LYMPHOID NEOPLASIA

High *BAALC* expression predicts chemoresistance in adult B-precursor acute lymphoblastic leukemia

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Overexpression of *BAALC* is an adverse prognostic factor in adults with cytogenetically normal acute myeloid leukemia and T-cell acute lymphoblastic leukemia (ALL). Here, we analyzed the prognostic significance of *BAALC* in B-precursor ALL. *BAALC* MRNA expression was determined in 368 primary adult B-precursor ALL patients enrolled on the 06/99 and 07/03 GMALL trials. Patients were grouped into tertiles according to *BAALC* expression (T1-T3). Higher *BAALC* expression (T3 vs T2 vs T1) was associated with higher age (*P* < .001), a higher white blood cell count (*P* = .008), CD34 (*P* = .001), *BCR-ABL* (*P* < .001), and *MLL-AF4* (*P* < .001). Higher *BAALC* expression predicted primary therapy resistance in the overall cohort (*P* = .002) and in the *BCR-ABL*⁻ and *MLL-AF4*⁻ subgroup (*P* = .01). In *BCR-ABL*⁻ and *MLL-AF4*⁻ patients, higher *BAALC* expression was associated with a shorter overall survival (OS; 5-year OS: T3, 38%; T2, 52%; T1, 70%; *P* = .004) and independently predicted OS in multivariate models (*P* = .03). Gene-expression profiling revealed an upregulation of stem cell markers and genes involved in chemoresistance (*TSPAN7* and *LYN*) in the high *BAALC* group. Thus, high *BAALC* expression is associated with an immature, chemoresistant leukemic phenotype and identifies patients with inferior OS. Determination of *BAALC* might contribute to risk assessment of molecularly undefined adult B-precursor ALL. (*Blood*. 2010;115(18):3737-3744)

Introduction

Outcome of adult B-precursor acute lymphoblastic leukemia (ALL) has considerably improved because of identification of clinical and genetic risk factors stratifying patients to different treatment groups. With the current risk-adapted treatment protocols, complete remission (CR) rates range between 74% and 93%; however, long-term survival remains limited to only 27% to 54% of adult patients.[1] Commonly accepted risk factors in B-precursor ALL include white blood cell (WBC) count, the immunophenotype of pro-B ALL, response to induction therapy, level of minimal residual disease, age, and cytogenetic as well as molecular genetic aberrations.[1,2] Thus far, high-risk cytogenetics in B-precursor ALL mainly compose the Philadelphia chromosome with the balanced translocation t(9;22)(q34;q11) and BCR-ABL fusion protein in approximately 20% to 36%,[1,3] and the translocation t(4;11)(q21;q23) with the MLL-AF4 fusion protein in approximately 6% to 9%[1,4] of cases. Patients lacking clinical and molecular risk factors are considered standard risk (SR); however, the definition of risk groups varies between different study protocols. Outcome for SR patients is still unsatisfactory with a long-term survival of only 40% to 60%,[1,4,5] indicating the clinical and biologic heterogeneity of these patients. Therefore, the identification of novel predictive molecular markers in B-precursor ALL might be particularly useful for patients yet unclassified by (molecular) risk factors and may improve treatment stratification of this subgroup.

The *Brain And Acute Leukemia, Cytoplasmic* (*BAALC*) gene is a marker of early hematopoietic progenitor cells and is aberrantly expressed in a subset of acute leukemias.[6,7] The *BAALC* gene is located on chromosome 8q22.3. The protein sequence shows no homology to any other known proteins or functional domains. Its role in hematopoiesis and leukemogenesis is unknown. *BAALC* has been associated with an immature leukemic subtype in adult cytogenetically normal (CN) acute myeloid leukemia (AML) and acute T-lymphoblastic leukemia (T-ALL).[6,8,9] The prognostic significance of *BAALC* was first shown for CN-AML, with a poor CR rate, overall survival (OS), and disease-free survival for patients with high *BAALC* expression.[8,10] In adult T-ALL, high *BAALC* expression was associated with inferior overall and relapse-free survival (RFS).[9] Because of the prognostic relevance of *BAALC* in different leukemic subgroups, we hypothesized that *BAALC* might also show an aberrant expression pattern and prognostic significance in adult B-lineage ALL and may help to risk-stratify molecularly unclassified patients with B-precursor ALL.

To assess this, we analyzed *BAALC* mRNA expression in 368 adult patients with B-precursor ALL enrolled on multicenter treatment protocols of the German Multicenter ALL (GMALL) study group in the context of established risk factors. Moreover, gene-expression profiling (GEP) was performed in 51 adult B-precursor ALL specimens to gain insights into biologic pathways of *BAALC* and mechanisms of chemoresistance.

