LMO2 expression reflects the different stages of blast maturation and genetic features in B-cell acute lymphoblastic leukemia and predicts clinical outcome

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Supplementary Design and Methods

Cytogenetic and molecular analysis of patient samples

G-banding karyotype analysis was performed in all cases according to standard methods, defining the numerical and structural chromosomal changes for each sample, including the ploidy state (hyperdiploidy >50 chromosomes and hypodiploidy <46 chromosomes). Other structural alterations, such as the t(1;19)(q23;p13) or variant translocations, and some less common or non-recurrent abnormalities were also identified by karyotype analysis. In addition, all B-ALL patient samples were analyzed by molecular analysis and/or FISH for the presence of *TEL-AML1* and *BCR-ABL* gene fusions. *MLL* rearrangements (studied either by FISH and/or Southern blot with an *MLL* consensus probe) were conducted in patients under the age of two years, in those with any 11q23 chromosomal rearrangement shown by cytogenetics, and in those with CD10+ B-ALL that were negative for *TEL-AML1* and *BCR-ABL* fusions and did not show any non-random cytogenetic alteration by karyotype analysis.

Accordingly, a sample was included in the normal karyotype subgroup when it displayed a normal cytogenetic study (46,XX or 46,XY) and was negative for the molecular/FISH tests.

Immunophenotyping of patient samples

Expression of cell surface antigens was examined in acute leukemia samples by direct immunofluorescence flow cytometry analysis with Coulter Profile II (Beckman Coulter) using a panel of fluorescein isothiocyanate or phycoerytrin labeled monoclonal antibodies reactive with lymphoid and myeloid antigens (CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD22, CD13, CD14, CD33, CD34, CD79a, cμ, sIgM and HLA-DR) (BD Biosciences). For intracytoplasmic detection of myeloperoxidase, CD22, CD3 and Tdt, immunocytochemical staining was performed using the alkaline phosphatase technique and specific monoclonal antibodies MPO-7 (Dako), CD22 and CD3 (BD Biosciences) and Tdt (Beckman Coulter).

ALL samples were classified into 208 B-ALL (CD19+ and/or CD22+ and CD2-CD3-) and 39 T-ALL (CD2+ and/or CD3+ and CD19- CD22-) cases. More detailed immunophenotype analyses were available for 145 B-ALL samples (144 with survival data available): based on the maturation sequence of the normal bone marrow B cells, B-ALL patients were classified into four major groups: Pro-B-ALL (CD19+, cCD79a+), Common-CD10+ ALL (CD10+), Pre-B-ALL (cμ+), and Mature B-ALL (sIgM+).[1]

Sorting of normal lymphocyte subpopulations

B cells were isolated from surgically removed reactive tonsils using B Cell Isolation Kit II and the autoMACS immunomagnetic separation system (Miltenyi Biotec). Three samples each of GC B cells, memory B cells and naïve B cells were enriched by positive selection using CD71 MicroBeads (Miltenyi Biotec, clone AC108.1), CD27 MicroBeads (Miltenyi Biotec) and FITC Mouse Anti-Human IgD (BD, clone IA6-2) plus Anti-FITC MicroBeads (Miltenyi Biotec) respectively. One sample of T cells was also isolated from a reactive tonsil as previously described.[2]

Two samples each of hematopoietic stem cells, early-B, pro-B, pre-B and immature B cells were isolated from bone marrow of healthy donors by immunomagnetic separation, followed by FACS sorting as previously described.[3] The immunophenotypes of all the purified cell subpopulations are summarized in the *Online Supplementary Table S1*.

