	AML M1	t(8;21)	100%	100%	100%

FISH analysis was performed as described in the text in this supplement. The percentage of interphases expressing the abnormal karyotype defined by FISH probes was determined by microscopy. AML samples obtained at diagnosis. Patients´ numbers (#) refer to the patients´ numbers shown in Table S3. Abbreviations: AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; No. #, patient number; MNC, mononuclear cells; sAML, secondary AML. The FAB category of AML (M0, M1, M4, M4eo) is also shown.

Table S9A

Expression of cytokine receptors on leukemic stem- and progenitor cells and comparison to normal bone marrow (BM) cells

		Expression of surface antigens on progenitor cells and stem cells (% positive cases in brackets)											
		Normal control BM				AML				CML			
Antigen	CD	$CD34^+/CD38^+$		$CD34^+/CD38^-$		$CD34^+/CD38^+$		$CD34^+/CD38^-$		$CD34^+/CD38^+$		$CD34^+/CD38^-$	
IL-2RA	CD25	−	(12)	−	(8)	+	(43)	+	(48)	+/−	(28)	+	(93)
Endoglin	CD105	+	(100)	+	(100)	+	(85)	+	(93)	+	(100)	+	(100)
TPOR	CD110	−/+	(67)	−/+	(56)	−	(0)	−	(5)	−	(22)	−	(11)
G-CSFR	CD114	+	(50)	+	(64)	+	(88)	+	(64)	+	(68)	+	(75)
M-CSFR	CD115	−	(0)	−	(0)	−	(14)	−	(17)	−	(20)	−	(0)
GM-CSFR	CD116	+/−	(75)	−/+	(38)	+/−	(67)	+/−	(47)	+/−	(46)	+/−	(50)
KIT/SCFR	CD117	+	(100)	+	(100)	+	(99)	+	(99)	+	(98)	+	(96)
IL-2RB	CD122	−	(0)	−	(0)	−	(6)	−	(6)	−	(10)	−	(10)
IL-3RA	CD123	+	(91)	+	(95)	+	(99)	+	(98)	+	(96)	+	(100)
CR-ß-chain	CD131	−	(17)	−	(0)	−	(6)	−	(0)	−	(25)	−	(0)
FLT3	CD135	+/−	(45)	+/−	(40)	+	(93)	+	(91)	+/−	(64)	+	(64)
CXCR4	CD184	+/−	(68)	+/−	(55)	+	(75)	+	(72)	+	(82)	+	(82)
IGF-1R	CD221	−/+	(22)	−/+	(25)	−	(19)	−	(18)	−/+	(42)	−/+	(42)
NGFR	CD271	−	(0)	−	(0)	−	(0)	−	(0)	−	(11)	−	(11)
KDR/VEFGR2	CD309	−	(0)	−	(0)	−	(0)	−	(3)	−	(10)	−	(7)
FGFR2	CD332	−/+	(20)	−/+	(20)	−/+	(33)	−/+	(33)	+/−	(44)	+/−	(44)
EPOR	n.c.	+/−	(50)	+/−	(50)	−	(3)	−	(4)	−	(25)	−	(8)
OSMRB	n.c.	−	(13)	−	(14)	+/−	(52)	+/−	(41)	−/+	(32)	+/−	(44)
IL-1RAP	n.c.	−/+	(36)	−	(0)	+	(79)	+	(65)	+	(95)	+	(77)
MET/HGFR	n.c.	−	(0)	−	(0)	−	(0)	−	(0)	−	(0)	−	(0)

Expression of surface antigens on $CD34^+/CD38^-$ and $CD34^+/CD38^+$ bone marrow (BM) stem- and progenitor cells was examined by multi-color flow cytometry. Leukemic samples were obtained at diagnosis (AML: at least 6 cases per marker tested; CML: at least 5 cases per marker tested).

Control samples (normal BM; at least 5 cases per marker tested) included purchased $CD34^+$ BM cell subsets and BM cells obtained from cases with suspected hematologic neoplasm without persistent cytopenia. Results show the levels of expression of surface markers (as per score defined below) and as % of positive cases in each group (in brackets). Score of antibody reactivity: +, clear expression in majority of cases; +/−, weak expression in majority of cases; −/+, expression in minority of cases; −, no expression in a vast majority of cases. Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; n.c., not yet clustered.

