High CD45 surface expression determines relapse risk in children with precursor B-cell and T-cell acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol

Gunnar Cario,[1] Peter Rhein,[2]* Rita Mitlöhner,[2]* Martin Zimmermann,[3] Obul R. Bandapalli,[4] Renja Romey,[1] Anja Moericke,[1] Wolf-Dieter Ludwig,[5] Richard Ratei,[5] Martina U. Muckenthaler,[4] Andreas E. Kulozik,[4] Martin Schrappe,[1] Martin Stanulla,[1,3] and Leonid Karawajew[2]

[1]Pediatrics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel; [2]Department of Pediatric Oncology/Hematology, Charité – Universitätsmedizin Berlin; [3]Department of Pediatric Hematology/Oncology, Medical School Hannover; [4]Department of Pediatric Oncology, Hematology and Immunology, Children's Hospital, University of Heidelberg; and [5]Department of Hematology, Oncology and Tumor Immunology, HELIOS-Clinic Berlin-Buch, Berlin, Germany

*PR and RM contributed equally to this work.

ABSTRACT

Further improvement of outcome in childhood acute lymphoblastic leukemia could be achieved by identifying additional high-risk patients who may benefit from intensified treatment. We earlier identified *PTPRC* (CD45) gene expression as a potential new stratification marker and now analyzed the prognostic relevance of CD45 protein expression. CD45 was measured by flow cytometry in 1065 patients treated according to the ALL-BFM-2000 protocol. The 75th percentile was used as cut-off to distinguish a CD45-high from a CD45-low group. As mean CD45 expression was significantly higher in T-cell acute lymphoblastic leukemia than in B-cell-precursor acute lymphoblastic leukemia (P<0.0001), the analysis was performed separately in both groups. In B-cell-precursor acute lymphoblastic leukemia we observed a significant association of a high CD45 expression with older age, high initial white blood cell count, *ETV6/RUNX1* negativity, absence of high hyperdiploidy (P<0.0001), *MLL/AF4* positivity (P=0.002), *BCR/ABL1* positivity (P=0.007), prednisone poor response (P=0.002) and minimal residual disease (P<0.0001). In T-cell acute lymphoblastic leukemia we observed a significant association with initial white blood cell count (P=0.0003), prednisone poor response (P=0.01), and minimal residual disease (P=0.02). Compared to CD45-low patients, CD45-high patients had a lower event-free survival rate (B-cell-precursor acute lymphoblastic leukemia: 72±3% *versus* 86±1%, P<0.0001; T-cell acute lymphoblastic leukemia: 60±8% *versus* 78±4%, P=0.02), which was mainly attributable to a higher cumulative relapse incidence (B-cell-precursor acute lymphoblastic leukemia: 22±3% *versus* 11±1%, P<0.0001; T-cell acute lymphoblastic leukemia: 31±8% *versus* 11±3%, P=0.003) and kept its significance in multivariate analysis considering sex, age, initial white blood cell count, and minimal residual disease in B-cell-precursor- and T-cell acute lymphoblastic leukemia, and additionally presence of *ETV6/RUNX1*, *MLL/AF4* and *BCR/ABL1* rearrangements in B-cell-precursor acute lymphoblastic leukemia (P=0.002 and P=0.025, respectively). Consideration of CD45 expression may serve as an additional stratification tool in BFM-based protocols. (*ClinicalTrials.gov identifier: NCT00430118)*

Introduction

Although treatment of children with acute lymphoblastic leukemia (ALL) has improved continuously over the last decades, first-line therapy still fails in approximately 20% of cases and children suffer from disease recurrence.[1-3] As yet, only some of these children with relapsed ALL can be cured, mainly depending on the time point and site of relapse, and on the ALL-immunophenotype. Patients with T-cell ALL (T-ALL), in particular, have a very poor prognosis once they have relapsed[4,5] and further improvement of front-line treatment for such patients is, therefore, continuously needed. Further reduction of relapses in childhood ALL could be achieved by identifying additional high-risk patients who may benefit from treatment intensification. Ideal stratification parameters are available at diagnosis in order to adapt treatment as early as possible and are easy to standardize and to assess by routine laboratories worldwide. Moreover, in order for the parameter to be used not only in developed countries, its analysis should be inexpensive.