Methods

Patients

We analyzed 368 patients with newly diagnosed adult B-precursor ALL enrolled between 1999 and 2006 on the 06/99 (n = 199) and 07/03 (n = 169) GMALL multicenter trials.[11] Specimens were selected from consecutive patients who had sufficient material available. Patient characteristics and outcome were comparable with those from the total study population.[12,13] The 06/99 and 07/03 study protocols included intensive chemotherapy, radiotherapy, and autologous or allogeneic stem cell transplantation (SCT) according to risk-adapted classification, using the following risk factors: WBC more than 30×10^9/L, late CR (> 3 weeks), pro-B ALL, t(9;22)/*BCR-ABL*, and t(4;11)/*MLL-AF4*. Risk groups were assigned as follows: SR (no risk factor), high risk (≥ 1 risk factor), and very high risk (presence of t(9;22)/*BCR-ABL*). High-risk and very-high-risk patients with a sibling or matched unrelated donor were scheduled for allogeneic SCT in first CR (CR1). The short form of the study protocol in the European Leukemia Trial Registry contains details.[14]

The studies were approved by the ethics board of the Johann Wolfgang Goethe-Universität Frankfurt/Main, Germany, and informed consent was given according to the Declaration of Helsinki.

Immunophenotyping

Immunophenotypic analyses were performed by flow cytometry on fresh pretreatment bone marrow (BM) and peripheral blood (PB) samples at the GMALL central reference laboratory at the Charité Berlin, Germany, as previously described.[15] A cell-surface antigen was considered positive when at least 20% of cells showed fluorescence intensity greater than the negative control.

Molecular analyses

The analyses of *BCR-ABL* and *MLL-AF4* were performed at the GMALL central reference laboratory at the Charité Berlin, Germany, as previously reported.[15,16] *MLL-AF4* was only determined in CD10⁻ samples as presence of *MLL-AF4* in B-precursor ALL is largely restricted to this subgroup.[17]

RNA isolation and synthesis of complementary DNA

Total RNA isolation from mononuclear cells was carried out from diagnostic BM samples using RNeasy Mini Kit (QIAGEN) following the manufacturer's instruction. Synthesis of complementary DNA was performed using 500 ng of total RNA, avian myeloblastosis virus-reverse transcriptase, RNase inhibitor, and oligo dT20-primer at 50°C for 60 minutes (Roche Diagnostics).

Real-time reverse-transcribed polymerase chain reaction assays

BAALC expression was analyzed by multiplex quantitative real-time reverse transcribed polymerase chain reaction (PCR) on the Rotorgene RG-3000 real time cycler (Corbett Research) with coamplification of the housekeeping gene *Glucose-Phosphate Isomerase* (*GPI*) as previously reported.[18] Relative *BAALC* expression was calculated with the mean of the cycle number difference of the 2 replicates ($\Delta C_T = GPI-BAALC$), expressed as $2^{\mu(\Delta CT)}$. For each sample, polymerase chain reaction assays were performed in duplicate. Positive and negative controls were included in each assay. RNA from the cell line KG1a was included in each run for calibration between runs. If amplification of *GPI* did not reach the threshold within 30 cycles, samples were excluded from this study.

Oligonucleotide microarray studies

Microarray-based GEP was performed on an additional set of 51 BM samples from adult patients with newly diagnosed t(9;22)/*BCR-ABL*⁻ and t(4;11)/*MLL-AF4*⁻ B-precursor ALL, which had been included in the Microarray Innovations in LEukemia multicenter study (MILE).[19] Oligonucleotide microarray hybridization (HG-U133 Plus 2.0; Affymetrix) was performed as described previously.[20]

GeneSpring software, Version 4.2 (Silicon Genetics), was used for data analyses. All microarrays fulfilled the MIAME standards.[21] *BAALC* expression was represented by 2 probe sets (218899_s_at, 222780_s_at), and samples were grouped into tertiles (t1-t3) according to the median expression level of the 2 probe sets. To obtain gene-expression signatures that sufficiently discriminated between high and low *BAALC* expressers, samples in the upper *BAALC* expression tertile were compared with samples in the lower tertile (t3 vs t1) and named high and low *BAALC*, respectively. Genes were considered to be differentially expressed if their expression showed an at least 3-fold change and probe sets were called present by the Affymetrix data analysis in at least 75% of samples. Statistical significance of changes was calculated by the nonparametric t test ($P \leq .05$). The microarray data can be accessed at the Gene Expression Omnibus public database under accession number GSE13204 (http://www.ncbi.nlm.nih.gov/geo/).