Table S9B

Estimation of sites per cell of cytokine receptors on leukemic stem cells

		AML $CD34^+/CD38^-$			CML $CD34^+/CD38^-$		
Antigen	CD	median SI	median sites/cell (25^{th}–75^{th} quantile)		median SI	median sites/cell (25^{th}–75^{th} quantile)	
IL-2RA	CD25	1.4	<100	(49-261)	10.4	625	(133-1081)
Endoglin	CD105	4.2	258	(164-579)	10.9	658	(466-995)
TPOR	CD110	1.0	<100	(53-96)	1.2	<100	(54-81)
G-CSFR	CD114	2.2	136	(84-226)	2.8	171	(114-327)
M-CSFR	CD115	1.1	<100	(62-76)	0.8	<100	(64-66)
GM-CSFR	CD116	1.3	<100	(59-126)	2.3	138	(46-235)
KIT/SCFR	CD117	32.1	1932	(867-4026)	15.5	939	(401-2174)
IL-2RB	CD122	0.9	<100	(46-66)	0.8	<100	(45-71)
IL-3RA	CD123	12.2	738	(356-2173)	9.9	598	(344-875)
CR-ß-chain	CD131	1.0	<100	(55-62)	0.8	<100	(38-54)
FLT3	CD135	7.6	464	(264-1002)	2.6	161	(106-222)
CXCR4	CD184	2.2	133	(98-235)	3.8	232	(120-422)
IGF-1R	CD221	1.1	<100	(59-112)	1.7	104	(81-120)
NGFR	CD271	0.7	<100	(37-50)	0.6	<100	(34-48)
KDR/VEFGR2	CD309	1.1	<100	(56-76)	1.1	<100	(60-92)
FGFR2	CD332	1.5	<100	(83-129)	1.8	112	(92-152)
EPOR	n.c.	1.1	<100	(61-78)	1.4	<100	(76-90)
OSMRB	n.c.	1.2	<100	(57-275)	1.5	<100	(79-122)
IL-1RAP	n.c.	3.0	186	(91-698)	3.1	190	(103-404)
MET/HGFR	n.c.	0.9	<100	(48-64)	1.0	<100	(60-66)

Cytokine receptors on leukemic $CD34^+/CD38^-$ cells in patients with AML and CML (leukemic samples obtained at diagnosis – see Table S9A) were analyzed by flow cytometry using PE-labeled mAb (specification of mAb is shown in Table S1). For estimation of the number of PE-molecules bound to the cell surface, the Quantibrite kit (BD Biosciences) was applied and staining indices were correlated with absolute numbers of PE-molecules as described in the supplemental methods. The PE-molecules on the cell surface correspond to sites per cell, assuming a ratio 1:1 for

PE-molecules per mAb. Results show the median staining index (ratio of PE-labeled mAb/isotype control mAb) and sites per cell including the range of the 25^{th}–75^{th} quantile of all tested patients in brackets. The detection limit was defined as 100 sites/cell which corresponds to a staining index of 1.6. Abbreviations: SI, staining index, AML, acute myeloid leukemia; CML, chronic myeloid leukemia; n.c., not yet clustered.

Table S10

Flow cytometry staining results of differentially expressed markers on normal $CD34^{+}/CD38^{-}$ bone marrow (BM) HSC and cord blood (CB) $CD34^{+}/CD38^{-}$ stem cells

Antigen	Normal Bone Marrow $CD34^{+}/CD38^{-}$ HSC		Cord Blood $CD34^{+}/CD38^{--}$	
	% of patients positive (No. of patients tested)	Mean staining index	% of patients positive (No. of patients tested)	Mean staining index
CD25	8% (25)	0.9	22% (9)	1.2
CD33	100% (15)	14.3	100% (14)	32.2
CD52	54% (24)	3.2	100% (9)	12.7
CD93	63% (8)	2.3	100% (2)	13.5
CD123	95% (21)	4.3	100% (14)	8.3
CD221	25% (8)	2.0	100% (14)	6.3
IL-1RAP	0% (11)	1.0	89% (9)	3.4
HLA-DR	83% (6)	3.3	100% (6)	22.6

Normal BM stem cells were purchased from Lonza. Abbreviations: CD, cluster of differentiation; No., number; CB, cord blood; HSC, hematopoietic stem cells; IL-1RAP, interleukin-1 receptor accessory protein; HLA-DR, human leukocyte antigen.