Cell lines

LMO2 expression was also measured in 11 cell lines derived from patients with B-ALL (CEMO-1,[4] RCH-ACV,[5] MHH-CALL-4,[6] LK63,[7] KOPN-8,[8] SEM,[9] PER-377,[10] MUTZ-5,[11] NALM-27,[12] TOM-1[13] and NALM-20),[14] 4 T-ALL cell lines (JURKAT,[15] HSB-2,[16] LOUCY[17] and MOLT-4)[18], 6 activated B-cell-like (ABC)-DLBCL cell lines (SU-DHL-8, RIVA, HLY-1, OCI-LY-10, OCI-LY-3 and MD901) and 14 cell lines of germinal-center B-cell-like (GCB)-DLBCL origin (OCI-LY-1, OCI-LY-8, DB, ROS-50, SU-DHL-4, SU-DHL-6, OZ, SU-DHL-16, VAL, SC-1, RL, PR-1, SU-DHL-10 and OCI-LY-19). Cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal calf serum (FCS); with exception of OCI-LY-10 and OCI-LY-3, that were grown in IMDM medium (Invitrogen) supplemented with 20% human plasma.

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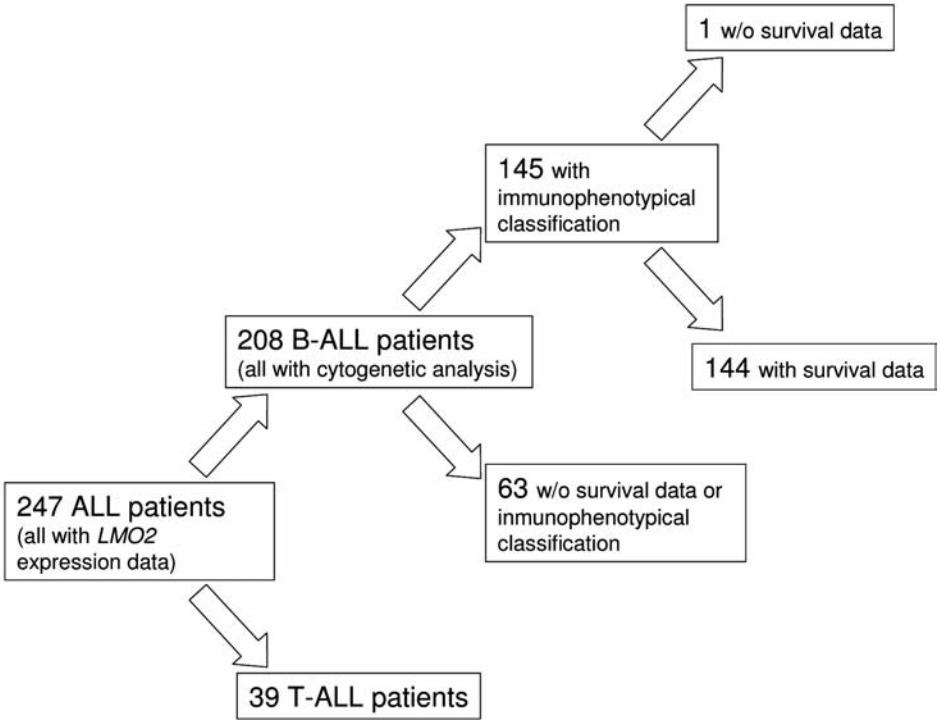
Online Supplementary Table S1. Number of B-ALL patients included in the different cytogenetic and immunophenotypic subgroups.

Classification	Total	With survival data
Cytogenetic	**208**	**144**
BCR-ABL	41	21
TEL-AML1	32	25
MLL rearrangements	16	6
Hyperdiploid	9	3
Normal karyotype	89	76
Non-recurrent alterations	21	13
Immunophenotypic	**145**	**144**
Pro-B	35	35
Common-CD10$^+$	94	93
Pre-B	12	12
Mature B-ALL	4	4

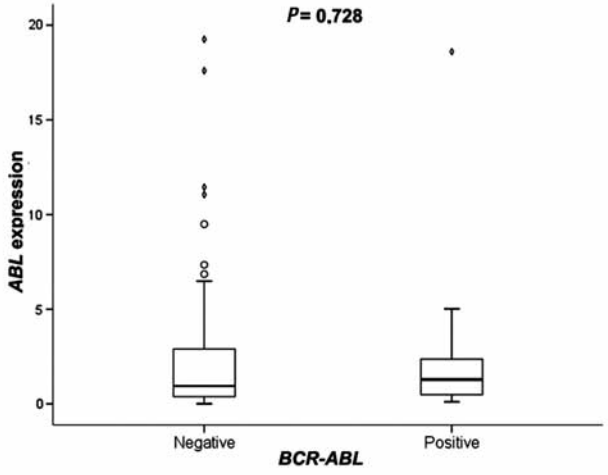
Online Supplementary Table S2. Isolation of normal hematopoietic and lymphoid cell subpopulations.