Statistical analyses

BAALC mRNA expression levels determined by real-time reverse-transcribed polymerase chain reaction were grouped into tertiles (T1-T3) based on the trend observed in clinical outcome (Figure 1). *BAALC* expression ranged between 0 and 91 $2^{\mu(\Delta CT)}$ (median T1, 0.2; median T2, 2.0; median T3, 9.0). T1, T2, and T3 are capitalized to distinguish the data from microarray-based expression (t1, t2, and t3).
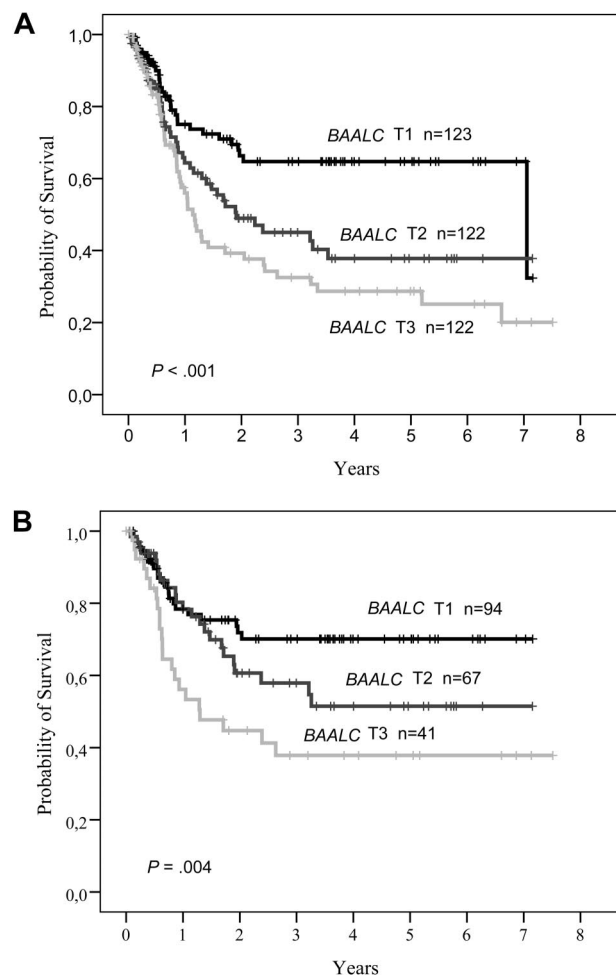
Figure 1. Kaplan-Meier analyses of OS according to *BAALC* expression. Patients undergoing SCT in CR1 were censored at the time of transplantation. (A) Overall cohort of B-precursor ALL. (B) *BCR-ABL*⁻ and *MLL-AF4*⁻ ALL.

Remission status was assessed after completion of induction chemotherapy. CR was defined as follows: granulocyte count of at least 1.5×10^9/L, platelet count of at least 100×10^9/L, no PB blasts, BM cellularity of at least 20% with maturation of all cell lines and less than 5% blasts, and no extramedullary leukemia. Primary therapy failure was defined as persistence of PB blasts or at least 25% blasts in BM after induction therapy. Relapse was defined as reappearance of PB blasts, more than 5% blasts in BM, or appearance of extramedullary manifestations after CR was achieved.

OS was determined from the first day of therapy until death or date of last follow-up. RFS was measured from the date of first CR until the date of relapse. Patients without reported relapse were censored by the end of the follow-up. Patients with withdrawal or death in CR were censored at the respective dates. Survival curves were calculated using the Kaplan-Meier method, with the log-rank comparing differences between survival curves. The median follow-up for living patients was 44 months. For 1 patient, no clinical data were available. Patients who received SCT in CR1 were censored at the time of transplantation for survival analyses.

Comparisons of molecular and clinical features across groups were made using the χ^2 or a 2-sided Fisher exact test for categorical data and the nonparametric Mann-Whitney U test for continuous variables. A *P* value less than or equal to .05 (2-sided) was considered significant. Multivariate analyses were performed according to the Cox proportional hazards model for OS and with logistic regression for response to induction therapy, both with stepwise forward selection, including the following variables in the full model: *BAALC* expression (T1 vs T2 vs T3), WBC ($> 30 \times 10^9$/L vs $\leq 30 \times 10^9$/L), age (10-year increase), CD20 (positive vs negative), *BCR-ABL* (presence vs absence), *MLL-AF4* (presence vs absence), and immunophenotype (pre-B vs common/pro-B). When analyzing SR patients, WBC count was treated as a categorical variable with 1×10^9/L increase. SPSS software package (Version 17.0 for Windows; SPSS) was used for all calculations.

Results

BAALC expression and correlation to clinical and molecular features

BAALC mRNA expression was analyzed in pretreatment BM samples of 368 patients with newly diagnosed B-precursor ALL.

Patients were divided into 3 *BAALC* expression groups as described in "Statistical analyses" (T1-T3). Patients with higher *BAALC* expression (T3 vs T2 vs T1) were significantly older ($P < .001$) and had higher WBC ($P = .008$) at initial diagnosis compared with patients with lower *BAALC* expression (Table 1). Higher *BAALC* expression was associated with the presence of *BCR-ABL* ($P < .001$) and *MLL-AF4* ($P < .001$). In addition, higher *BAALC* expression correlated with a more immature CD34+ leukemic phenotype ($P = .001$). There was no significant association between *BAALC* expression level and CD20 positivity, coexpression of myeloid markers, or immunophenotypic subgroups of B-precursor ALL (Table 1).