Table S11

Flow cytometry staining results of normal BM $CD34^{+}/CD38^{-}$ and $CD34^{+}/CD38^{+}$ cells

Antigen	Normal Bone Marrow $CD34^{+}/CD38^{-}$		Normal Bone Marrow $CD34^{+}/CD38^{+-}$	
	% of patients positive (No. of patients tested)	Mean staining index	% of patients positive (No. of patients tested)	Mean staining index
CD18	71% (7)	2.5	100% (7)	5.4
CD33	100% (15)	14.3	100% (15)	20.9
CD90	85% (13)	3.7	8% (13)	0.9
CD114	64% (11)	2.2	50% (12)	4.4
CD117	100% (20)	35.7	100% (21)	34.5
CD371	0% (9)	0.8	100% (9)	6.1
HLA-DR	83% (6)	3.3	100% (6)	10.7
IL-1RAP	0% (11)	1.0	36% (11)	1.5
NPDC1	50% (4)	1.7	0% (4)	1.4
ROBO4	100% (6)	3.6	50% (6)	1.7

Normal BM stem cells were purchased from Lonza or obtained from patients with suspected BM neoplasm. Abbreviations: CD, cluster of differentiation; No, number; BM, bone marrow; HSC, hematopoietic stem cells; IL-1RAP, interleukin-1 receptor accessory protein; ROBO4, roundabout 4; HLA-DR, human leukocyte antigen.

Table S12

Antigen expression on mature cells as determined by flow cytometry

Antigen	Basophils	Eosinophils	T cells	B cells	Monocytes	Neutrophils
CD9	+	+	+/–	+/–	+	+
CD25	+	–	+/–	+/–	–	–
CD26	+	–	+	–	–	–
CD33	+	+	–	–	+	+
CD36	+	+	+	+	+	+
CD44	+	+/–	+	+	+	+
CD51/61	–	–	–	–	+/–	–
CD52	+/–	+/–	+	+	+	–
CD56	–	–	–	–	–	–
CD61	+/–	+/–	+/–	+/–	+	+/–
CD90	–	+/–	–	+	+/–	+/–
CD95	+	–	+/–	–	+	+
CD96	–	–	+	+/–	–	–
CD105	+/–	–	–	+/–	+	+/–
CD114	–	–	–	–	+/–	+
CD115	–	–	–	–	+	+/–
CD117	–	–	–	–	–	–
CD122	–	–	+/–	+	–	–
CD123	+	+/–	–	+/–	+	+/–
CD129	–	–	–	–	+/–	+/–
CD131	+	+	–	–	+/–	+
CD133	–	–	–	–	–	–
CD135	+/–	–	–	–	–	–
CD150	–	–	+/–	+	–	–
CD203c	+	–	–	–	–	–
MET	–	–	–	–	–	–
EPOR	–	–	–	–	–	–
OSMRß	–	–	–	–	+	–

Peripheral blood cells from healthy donors (n=5) were stained with fluorochrome-labeled monoclonal antibodies (mAb) and analyzed by flow cytometry on FACSCanto II. Basophils were defined as $CD123^+/CD203c^+$ cells, eosinophils were defined as forward and side-scatter high and highly ´auto-fluorescent´ cells, T cells were defined as $CD3^+/CD19^-$, B cells were defined as $CD19^+/CD3^-$, Monocytes were defined as $CD14^+$, and neutrophil granulocytes were defined as $CD45^+$ and side scatter high with exclusion of eosinophils and monocytes.
Antigen expression of tested mAb: +: most of cells positive in most donors, +/–: positive subpopulation or weak expression; –: negative staining in most donors

Table S13A

Expression of cell surface antigens on normal BM HSC and LSC in AML and CML as determined by flow cytometry

Antigen	Normal Bone Marrow $CD34^+/CD38^-$ HSC		AML $CD34^+/CD38^-$		CML $CD34^+/CD38^-$	
	% of patients positive (No. of patients tested)	Mean staining index	% of patients positive (No. of patients tested)	Mean staining index	% of patients positive (No. of patients tested)	Mean staining index
CD9	14% (7)	1.3	63% (27)	4.2	100% (30)	8.9
CD25	8% (25)	0.9	48% (164)	6.5	93% (72)	13.3
CD26	0% (22)	0.8	10% (164)	2.7	97% (74)	9.7
CD33	100% (15)	14.3	96% (90)	49.3	100% (34)	49.6
CD36	57% (7)	2.6	45% (22)	7.8	55% (38)	5.6
CD44	100% (15)	113.6	98% (63)	44.3	100% (36)	91.1
CD47	100% (8)	44.6	100% (23)	43.1	100% (33)	54.8
CD52	54% (24)	3.3	51% (172)	3.2	81% (53)	3.2
CD56	8% (13)	0.9	18% (40)	5.0	91% (33)	6.6
CD69	33% (3)	2.1	86% (7)	5.6	100% (3)	3.4
CD90	85% (13)	3.7	13% (24)	1.4	91% (11)	15.4
CD93	63% (8)	2.3	72% (32)	6.0	86% (14)	9.7
CD96	0% (10)	1.0	40% (42)	4.1	0% (11)	0.9
CD105	100% (10)	6.6	93% (45)	8.1	100% (51)	12.8
CD114	64% (11)	2.2	64% (87)	3.5	75% (24)	4.1
CD116	38% (8)	1.9	47% (17)	5.0	50% (24)	2.8
CD117	100% (20)	35.7	99% (158)	50.4	96% (46)	27.9
CD123	95% (21)	4.3	98% (167)	27.5	100% (24)	10.6
CD164	86% (7)	5.5	14% (22)	4.0	86% (7)	8.9
CD184	55% (22)	2.6	72% (78)	3.2	82% (38)	5.2
CD371	0% (9)	0.8	68% (80)	9.1	17% (6)	6.7
HLA-DR	83% (6)	3.3	71% (24)	7.0	100% (21)	82.7
IL-1RAP	0% (11)	1.0	65% (112)	8.8	77% (62)	5.3
ROBO4	100% (6)	3.6	29% (42)	1.8	85% (27)	2.9