In the ALL-BFM 2000 trial, the high-risk group was defined by inadequate initial response to induction treatment (poor prednisone response on treatment day 8, non-remission on treatment day 33, and/or a high load of minimal residual disease (≥10^{-3}) after 12 weeks of treatment) and/or by positivity for a translocation t(4;11) or t(9;22) or their molecular counterparts. Absence of minimal residual disease already on treatment day 33 defined standard-risk patients, whereas measurable minimal residual disease at a low level characterized the intermediate-risk group. Of importance, a very high

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Correspondence: g.cario@pediatrics.uni-kiel.de

number of relapses occurred within this heterogeneous group of patients.[1-3]

In a previous study, we compared gene expression profiles of minimal residual disease-resistant and -sensitive ALL in a case-control setting in order to identify potential new stratification markers.[6] Subsequently, we tried to confirm the potential prognostic relevance of genes identified and their respective proteins in representative study populations. The protein tyrosine phosphatase CD45, encoded by The *PTPRC* (protein-tyrosine phosphatase, receptor-type, C) gene, encoding for protein-tyrosine phosphatase CD45 was one of these candidate genes.

CD45 is a common surface molecule on hematopoietic cells and included in routine flow cytometric diagnostics in leukemias. It is a key regulator of antigen receptor signaling in T and B cells by dephosphorylation of Src kinases and inhibits cytokine receptor signaling by negatively regulating JAK kinases.[7-10] There is increasing surface expression as lymphoid cells mature; however, a bright expression was also seen in *MLL-AF4*-positive pro-B-ALL.[11,12] Notably, several years ago, M. Borowitz and colleagues already showed in a Pediatric Oncology Group study that bright CD45 expression was associated with a poor prognosis in B-cell-precursor ALL (BCP-ALL) in their study population.[11] Very recently, inactivating mutations in the *PTPRC* gene were identified in T-ALL and it was shown that CD45 might act as a tumor suppressor, suggesting that CD45 expression could be of prognostic relevance also in T-ALL.[13]

In the present study, we assessed the prognostic role of CD45 expression in a large cohort of 1065 pediatric ALL patients treated according to the ALL-BFM 2000 protocol and found that high CD45 expression was associated with an inferior outcome not only in BCP-ALL but also in T-ALL. As CD45 is already included in routine diagnostic panels, it constitutes an interesting candidate marker to be included in future treatment stratification strategies on ALL-BFM-based protocols.

Methods

Patients

In accordance with institutional review board regulations, clinical samples were obtained from children with ALL before treatment. The study was approved by the institutional review board of Hannover Medical School, Hannover, Germany, and informed consent was obtained from patients and/or their legal guardians in accordance with the Declaration of Helsinki. Diagnostics, risk group assignment, and treatment were performed according to the ALL-BFM 2000 protocol.[1,3] Details are given in the *Online Supplementary Methods*. Patients consecutively enrolled from April 2003 to January 2008 in the ALL-BFM 2000 trial were included in our present study.

Flow cytometric measurement and quantification of CD45 surface expression

CD45 expression was routinely assessed at diagnostic immunophenotyping, which was performed centrally in the immunodiagnostic reference laboratory of the ALL-BFM 2000 trial. Samples were taken prior to the initiation of treatment and shipped overnight. They were analyzed and reported according to the guidelines proposed by the European Group for the Immunological Characterization of Leukemias (EGIL).[14] Accordingly, antigen expression was quantified in terms of percentage of positive cells as compared to an antigen-negative subpopulation or, in the case of CD45, in comparison to a negative control with an isotype- and fluorochrome-matched antibody. Details on the methods applied are explained in the *Online Supplementary Methods*.[15]

In the study presented here, we re-analyzed the "raw" flow cytometric data and quantified CD45 expression in order to provide a full-scale estimation of CD45 expression. To control for technical variation, we normalized the measured expression values by use of normal mature B- and T-lymphocytes within the same sample, which are known to express high levels of CD45 stably.[16,17] The resulting expression values are described as relative ratios of CD45 expression in leukemic *versus* normal cells in percentages.

Detection of the P2RY8-CRLF2 fusion

Data on *P2RY8-CRLF2* status were available for 250 patients with BCP-ALL. The *P2RY8-CRLF2* fusion was investigated as previously described and detailed in the *Online Supplementary Methods*.[18]

Analysis of IL7R mutations

In 107 patients with T-ALL data on the mutation status of the interleukin-7 receptor gene (*IL7R*) were available. The analysis was performed as previously described and details are given in the *Online Supplementary Methods*.[19]

Statistical analysis

Event-free survival was calculated from the date of diagnosis to last follow-up or to the first event (no complete remission as an event on day 0, relapse, secondary malignancy, or death of any cause). Rates were calculated according to Kaplan-Meier and compared by the log-rank test. Cumulative relapse incidence functions were constructed using the method of Kalbfleisch and Prentice and compared by the Gray test. Multivariate cox regression was used to calculate the hazard ratio for an event, mediated by CD45-high expression. Proportional differences between groups of patients were analyzed by a χ^2 or Fisher's exact test. Statistical analyses were carried out using the SPSS statistical package (IBM, Chicago, IL, USA; release XY) and two-sided *P*-values below 0.05 were considered to be statistically significant.