Within the subgroup of *BCR-ABL*− and *MLL-AF4*− B-precursor ALL, higher *BAALC* expression significantly correlated with CD34 positivity ($P = .03$). No other association was seen between *BAALC* expression and clinical features in this subgroup (data not shown).

Outcome in B-precursor ALL patients with respect to *BAALC* expression (overall cohort)

Patients with higher *BAALC* expression showed a significantly lower CR rate (*BAALC* T3, 79%; *BAALC* T2, 86%; *BAALC* T1, 92%; $P = .03$) and a higher incidence of primary resistant disease (*BAALC* T3, 15%; *BAALC* T2, 8%; *BAALC* T1, 3%; $P = .004$). In multivariate analysis, *BAALC* remained the only predictive factor for primary therapy resistance ($P = .002$; odds ratio = 2.4; 95% confidence interval [CI], 1.4-4.3).

OS was significantly shorter for patients with higher *BAALC* expression compared with lower *BAALC* expression (5-year OS, 95% CI: *BAALC* T3, 29%, 18%-40%; *BAALC* T2, 38%, 25%-50%; *BAALC* T1, 65%, 54%-75%; $P < .001$; Figure 1A). However, *BAALC* expression was not independently predictive for OS in multivariate analysis. Significant adverse factors for OS were WBC and age (data not shown). No influence on the relapse rate ($P = .94$) or RFS ($P = .52$) was observed with respect to *BAALC* expression. There were no significant differences regarding deaths

Table 1. Clinical and molecular characteristics at diagnosis according to *BAALC* expression in B-precursor ALL

Characteristic	*BAALC* T1 (n = 123)	*BAALC* T2 (n = 123)	*BAALC* T3 (n = 122)	*P*
Median age, y (range)	32 (15-64)	36 (15-65)	42 (18-64)	< .001
Male sex, percentage	54	57	49	.51
WBC, ×10⁹/L (n = 342)				
Median (range)	10 (0.4-317)	15 (0.8-472)	20 (0.5-594)	.008
More than 30 × 10⁹/L, no. (%)	34 (29)	43 (37)	46 (43)	.09
CNS involvement, no. /total (%) (n = 272)	4/96 (4)	6/96 (6)	3/80 (4)	.77
Molecular genetics				
BCR-ABL, no. (%)				< .001
Positive	29 (24)	41 (33)	70 (57)	
Negative	94 (76)	82 (67)	52 (43)	
MLL-AF4, no. (%)				< .001
Positive	0 (0)	14 (11)	11 (9)	
Negative	123 (100)	109 (89)	111 (91)	
CD34 positivity, no. (%)	79 (64)	103 (84)	95 (78)	.001
CD20 positivity, no. (%)	43 (35)	49 (40)	36 (30)	.23
Myeloid markers, no. (%)*	50 (41)	49 (40)	58 (48)	.42
Immunophenotypic subtype, no. (%)				.10†
Common ALL	77 (63)	81 (66)	89 (73)	
Pre-B ALL	37 (30)	25 (20)	23 (19)	
Pro-B ALL	9 (7)	17 (14)	10 (8)	

CNS indicates central nervous system.

*Coexpression of CD13, CD33, CD65s, or CD15.

†Overall *P* value for the frequency of the 3 immunophenotypes across the *BAALC* tertiles.

Table 2. Clinical outcome according to *BAALC* expression in *BCR-ABL*⁻ and *MLL-AF4*⁻ patients

Clinical outcome	*BAALC* T1 (n = 94)	*BAALC* T2 (n = 67)	*BAALC* T3 (n = 41)	*P*
Complete remission, no./total (%) (n = 193)	86/92 (94)	59/65 (91)	29/36 (81)	.09‡
Refractory disease, no./total (%) (n = 184)	1/87 (1)	4/63 (6)	5/34 (15)	.009
Overall survival at 5 y, percentage (95% CI) (n = 202)*	70 (59-81)	52 (36-67)	38 (22-54)	.004
Relapse rate, no./total (%) (n = 114)†	26/53 (49)	16/36 (44)	10/25 (40)	.76

*Patients who received SCT in CR1 were censored at the time of transplantation.

†Patients who received SCT in CR1 were excluded.

‡*P* value across the 3 *BAALC* tertiles. Statistical significance was reached comparing *BAALC* T1 vs T3 (*P* = .047).

in induction therapy (*P* = .96) or deaths in CR (*P* = .28) between the 3 *BAALC* groups.