Expression of surface antigens on $CD34^+/CD38^-$ stem cells was examined by multi-color flow cytometry. All leukemic samples obtained at diagnosis. Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CD, cluster of differentiation; No, number; HSC, hematopoietic stem cells; LSC, leukemic stem cells; IL-1RAP, interleukin-1 receptor accessory protein; ROBO4, roundabout 4; HLA-DR, human leukocyte antigen.

Table S13B

Expression of antigens on purified normal hematopoietic stem cells, cord blood $CD34^+/CD38^-$ and leukemic $CD34^+/CD38^-$ cells as determined by qPCR

Antigen	Normal BM $CD34^+/CD38^-$	Cord Blood $CD34^+/CD38^-$	AML $CD34^+/CD38^-$	CML $CD34^+/CD38^-$
CD9	+	+	n.t.	+
CD25	+	+	+	+
CD26	n.t.	–	–	+
CD33	–	+	+	+
CD36	–	+	n.t.	+
CD44	+	+	+	+
CD52	+	+	+	+
CD90	+	+	–	–
CD96	–	–	+	–
CD114	–	–	+	+
CD116	–	–	+	–
CD117	+	+	+	+
CD123	–	–	+	+
CD164	+	+	n.t.	+
CD184	+	+	+	+
CD371	+	+	+	–
IL-1RAP	–	+	+	–
ROBO4	–	–	–	+
NPDC1	–	–	+	+

$CD34^+/CD38^-$ cells from patients with AML (n=14), CML (n=4) (leukemic samples obtained at diagnosis or relapse) and from normal bone marrow (n=3) and cord blood (n=4) were sorted by flow cytometry and then conducted to qPCR analysis. Results are expressed as + (relative ′over-expression′) and – (relative ′under-expression′). Abbreviations: qPCR, quantitative polymerase chain reaction; BM, bone marrow; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; n.t., not tested.

Table S14A

Expression of novel and established stem cell antigens on AML stem cells – comparison between flow cytometry data and gene chip results

		Expression of cell surface markers on stem cells (mRNA and protein level)					
		AML #210* $CD34^+/CD38^-$		AML #173* $CD34^+/CD38^-$		AML #102* $CD34^+/CD38^-$	
Antigen	CD	gene chip	flow	gene chip	flow	gene chip	flow
IL-1RAP	n.c.	+	+/−	+	−	+	+
IL-2RA	CD25	−/+	−	+/−	+/−	+	+
DPPIV	CD26	+/−	−	+/−	−	+/−	−
Campath-1	CD52	+/−	+	+/−	+/−	−	−

Comparison of expression of stem cell markers in $CD34^+/CD38^-$ AML stem cells by multi-color flow cytometry and microarray studies. Leukemic samples obtained at diagnosis. Gene chip results were scored and normalized to normal $CD34^+/CD38^-$ bone marrow cells: −, less than 50% expression compared to normal cells; −/+, 50-100%; +/−, 101-200%; +, >200% compared to normal cells. Flow cytometry results are presented as staining index (SI) and were graded using the following score: −, 0-1.3; +/−, 1.31-3; +, 3.01-10.

*Patients´ numbers (#) refer to the patients´ numbers defined in Table S3. Abbreviations: AML, acute myeloid leukemia; n.c., not yet clustered; IL-1RAP, interleukin-1 receptor-associated protein; IL-2RA, IL-2 receptor alpha chain; DPPIV; dipeptidyl peptidase IV; flow, flow cytometry.