Results

Association of CD45 expression with immunophenotype and genetic aberrations

CD45 expression was measured in 1065 leukemic samples from children with ALL consecutively enrolled in the ALL-BFM 2000 trial (*Online Supplementary Table S1*). Bone marrow was analyzed in 92% of cases and peripheral blood in the remaining 8%. CD45 expression was significantly higher in T-ALL than in BCP-ALL (mean±SD: 51.05±30.33% *versus* 8.61±9.75%, $P<0.0001$). In T-ALL we observed that CD45 expression increased according to maturation arrest stages (*Online Supplementary Table S2*). In contrast, in BCP-ALL CD45 expression was significantly higher in pro-B ALL than in common or pre-B-ALL which was not only attributable to the high frequency of an *MLL/AF4* rearrangement in this BCP-ALL subgroup (*Online Supplementary Table S3*). Among common molecular subtypes, CD45 expression was low in *ETV6/RUNX1*-positive and hyperdiploid good prognosis patients. Conversely, high CD45 expression was seen in unfavor-

able groups with a *BCR/ABL1* or *MLL/AF4* rearrangement. Samples negative for these common aberrations ('B-other') were characterized by a CD45 expression comparable to that in *BCR/ABL1*-positive ALL cases (*Online Supplementary Table S4*).

Association of CD45 expression with patient and biological characteristics and response to induction therapy

As CD45 expression was significantly different in BCP-ALL and T-ALL, further analysis was performed separately in both groups. Analyzing 5-year event-free survival and cumulative incidence of relapse according to CD45 expression in quartiles, we observed that patients with a CD45 expression up to the 75th percentile showed only little differences in outcome, but those with expression within the 4th quartile had a much higher risk of treatment failure (*Online Supplementary Figure S1*). This observation is similar to that of Borowitz and colleagues, and we, therefore, also used the 75th percentile as a cut-off to distinguish a CD45-high from a CD45-low expression group in our study.[11] In BCP-ALL, the 75th percentile corresponds to a CD45-expression ratio of leukemic blasts *versus* normal lymphocytes of 11%, whereas in T-ALL it corresponds to a ratio of 70%.

Comparing the CD45-high and –low expression groups, no significant differences were observed for sex in either immunophenotype or for age at diagnosis in T-ALL. In BCP-ALL, there was a positive association of CD45 expression with older age (P<0.0001), positivity for a *BCR/ABL1* rearrangement (P=0.007) and a *MLL/AF4* rearrangement (P=0.002), absence of high hyperdiploidy (P<0.0001), as well as negativity for an *ETV6/RUNX1* rearrangement (P<0.0001) (Table 1).

In BCP-ALL and T-ALL, a high CD45 expression was associated with a high white blood cell count at diagnosis (BCP-ALL: P<0.0001, T-ALL: P=0.0003). Analyzing the association of CD45 expression and treatment response in both immunophenotypes, there were significantly more patients with prednisone-poor response (BCP-ALL: P=0.002; T-ALL: P=0.01) and high-risk minimal residual disease (P<0.0001 and P=0.02, respectively) in the CD45-high expression group (Table 1).

Association of CD45 expression and treatment outcome

Patients with high CD45 expression had a worse 5-year event-free survival probability compared to patients with low expression (BCP-ALL: 72±3% *versus* 86±1%, P<0.0001; T-ALL: 60±8% *versus* 78±4%, P=0.02) (Figure 1A and E). This effect was mainly related to a higher cumulative incidence of relapse (BCP-ALL: 22±3% *versus* 11±1%, P<0.0001; T-ALL: 31±8% *versus* 11±3%, P=0.003) (Figure 1B and F).

Analyzing treatment outcome stratified according to current risk groups, these differences in outcome were mainly attributable to the effect of CD45 expression in immediate-risk patients: no significant differences in event-free survival or cumulative relapse incidence were seen in standard-risk patients (CD45-high *versus* low patients: BCP-ALL: 5-year event-free survival 92±2% *versus* 92±3%, P=0.96; cumulative incidence of relapse 6±3% *versus* 7±2%, P=0.66; T-ALL: no events in both groups) and only a trend towards poor event-free survival and high cumulative relapse incidence in patients with higher CD45 expression but not significant in high-risk patients (CD45-high *versus* low patients: BCP-ALL: 5-year event-free survival 50±7% *versus* 65±7%, P=0.14; cumulative incidence of relapse 38±7% *versus* 25±6%, P=0.10; T-ALL: 5-year event-free survival 57±10% *versus* 70±7%, P=0.22; cumulative relapse incidence 30±10% *versus* 18±6%, P=0.20). However, in intermediate-risk patients, event-free survival was lower (BCP-ALL: 67±5% *versus* 85±2%, P<0.0001; T-ALL: 64±15% *versus* 81±6%, P=0.18) (Figure 1C and G) and the corresponding cumulative relapse incidence significantly higher in CD45-high patients (BCP-ALL: 28±4% *versus* 11±2%, P<0.0001; T-ALL: 36±15% *versus* 8±4%, P=0.008) (Figure 1D and H).