BAALC expression and outcome in *BCR-ABL*⁻ and *MLL-AF4*⁻ B-precursor ALL

We next analyzed the prognostic impact of *BAALC* expression in the molecular low-risk subgroup of *BCR-ABL*⁻ and *MLL-AF4*⁻ B-precursor ALL (n = 202). In this group, patients with higher *BAALC* expression had a lower CR rate (*BAALC* T3, 81%; *BAALC* T2, 91%; *BAALC* T1, 94%; *P* = .09; Table 2). *BCR-ABL*⁻ and *MLL-AF4*⁻ patients with higher *BAALC* expression had a significantly higher frequency of refractory disease compared to patients with lower *BAALC* expression (*BAALC* T3, 15%; *BAALC* T2, 6%; *BAALC* T1, 1%; *P* = .009; Table 2). On multivariate analysis, higher *BAALC* expression was independently predictive for primary resistant disease (*P* = .01) as well as the immunophenotype (Table 3).

Higher *BAALC* expression was significantly associated with an inferior OS for *BCR-ABL*⁻ and *MLL-AF4*⁻ patients (5-year OS: *BAALC* T3, 38%; *BAALC* T2, 52%; *BAALC* T1, 70%; *P* = .004; Table 2; Figure 1B). In the Cox regression analysis, *BAALC* expression was of independent prognostic significance for OS (*P* = .03); the other factors in the final model predicting OS were WBC and age (Table 3). *BAALC* expression did not have an impact on relapse rate (*P* = .76; Table 2) or RFS (*P* = .94) in *BCR-ABL*⁻ and *MLL-AF4*⁻ patients.

Moreover, within the SR group as defined by GMALL (*BCR-ABL*⁻ and *MLL-AF4*⁻ patients with CR after first induction therapy and WBC ≤ 30 × 10⁹/L at initial diagnosis), higher *BAALC* expression remained predictive for a shorter OS (*P* = .04; hazard ratio = 1.5; 95% CI, 1.0-2.3) as well as age.

We further examined the clinical outcome of *BCR-ABL*⁻ and *MLL-AF4*⁻ patients undergoing allogeneic SCT in CR1 with respect to *BAALC* expression. Because of the small number of

Table 3. Multivariate analysis of *BAALC* expression for clinical outcome in *BCR-ABL*⁻ and *MLL-AF4*⁻ patients

Variable	HR	OR	95% CI	*P*
Refractory disease*				
BAALC expression, T1 vs T2 vs T3		4.2	1.4-12.3	.01
Immunophenotype				.01‡
Common vs pre-B		1.0	0.1-9.1	.98
Pro-B vs pre-B		14	1.1-176	.04
Overall survival*†				
BAALC expression, T1 vs T2 vs T3	1.4		1.0-2.0	.03
WBC more than 30 × 10⁹/L	3.9		2.2-6.9	< .001
Age, 10-year increase	1.5		1.3-1.8	< .001

HR indicates hazard ratio; and OR, odds ratio.

*Variables considered for model inclusion are described in "Statistical analyses." The final model is shown.

†Patients who received SCT in CR1 were censored at the time of transplantation.

‡Overall *P* value for the variable immunophenotype.

patients and the stronger discrimination of T1 versus T2 compared with T2 versus T3 in OS (Figure 1), *BAALC* T2 and T3 were defined as high *BAALC* and *BAALC* T1 was defined as low *BAALC* for this analysis. *BCR-ABL*⁻ and *MLL-AF4*⁻ patients were assigned to allogeneic SCT if they fulfilled one of the following high-risk criteria: late therapy response, the immunophenotype of pro-B ALL, or high WBC count ("Patients"). In this subgroup, *BAALC* was no longer an adverse factor regarding OS, as patients with high *BAALC* expression tended to have an improved survival with a 5-year OS of 62% (data not shown). No selection bias was found between the *BAALC* groups in terms of age, WBC count, sex, indication for SCT, or other clinical factors. Thus, allogeneic SCT might overcome the adverse impact of *BAALC* and thereby leveling the differences in survival curves between the *BAALC* groups.

GEP of *BCR-ABL*⁻ and *MLL-AF4*⁻ B-precursor ALL

A microarray-based GEP of 51 B-precursor ALL pretreatment BM samples was performed to gain insights into biologic mechanisms of *BAALC* and its potential role in B-lymphoblastic leukemia and chemoresistance. Because *BAALC* was of particular prognostic relevance in the *BCR-ABL*⁻ and *MLL-AF4*⁻ subgroup and both aberrations, *BCR-ABL* and *MLL-AF4*, have previously been shown to drive specific gene-expression patterns,[22,23] we restricted our GEP analysis to *BCR-ABL*⁻ and *MLL-AF4*⁻ samples. Samples were grouped into high and low *BAALC* (t3 vs t1) based on the microarray expression data as described in "Oligonucleotide microarray studies." Expression level of *BAALC* was 22-fold increased in t3 samples compared with t1. Between the high and low *BAALC* expression groups, 54 differentially expressed probe sets corresponding to 43 genes and hypothetical proteins were identified (Figure 2); 32 were up-regulated and 11 were down-regulated (Table 4).