Table S14B

Expression of novel and established stem cell antigens on CML stem cells – comparison between flow cytometry data and gene chip results

		Expression of surface markers on stem cells (mRNA and protein level)					
		CML #8 $CD34^+/CD38^-$		CML #11 $CD34^+/CD38^-$		CML #21 $CD34^+/CD38^-$	
Antigen	CD	gene chip	flow	gene chip	flow	gene chip	flow
IL-1RAP	n.c.	+/−	+	+/−	−	+	+
Tetraspanin 29	CD9	−/+	+	−/+	+/−	+/−	+
IL-2RA	CD25	+	+	+	+	+	+
DPPIV	CD26	+	+	+/−	+	+	+
Siglec-3	CD33	+/−	+	+/−	+	+/−	+
KIT/SCFR	CD117	−	+	−	−	−	+/−

Comparison of expression of stem cell markers on $CD34^+/CD38^-$ CML stem cells by multi-color flow cytometry and microarray studies. Leukemic samples obtained at diagnosis. Gene chip results were scored and normalized to normal $CD34^+/CD38^-$ bone marrow cells: −, less than 50% expression compared to normal cells; −/+, 50-100%; +/−, 101-200%; +, >200% compared to normal cells. Flow cytometry results are presented as staining index (SI) and were graded using the following score: −, 0-1.3; +/−, 1.31-3; +, 3.01-10.

*Patients´ numbers (#) refer to the patients´ numbers defined in Table S2. Abbreviations: CML, chronic myeloid leukemia; n.c., not yet clustered; IL-1RAP, interleukin-1 receptor-associated protein; IL-2RA, IL-2 receptor alpha chain; DPPIV; dipeptidyl peptidase IV; SCFR, stem cell factor receptor; flow, flow cytometry.

Table S15

Correlation between surface marker expression on $CD34^+/CD38^-$ cells and *FLT3* mutation status in AML

Marker	Mean staining index (n)			p value	Mean percentage of positive cells (n)			p value
	Normal BM	AML *FLT3* ITD	AML *FLT3* wt	*FLT3* ITD vs. wt	Normal BM	AML *FLT3* ITD	AML *FLT3* wt	*FLT3* ITD vs. wt
CD25	0.9 (25)	17.7 (39)	3.6 (115)	<0.001**	6.8 (25)	60.6 (39)	26.5 (115)	<0.001**
CD26	0.8 (22)	7.9 (40)	1.2 (115)	<0.001**	1.5 (22)	30.5 (40)	3.2 (115)	<0.001**
CD33	14.3 (15)	93.2 (21)	42.6 (62)	0.003 **	93.8 (15)	92.8 (21)	74.8 (62)	<0.001**
CD52	3.3 (24)	3.6 (41)	3.0 (119)	n.s.	43.1 (24)	43.9 (41)	28.9 (119)	0.052
CD61	1.0 (12)	0.7 (14)	1.1 (41)	n.s.	11.6 (12)	2.4 (14)	10.6 (41)	0.015 *
CD96	1.0 (10)	6.4 (11)	2.4 (28)	0.100	2.2 (10)	55.5 (11)	18.7 (28)	0.022 *
CD105	6.6 (10)	3.5 (12)	10.1 (30)	0.015 *	86.4 (10)	61.9 (12)	81.1 (30)	0.040 *
CD117	35.7 (20)	19.3 (36)	58.9 (111)	<0.001**	96.5 (20)	85.2 (36)	94.5 (111)	<0.001**
CD123	4.3 (21)	62.8 (37)	20.1 (118)	<0.001**	64.3 (21)	92.1 (37)	85.2 (118)	<0.001**
CD133	17.6 (15)	14.8 (34)	20.8 (98)	0.003 **	99.0 (15)	58.9 (34)	86.5 (98)	0.003 **
CD184	2.6 (22)	12.5 (17)	3.3 (59)	0.063	42.9 (22)	32.3 (17)	43.0 (59)	n.s.
IL-1RAP	1.0 (11)	17.4 (28)	6.3 (76)	<0.001**	2.0 (11)	82.0 (28)	43.0 (76)	<0.001**

Normal bone marrow $CD34^+/CD38^-$ cells and $CD34^+/CD38^-$ cells from AML patients (samples obtained at diagnosis or relapse) with mutated *FLT3* and wild type *FLT3* were analyzed for marker expression with flow cytometry. Pairwise Wilcoxon rank-sum test between subgroups was performed when Kruskal-Wallis test showed significant differences ($p<0.05$). P-values show results of pairwise Wilcoxon rank-sum-test between AML with mutated *FLT3* vs. wild type *FLT3* corrected for multiple testing with Benjamini-Hochberg method.

