

SUPPLEMENTARY MATERIAL AND METHODS

Protein extraction and western blot

Protein extracts were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA) and incubated with a monoclonal antibody against described proteins.

CDK6, Rb, Rb-P, PARP and β -actin protein expression were analyzed by western blot as described previously (8). β -actin was used as a loading control. For the study of acetylated-Histone H3, total Histone-3, acetylated-histone H4 and histone-2AX-phosphorylated, acid protein extraction was used. Cell pellets were resuspended in lysis buffer containing 10mM HEPES Ph 7.9 (Sigma), 1.5mM MgCl₂ (Sigma), 10mM KCl (Merck), 0.5 mM DTT (Sigma), 2 mM PMSF (Sigma) and 0.2M HCl (Merck) for 30 minutes at 4°C. After centrifugation at 11000g for 10 minutes at 4°C, supernatant was collected and introduced in a 0.2 cellulose membrane (Sigma). The dialysis tubing was washed for 1 hour with 0.1M acetic acid two times and three times with desionized water.

Tumor lysates were prepared using mechanical tissue homogenization by ultraturrax (IKA) in 200 μ l of the same lysis buffer used for the cell pellets. Primary antibody against acetyl-histone H3 (diluted 1:1000 for o/n at 4°C, Upstate), total histone H3 (diluted 1:50000 for 1 hour at RT, Abcam), anti-acetyl-histone H4 (diluted 1:10000 for o/n at 4°C, Upstate) or phospo-histone H2AX (Ser139) (diluted 1:10000 for o/n at 4°C, Cell signalling) were used. Secondary antibodies and western blot exposed were performed as described above. Total histone H3 was used as a loading control.

Bioinformatic data analysis

Background correction and normalization of gene expression microarray signals were done using RMA (Robust Multichip Average) algorithm (19). Then, a filtering process was performed to eliminate low expression probe sets. R and Bioconductor (20) were used for preprocessing and statistical analysis. LIMMA (Linear Models for Microarray Data) (21) was used to find out the probe sets that showed significant differential expression between experimental conditions. Genes were selected as significant using a B statistic cut off ($B > 0$).

For methylation microarrays, a filtering process based on the methylation index (MI) value was performed to focus the analysis on genes with large differences in their

methylation status. LIMMA was used to find out the probes that showed significant differential methylation patterns. Genes were selected as significant using a B statistic cut off ($B > 0$). In addition to this study, analysis of significant methylation changes in each sample was performed by a Z score transformation of fold-change distributions (22). A threshold of $|z| > 1.64$ ($p\text{-value} < 0.05$) was fixed to select genes for further analysis. SNP microarray data preprocessing, normalization, probe summarization and calculation of raw total copy-number estimates were done using the CRMAv2 method implemented in the *aroma.affymetrix* R package (23). Bioinformatic analysis of TLDA results started with a filtering process to remove undetermined detectors, followed by normalization using the geometric mean of the 4 detectors corresponding to the mammalian U6 endogenous control to calculation of DCt values. LIMMA was used to find out the detectors that showed significant differential expression experimental conditions. miRNAs were selected as significant using a cut off of $p\text{-value} < 0.05$. Functional enrichment analysis of Gene Ontology (GO) categories was carried out using standard hypergeometric test (24). The biological knowledge extraction was complemented through the use of Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com), which database includes manually curated and fully traceable data derived from literature sources.

Raw array data files were submitted to GEO and are available under the accession number GSE26807.

CD34 positive cell isolation and treatment

CD34⁺ cells were obtained from umbilical cord blood after the mother's informed consent was obtained. Samples were collected at dawn and processed during the next 12 hours post-partum. Mononuclear cells were obtained by layering the blood onto Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) and centrifuging at 400g for 30 minutes. The interface layer was then collected and subjected to immunomagnetic separation using the VarioMACS CD34 progenitor cell isolation kit (Myltenyi), following the manufacturer's recommendations obtaining a cell population with more than the 96% of purity of CD34⁺ cells. These CD34⁺ cells were cultivated with ISCOVE (Cultek) medium 20% FBS and 0,5% of penicilin supplemented with 100ng/ml of thrombopoietin (Biosource International), 300ng/ml of human stem cell factor (hSCF, Peprotech, London, UK), and 100ng/ml of Flt3 ligand (Flt3L; Biosource International). After 24 hours of culture, human CD34⁺ cells and cells from the ALL-T1 human acute lymphoblastic leukemia mice model were treated in vitro with LBH589, Vincristine plus Dexametasone combination or with LBH589, Vincristine and Dexametasone

combination. The doses used for the *in vitro* experiment were the IC₅₀ of LBH589 (30nM), Vincristine (2.6pM) and Dexamethasone (0.6mM) observed in most of the ALL cells lines we have used in this study.

Statistical analysis

All calculations were performed with the SPSS statistical package (SPSS, Chicago, IL.). Data represent the mean (\pm SD) of at least three independent experiments. Statistical analysis was performed by the Student t test for independent samples. Mouse survival was calculated using the Kaplan-Meier method, and survival curves were compared by log-rank test. *P* values below 0.05 were considered statistically significant.