Differentially up-regulated genes in patients with high *BAALC* expression included markers of hematopoietic stem cells (*CD34* and *CD99*)[24] and genes that have previously been associated with drug resistance in chronic myeloid leukemia and AML (*LYN* and *TSPAN7*).[25-27] Interestingly, *SERPINB9* was up-regulated in the high *BAALC* group (*BCR-ABL*⁻ patients), a gene overexpressed in adult *BCR-ABL* ALL[22] and differentially expressed in pediatric ALL.[28] Within the up-regulated genes, we further found the frizzled receptor family member *FZD6* that has already been associated with high *BAALC* expression in CN-AML.[10] Among the down-regulated genes in the high *BAALC* group, we identified genes that have been related to a favorable outcome in adult ALL and AML (*CDKN1C/p57^Kip2* and *RGS2*).[29,30] Thus, our *BAALC*-associated GEP signature supports the clinical observation of a chemoresistant disease with inferior survival defined by high *BAALC* expression.

Figure 2. Heat map of differentially expressed probe sets between high and low *BAALC* expression groups in *BCR-ABL⁻* and *MLL-AF4⁻* ALL. Columns represent samples and rows represent genes ordered by hierarchical cluster analysis. Colors indicate relative expression of each gene with respect to the median expression level: red indicates above median value; yellow, equal; blue, below median value. Heat map of differentially expressed probe sets between high and low *BAALC* expression groups in *BCR-ABL⁻* and *MLL-AF4⁻* ALL.
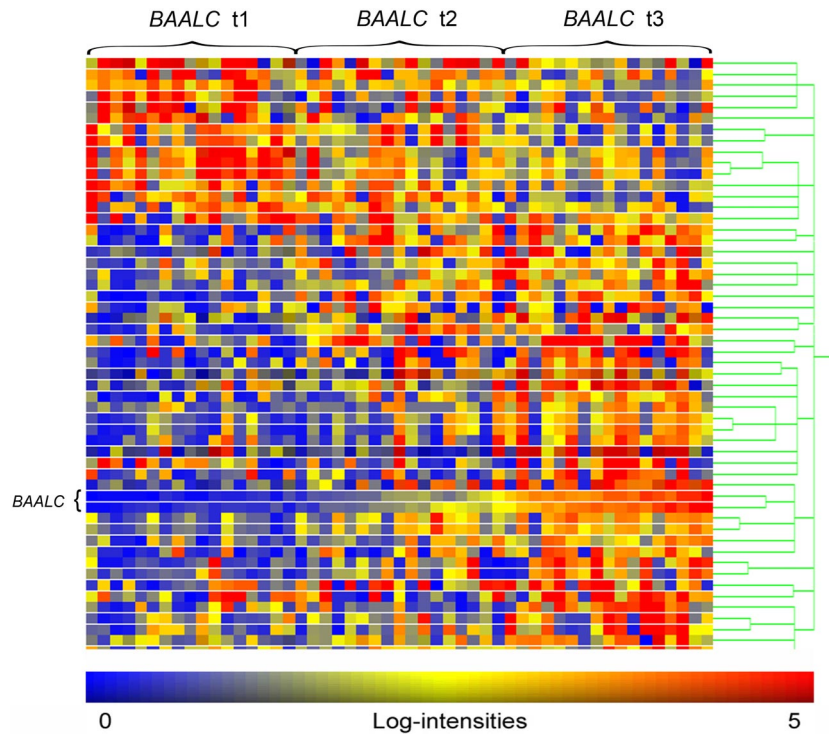
Discussion

The identification of novel predictive molecular markers in B-precursor ALL is particularly important for patients lacking high-risk markers (eg, *BCR-ABL*, *MLL-AF4*) and might in the future improve the outcome of this clinically and biologically heterogeneous subgroup. In this study, we have evaluated the prognostic significance of *BAALC* expression in B-precursor ALL, focusing on *BCR-ABL⁻* and *MLL-AF4⁻* patients.

As we have shown here, high *BAALC* expression independently predicted primary therapy resistance in adult B-precursor ALL patients. The risk of primary therapy failure in the overall cohort was 5 times higher for patients in the upper *BAALC* tertile (T3) compared with the lower tertile (T1). In *BCR-ABL⁻* and *MLL-AF4⁻* patients, the incidence of resistant disease was even 15-fold higher in *BAALC* T3 versus T1. In addition, higher *BAALC* expression was independently predictive for OS in *BCR-ABL⁻* and *MLL-AF4⁻* patients with a 5-year OS of only 38% in the *BAALC* T3 group.