*, significant difference with $p < 0.05$; **, highly significant with $p < 0.01$. Abbreviations: n, number of tested patients in each subgroup per marker; wt, wild type; n.s., not significant in Kruskal-Wallis test or pairwise Wilcoxon rank-sum test.

Table S16

AML $CD34^+/CD38^-$: marker expression differences in AML subgroups with *NPM1* mutation vs. *NPM1* wt

Marker	Mean staining index (n)			p value	Mean percentage of positive cells (n)			p value
	Normal BM	AML *NPM1* mut	AML *NPM1* wt	*NPM1* mut vs. wt	Normal BM	AML *NPM1* mut	AML *NPM1* wt	*NPM1* mut vs. wt
CD25	0.9 (25)	12.1 (47)	4.5 (122)	0.002 **	6.8 (25)	47.9 (47)	27.7 (122)	<0.001**
CD26	0.8 (22)	6.5 (47)	1.2 (122)	<0.001**	1.5 (22)	20.9 (47)	4.8 (122)	<0.001**
CD33	14.3 (15)	98.1 (19)	37.0 (46)	0.038 *	93.8 (15)	84.9 (19)	77.7 (46)	0.021*
CD96	1.0 (10)	5.7 (11)	1.9 (19)	0.011 *	2.2 (10)	50.3 (11)	14.8 (19)	0.016*
CD117	35.7 (20)	27.5 (42)	59.3 (98)	0.002 **	96.5 (20)	91.6 (42)	93.2 (98)	n.s.
CD123	4.3 (21)	54.2 (43)	19.6 (97)	0.002 **	64.3 (21)	89.0 (43)	85.1 (97)	0.006 **
CD371	0.8 (9)	4.1 (26)	11.3 (57)	0.042 *	4.6 (9)	33.0 (26)	46.8 (57)	0.169
IL-1RAP	1.0 (11)	11.6 (36)	7.8 (79)	0.098	2.0 (11)	64.7 (36)	46.9 (79)	0.007 **

Normal bone marrow $CD34^+/CD38^-$ cells and $CD34^+/CD38^-$ leukemic stem cells obtained from AML patients (samples obtained at diagnosis or relapse) with *NPM1* mutation and wild type *NPM1* were analyzed for surface marker expression by flow cytometry. Pair-wise Wilcoxon rank-sum test was performed as post-hoc analysis when Kruskal-Wallis test showed significant differences ($p<0.05$). P-values show results of pairwise Wilcoxon rank-sum-test between AML with mutated *NPM1* vs. wild type *NPM1* corrected for multiple testing with Benjamini-Hochberg method.

*, significant difference with $p < 0.05$; **, highly significant with $p < 0.01$.

Abbreviations: n, number of tested patients in each subgroup per marker; mut, mutated; wt, wild type; n.s., not significant in Kruskal-Wallis test or pairwise Wilcoxon rank-sum test.

Table S17

Correlations between surface marker expression on $CD34^+/CD38^-$ cells and the karyotype (complex vs. non-complex) in our patients with AML

Marker	Mean staining index (n)			p value	Mean percentage of positive cells (n)			p value
	Normal BM	AML complex karyotype	AML non-complex karyotype	Complex vs. non-complex	Normal BM	AML complex karyotype	AML non-complex karyotype	Complex vs. non-complex
CD26	0.8 (22)	0.8 (26)	2.9 (152)	0.017 *	1.5 (22)	2.4 (26)	10.0 (152)	0.013 *
CD117	35.7 (20)	72.3 (24)	47.4 (144)	0.013 *	96.5 (20)	96.0 (24)	90.8 (144)	n.s.
CD325	3.3 (10)	3.2 (11)	2.3 (39)	0.094	46.5 (10)	39.6 (11)	25.0 (39)	0.091

Normal bone marrow $CD34^+/CD38^-$ cells and $CD34^+/CD38^-$ leukemic stem cells obtained from AML patients (samples obtained at diagnosis or relapse) with complex karyotype (≥3 cytogenetic aberrations) and patients with a non-complex karyotype (0-2 cytogenetic aberrations) were analyzed for surface marker expression by flow cytometry. P values show results of pair-wise Wilcoxon rank-sum test that was performed as post-hoc analysis when Kruskal-Wallis test showed significant differences ($p<0.05$). P-values show results of pairwise Wilcoxon rank-sum-test between AML with mutated complex karyotype vs. AML with non-complex karyotype corrected for multiple testing with Benjamini-Hochberg method.

*, significant difference with $p < 0.05$;

Abbreviations: n, number of tested patients in each subgroup per marker; n.s., not significant in Kruskal-Wallis test or pairwise Wilcoxon rank-sum test.