Cell type	Markers used for isolation	Cell source
Hematopoietic stem cells	CD34$^+$CD38$^-$	Bone marrow
Early B cells	CD38$^+$CD34$^+$CD10$^+$CD19$^-$	Bone marrow
Pro-B	CD34$^+$CD10$^+$CD19$^+$sIgM$^-$	Bone marrow
Pre-B	CD34$^-$CD10$^+$CD19$^+$sIgM$^-$	Bone marrow
Immature B cells	CD34$^-$CD10$^+$CD19$^+$sIgM$^+$	Bone marrow
Naïve B cell	CD19$^+$IgD$^+$	Tonsil
Germinal center cells	CD19$^+$CD71$^+$	Tonsil
Memory B cells	CD19$^+$CD27$^+$	Tonsil
T cells	CD3$^+$	Tonsil



Online Supplementary Figure S1. Available data for the patients included in this study. Among the 144 patients with survival data, all of them had data on overall survival and 3 of them lacked information on event-free survival.

A



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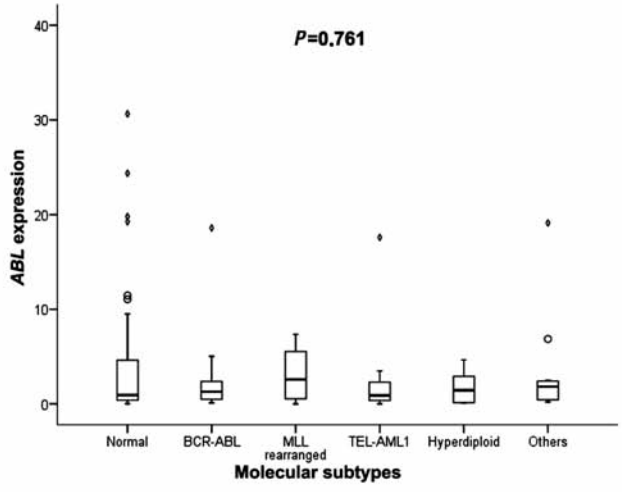

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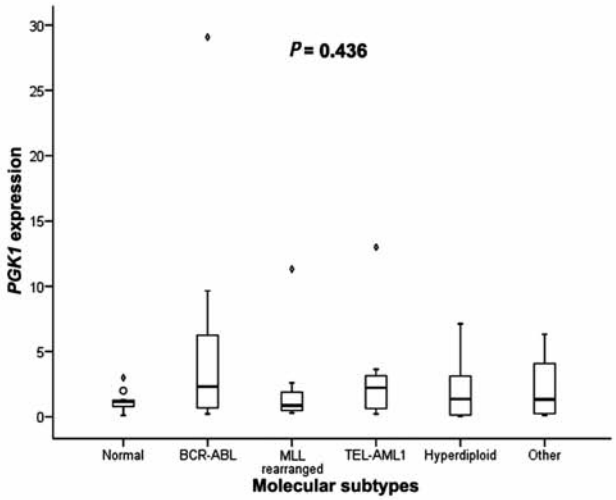

Online Supplementary Figure S2. Expression of the reference genes *ABL* and *PGK1* in the B-ALL cytogenetic subgroups. The level of expression of each gene was assessed as 2^{-dCt}, where dCt is the Ct obtained for the gene in the corresponding sample minus the mean Ct value of the gene among all samples. The comparison (A) between *BCR-ABL* positive and negative cases was performed with the Mann-Whitney U test, and (B) between the different molecular subgroups by using the Kruskal Wallis test. (C) The Kruskal Wallis test was also employed to test the suitability of *PGK1* as reference gene.