In previous studies, high *BAALC* expression has already been predictive for an unfavorable therapy response and inferior long-term survival in adult CN-AML and T-ALL.[8-10,18] Our results in B-precursor ALL further highlight the adverse prognostic significance of aberrant *BAALC* expression in acute leukemias independent of the lineage. Moreover, *BAALC* is associated with an immature CD34⁺ leukemic subtype throughout the leukemic lineages. In B-precursor ALL, higher *BAALC* expression was further strongly correlated with known risk factors, such as high WBC count, *BCR-ABL*, and *MLL-AF4*. Thus, *BAALC* characterizes a more aggressive, immature, highly proliferative, and chemoresistant leukemic phenotype. *BAALC* as marker for normal hematopoietic progenitor cells may thereby reflect a marker of putative leukemic stem cells conferring drug resistance. Although the functional role of *BAALC* remains unknown, these findings support the possible involvement of *BAALC* in leukemogenesis.

These clinical observations were further supported by the GEP signature associated with high *BAALC* expression. In the high *BAALC* group, we observed up-regulation of genes associated with hematopoietic stem and progenitor cells (*CD34*, *CD99*, and *FZD6*)[24,31] and genes involved in therapy resistance in myeloid leukemias (*LYN* and *TSPAN7*).[25-27] This is similar to findings of Langer et al,[10] who reported an up-regulation of *CD34*, *FZD6*, and the multidrug resistance gene *ABCB1* in CN-AML with high *BAALC* expression. Overexpression of the tyrosine kinase LYN appears to be important for proliferation and survival of leukemic blasts.[32] *LYN* signaling was also shown to play a crucial role in AML with *FLT3* internal tandem duplication (ITD).[33,34] In addition, *RGS2*, one of the differentially down-regulated genes in the high *BAALC* group, was demonstrated to be repressed by *FLT3*-ITD in AML.[30]

The cyclin-dependent kinase inhibitor *CDKN1C/p57^Kip2*, a cell-cycle regulatory gene and putative tumor suppressor,[35] was down-regulated in patients with high *BAALC* expression. *CDKN1C/p57^Kip2* is aberrantly methylated in adult ALL, and inactivation of *CDKN1C/p57^Kip2*, *p73*, and *p15* has been related to a worse outcome in *BCR-ABL⁻* patients.[36] Thus, the GEP signature associated with high *BAALC* expression designates a highly proliferative subgroup of B-precursor ALL. This is further strengthened by similarities with molecular pathways and expression profiles found in other highly proliferative and resistant leukemic subtypes with inferior prognosis, such as *BCR-ABL* ALL and *FLT3*-ITD AML. In addition, the overlap of *BAALC*-associated signatures in B-precursor ALL and CN-AML underscores the lineage-independent impact of *BAALC* in acute leukemias.

For patients with primary therapy failure, new strategies during induction therapy are needed to overcome chemoresistance and to improve long-term outcome. General approaches to increase initial treatment response in B-lineage ALL include dose intensification of cytostatic agents[37,38] and the addition of monoclonal antibodies.[39-41] Intensification of myelosuppressive therapy has to be carefully considered as the rate of early deaths is increased and

Table 4. Differentially expressed genes in *BCR-ABL*⁻ and *MLL-AF4*⁻ patients between *BAALC* expression groups (t3 vs t1)

Gene symbol	Description	Fold change
Genes overexpressed in the high *BAALC* group (t3 vs t1)		
BAALC*	Brain and acute leukemia, cytoplasmic	22.8
ARHGEF17*	Rho guanine nucleotide exchange factor (GEF) 17	10.2
KCNK12	Potassium channel, subfamily K, member 12	8.2
IGJ	Immunoglobulin J polypeptide, linker protein for immunoglobulin-α and -μ polypeptides	7.7
LOC285628	Hypothetical protein LOC285628	6.3
STARD13	Start domain containing 13	6.0
LOC26010	Viral DNA polymerase-transactivated protein 6	5.6
SEMA6A	Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	5.6
MMP28	Matrix metallopeptidase 28	5.2
SDC2	Syndecan 2	5.0
STYK1	Serine/threonine/tyrosine kinase 1	5.0
KLF9*	Kruppel-like factor 9	4.8
CD34	CD34 antigen	4.1
CD99*	CD99 antigen	3.9
CYTL1	Cytokine-like 1	3.9
LOC144481	Hypothetical protein LOC144481	3.8
NRN	Neuritin 1	3.8
SESN1*	Sestrin 1	3.7
EMP1*	Epithelial membrane protein 1	3.7
NUDT4	NUDIX (nucleoside diphosphate-linked moiety X)-type motif 4	3.5
TBXA2R	Thromboxane A₂ receptor	3.5
TMEM156	Transmembrane protein 156	3.5
PPM1F*	Protein phosphatase (PP2C domain containing)	3.3
TSPAN7	Tetraspanin 7	3.3
MDFIC	MYOD family inhibitor domain containing	3.3
LYN	V-YES-1 Yamaguchi sarcoma viral oncogene homolog 1	3.1
CYGB	Cytoglobin	3.1
SERPINB9	Serpin peptidase inhibitor, clade B (ovalbumin), member 9	3.1
CLEC14A	C-type lectin domain family 14, member A	3.1
FHL1	Four-and-a-half LIM domains 1	3.1
MADD	MAP-kinase activating death domain	3.1
FZD6	Frizzled homolog 6 (*Drosophila*)	3.0
Genes underexpressed in the high *BAALC* group (t3 vs t1)		
BCL11B*	B-cell CLL/lymphoma 11B (zinc finger protein)	5.1
CAMK2D	Calcium/calmodulin-dependent protein kinase (CAM kinase) IIδ	4.6
CDKN1C	Cyclin-dependent kinase inhibitor 1C	3.8
FLJ34870	Hypothetical protein FLJ34870	3.6
MGC10744*	Transmembrane protein 107	3.4
COL27A1	Collagen, type 27, α 1	3.3
GATA3	GATA binding protein 3	3.2
AEBP1	AE binding protein 1	3.1
GLT1D1	Glycosyltransferase 1 domain containing 1	3.1
PYCARD	PYD and CARD domain containing	3.0
RGS2	Regulator of G-protein signaling 2, 24 kDa	3.0