Table S18

Expression of surface target antigens on leukemic stem- and progenitor cells and comparison to normal bone marrow (BM) cells

		Expression of surface antigens on progenitor cells and stem cells (% positive cases in brackets)											
		Normal control BM				AML				CML			
Antigen	CD	$CD34^+/CD38^+$		$CD34^+/CD38^-$		$CD34^+/CD38^+$		$CD34^+/CD38^-$		$CD34^+/CD38^+$		$CD34^+/CD38^-$	
IL-2RA	CD25	−	(12)	−	(8)	+	(43)	+	(48)	+/−	(28)	+	(93)
DPPIV	CD26	−	(0)	−	(0)	−/+	(8)	−/+	(10)	+/−	(30)	+	(97)
Siglec-3	CD33	+/−	(100)	+/−	(100)	+	(98)	+	(96)	+	(100)	+	(100)
Pgp-1	CD44	+	(100)	+	(100)	+	(98)	+	(98)	+	(100)	+	(100)
Campath-1	CD52	+/−	(48)	+/−	(54)	+/−	(44)	+/−	(51)	−	(11)	+	(81)
MXRA4	CD93	+/−	(75)	+/−	(63)	+	(81)	+	(72)	+/−	(71)	+	(86)
TACTILE	CD96	−	(0)	−	(0)	+	(51)	+	(40)	−	(9)	−	(0)
KIT/SCFR	CD117	+	(100)	+	(100)	+	(99)	+	(99)	+	(98)	+	(96)
IL-3RA	CD123	+	(91)	+	(95)	+	(99)	+	(98)	+	(96)	+	(100)
FLT3	CD135	+/−	(45)	+/−	(40)	+	(93)	+	(91)	+/−	(64)	+	(64)
CXCR4	CD184	+/−	(68)	+/−	(55)	+	(75)	+	(72)	+	(82)	+	(82)
PD1	CD279	−	(0)	−	(0)	−	(9)	−	(18)	−	(0)	−	(7)
PD-L1	CD274	+	(100)	+	(100)	+	(84)	+	(95)	+	(89)	+	(100)
PD-L2	CD273	−	(0)	−	(0)	−	(0)	−	(0)	−	(0)	−	(0)
CLL-1	CD371	+	(100)	−	(0)	+	(92)	+	(68)	+	(67)	−	(17)
IL-1RAP	n.c.	−/+	(36)	−	(0)	+	(79)	+	(65)	+	(95)	+	(77)

Expression of surface antigens on $CD34^+/CD38^-$ and $CD34^+/CD38^+$ bone marrow (BM) stem- and progenitor cells was examined by multi-color flow cytometry. All leukemic samples obtained at diagnosis (AML: at least 11 cases per marker tested; CML: at least 6 cases per marker tested). Control samples (normal BM; at least 7 cases per marker tested) included purchased $CD34^+$ BM cell subsets and BM cells obtained from cases with suspected hematologic neoplasm without persistent cytopenia. Results show the levels of expression of surface markers (as per score defined below) and as % of positive cases in each group (in brackets). Score of antibody reactivity: +, clear expression

in majority of cases; +/−, weak expression in majority of cases; −/+, expression in minority of cases; −, no expression in a vast majority of cases. Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; n.c., not yet clustered.

Table S19

Target spectrum of kinase inhibitors, fusion proteins and targeting antibodies

Drug	Major targets	References
a) Kinase Inhibitors		
Midostaurin	ACK1, FLT3, JAK3, KIT, PDGFRb, RET, TNK1, TRK-A/C, VEGFR2, BLK, CSF1R, FGFR2, IGF-1R, JAK2, LCK, LTK, LYN, ROS, SYK, TRK-B, FER, FYN, HCK, MUSK, PDGFRa, SRC, TYK2	34
Gilteritinib	FLT3, LTK, AXL, ELM4-ALK	35
b) Fusion proteins		
Denileukin-diftitox	CD25 positive cells	36
c) Targeting antibodies		
Gemtuzumab/ozogamicin	CD33 positive cells	37
Alemtuzumab	CD52 positive cells	38

Abbreviations: ACK1, activated CDC42 kinase 1; FLT3, fms like tyrosine kinase 3; JAK, janus kinase; PDGFR, platelet-derived growth factor receptors; TNK1, tyrosine kinase non-receptor 1; TRK, tropomyosin receptor kinase; VEGFR, vascular endothelial growth factor receptors; BLK, B lymphoid tyrosine kinase; CSFR1, colony stimulating factor 1 receptor; FGFR, fibroblast growth factor receptor; IGR-1R, insulin-like growth factor receptor; LCK, lymphocyte-specific protein tyrosine kinase; LTK, leukocyte tyrosine kinase;

LYN, Lck/Yes novel tyrosine kinase; SYK, spleen tyrosine kinase; HCK, The hematopoietic cell kinase; MuSK, muscle-specific kinase; scr, sarkoma.

Table S20

Aberrantly expressed surface markers on $CD34^+/CD38^-$ cells in AML or CML cells injected in NSG mice

			Expression on $CD34^+/CD38^-$ cells as staining index (SI*)							
disease	**No. #**	**sample at**	**CD25 IL-2RA**	**CD26 DPPIV**	**CD33 Siglec-3**	**CD52 Campath-1**	**CD117 KIT**	**CD123 IL-3RA**	**CD135 FLT3**	**IL-1RAP**
AML	222	D	+/–	–	n.t.	+/–	++	++	n.t.	++
AML	211	D	+	–	++	+/–	++	+	n.t.	n.t.
AML	210	D	–	–	++	+	++	+	+/–	+/–
AML	126	D	++	++	++	+	n.t.	++	n.t.	++
AML	179	D	+/–	–	n.t.	+/–	n.t.	n.t.	n.t.	n.t.
CML	21	D	+	+	++	+/–	+/–	n.t.	+/–	+
CML	11	D	++	+	++	–	–	n.t.	+/–	–
CML	8	D	++	++	++	+/–	+	n.t.	n.t.	+

$CD34^+/CD38^-$ cells obtained from AML and CML patients were analyzed for surface marker expression by multicolor flow cytometry. The score is related to the expression of surface markers on AML and CML $CD34^+/CD38^-$ cells. For NSG experiments, T-cell depleted AML MNC or $CD34^+$ enriched CML cells were injected in NSG mice. SI* was calculated as ratio of median fluorescence intensities (MFI) obtained with specific monoclonal antibody (mAb) and control mAb (isotype control). SI values were graded using the following score: –, 0-1.3; +/–, 1.31-3; +, 3.01-10; ++, >10. Patients´ numbers (#) refer to the patients´ numbers defined in Table S2 and S3. Abbreviations: No. # , patient number; SI, staining index; D, diagnosis; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; n.t., not tested.

Table S21

Phenotype of pre-leukemic neoplastic stem cells (pre-L-NSC) in patients with MDS and comparison to AML LSC as determined by flow cytometry

Antigen	Normal Bone Marrow $CD34^+/CD38^-$		MDS $CD34^+/CD38^-$		AML $CD34^+/CD38^-$	
	% of patients positive (No. of patients tested)	Mean staining index	% of patients positive (No. of patients tested)	Mean staining index	% of patients positive (No. of patients tested)	Mean staining index
CD25	8% (25)	0.9	41% (34)	2.9	48% (164)	6.5
CD26	0% (22)	0.8	6% (32)	0.8	10% (164)	2.7
CD33	100% (15)	14.3	100% (18)	40.7	96% (90)	49.3
CD44	100% (15)	113.6	100% (15)	37.4	98% (63)	44.3
CD52	54% (24)	3.3	56% (41)	3.0	51% (172)	3.2
CD90	85% (13)	3.7	100% (5)	12.2	13% (24)	1.4
CD96	0% (10)	1.0	14% (7)	1.2	40% (42)	4.1
CD105	100% (10)	6.6	100% (13)	14.7	93% (45)	8.1
CD114	64% (11)	2.2	94% (18)	8.5	64% (87)	3.5
CD117	100% (20)	35.7	100% (29)	95.3	99% (158)	50.4
CD123	95% (21)	4.3	100% (34)	11.0	98% (167)	27.5
CD135	40% (10)	3.4	100% (8)	16.6	91% (35)	13.2
CD184	55% (22)	2.6	86% (14)	4.0	72% (78)	3.2
CD221	25% (8)	2.0	33% (9)	1.9	18% (28)	2.0
CD371	0% (9)	0.8	54% (13)	2.0	68% (80)	9.1
IL-1RAP	0% (11)	1.0	40% (20)	2.0	65% (112)	8.8
NPDC1	50% (4)	1.7	67% (3)	4.2	76% (21)	3.5
ROBO4	100% (6)	3.6	78% (9)	3.0	29% (42)	1.8

Expression of surface antigens on $CD34^+/CD38^-$ stem cells was examined by multi-color flow cytometry. All leukemic samples obtained at diagnosis. Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CD, cluster of differentiation; No., number; LSC, leukemic stem cells; HSC, hematopoietic stem cells; IL-1RAP, interleukin-1 receptor accessory protein; ROBO4, roundabout 4.

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