*Gene was represented by more than one probe set. The fold change corresponds to the mean value of all probe sets.

dose intensity of subsequent treatments may be compromised. In this regard, determination of *BAALC* expression may help to better select patients at diagnosis with a poor response to induction therapy for which dose intensification or the use of new agents without multidrug resistance may be advantageous.

We demonstrate that higher *BAALC* expression independently predicts a shorter OS in *BCR-ABL*⁻ and *MLL-AF4*⁻ patients as well as in the SR group. Our findings indicate that determination of *BAALC* expression may contribute to a more detailed risk stratification of these patients. We focused on *BCR-ABL*⁻ and *MLL-AF4*⁻ patients for survival analyses because *BCR-ABL* and *MLL-AF4*, as confirmed unfavorable molecular markers, already direct specific treatment strategies.[1,42-44] We did not consider other high-risk cytogenetics, such as complex karyotypes,[44] because these aberrations are less frequent in B-precursor ALL and are not yet consistently integrated in ALL risk stratification throughout different study groups.

Interestingly, in *BCR-ABL*⁻ and *MLL-AF4*⁻ high-risk patients undergoing allogeneic SCT, high *BAALC* expressers showed a tendency toward an improved outcome compared with the low *BAALC* group (data not shown). Similar findings have been made in CN-AML, where the beneficial effect of allogeneic SCT was most evident in patients with high *BAALC* expression.[18] The adverse impact of *BAALC* might be overcome by intensive treatment strategies, such as allogeneic SCT. Further prospective clinical trials and experimental studies are necessary to address the impact of *BAALC* expression on outcome of patients undergoing allogeneic SCT.

Identification of molecular markers in acute leukemias is important to elucidate molecular pathways of leukemogenesis and

to direct the development of targeted therapies. The integration of prognostically relevant molecular markers into the risk assignment of ALL may improve clinical outcome by attributing to a precise risk-adapted treatment strategy. Recent approaches to identify new risk groups in B-precursor ALL included the determination of *CTGF* expression,[45] analyses of methylation status,[46] the integration of rare cytogenetic subgroups,[44,47] and genome-wide profiling of genetic alterations.[48,49] Deletions or mutations of the *IKAROS* gene have been associated with poor outcome in *BCR-ABL*⁻ high-risk childhood B-precursor ALL.[49] However, prospective studies are needed to confirm the prognostic significance of these new markers in the context of established risk factors.

This is the first study that investigated *BAALC* expression with respect to clinical features, treatment outcome, and GEP in adult B-precursor ALL. Our data suggest that *BAALC* may provide a new marker that identifies patients with an immature, chemoresistant leukemic phenotype associated with an unfavorable therapy response and inferior survival. Determination of *BAALC* expression might in the future facilitate treatment stratification for molecularly undefined subgroups of adult B-precursor ALL.

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Authorship

Contribution: A.K. performed research, analyzed data, and wrote the manuscript; N.G. supervised the GMALL study center and reviewed the manuscript; A.S. performed statistical analyses; T.B. provided diagnostic molecular genetic data; M.N. and S.H. contributed to study analysis; T.H. provided samples and expression data; D.H. supervised the GMALL study group; W.-K.H. contributed to the study analysis and reviewed the manuscript; E.T. supervised the central immunophenotyping and molecular genetics laboratory and reviewed the manuscript; and C.D.B. designed the research, contributed to study analysis, and reviewed the manuscript.

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