Analyzing outcome after exclusion of samples with *BCR/ABL1* and *MLL/AF4* rearrangements, which are known to have a poor prognosis, a high CD45 expression kept its association with an inferior event-free survival (*Online Supplementary Figure S2*). Similarly, we looked at those cases without any of the common genetic aberrations known to be associated with a distinct prognosis (no *ETV6/RUNX1* rearrangement, no high hyperdiploidy, no *BCR/ABL1*, no *MLL/AF4* rearrangement, so called 'B-other'). Applying the 75th percentile cut-off when determined using the entire group of BCP-ALL, no significant differences in outcome were observed comparing CD45-high *versus* CD45-low expressing cases (*data not shown*). As CD45 expression was significantly higher in this group than in the remaining samples (Table 1), we next determined the 75th percentile within the 'B-other' group, resulting in a cut-off of 16%. Applying this cut-off, a high CD45 expression was again found to be significantly associated with a lower probability of event-free survival (67±5% *versus* 76±3%, P=0.039); however cumulative incidence of relapse did not differ significantly (25±5% *versus* 20±3%, P=0.15) (*Online Supplementary Figure S3*).

In a multivariate analysis considering sex, initial WBC count, age at diagnosis, presence of *ETV6/RUNX1* rearrangement, presence of *BCR-ABL1* or *MLL-AF4* rearrangement, and minimal residual disease risk group in addition to high CD45 expression in BCP-ALL, a high CD45 expression provided independent prognostic information (hazard ratio for relapse: 1.93, 95% confidence interval 1.28-2.91, P=0.002) (Table 2). High hyperdiploidy was not considered in multivariate analysis as data regarding this feature were incomplete in our data set. In T-ALL, multivariate analysis was performed including sex, initial WBC count, age at diagnosis and minimal residual disease risk group in addition to high CD45 expression; once again, high CD45 expression provided independent prognostic information (hazard ratio for relapse: 2.86, 95% confidence interval 1.14-7.21, P=0.025) (Table 3). Similar results were obtained considering intermediate-risk patients only (Tables 2 and 3). Remarkably, in T-ALL CD45 expression had a greater significance than all other parameters.

CD45 expression and presence of P2RY8-CRLF2 in B-cell precursor acute lymphocytic leukemia and IL7R mutations in T-cell acute lymphocytic leukemia

CD45 is a negative regulator of cytokine receptor signaling. A cytokine receptor, cytokine receptor-like factor 2 (CRLF2), has been recently implicated in lymphoid transformation in BCP-ALL. In particular, the *P2RY8-CRLF2* fusion leads to elevated CRLF2 expression and consecutively to activation of downstream JAK/STAT signaling.[20-22]

We, therefore, analyzed the association of CD45 expression and the presence of the *P2RY8-CRLF2* fusion in BCP-ALL. Information on the presence of a *P2RY8-CRLF2* fusion was available for 250 patients with BCP-ALL. Comparing samples analyzed for the *P2RY8-CRLF2* fusion and those not analyzed, no significant differences were seen with respect to sex, age, presence of *ETV6/RUNX1* and *BCR/ABL1* rearrangements, minimal residual disease, event-free survival, as well as CD45 expression. Samples analyzed for the *P2RY8-CRLF2* fusion had a higher WBC at diagnosis ($P<0.001$). We observed significantly more *P2RY8-CRLF2*-positive patients in the CD45-high group than in the CD45-low group (12.9% *versus* 2.1%, $P=0.002$), suggesting a relationship between presence of the *P2RY8-CRLF2* fusion, consecutive activation of CRLF2 signaling, and up-regulation of CD45 expression.

Similarly, we analyzed the association of high CD45 expression and activating *IL7R* mutations in T-ALL.[19,23] Comparing samples analyzed for *IL7R* mutations and those not analyzed, no significant differences were seen with respect to sex, age, WBC at diagnosis, minimal residual disease, or event-free survival. Samples analyzed for

Table 1. Patient and biological characteristics and response to treatment according to CD45 expression in BCP-ALL and in T-ALL.

	BCP ALL[1]			T-ALL[1]		
	CD45-low n. (%)	CD45-high n. (%)	P[2]	CD45-low n. (%)	CD45-high n. (%)	P[2]
N. of patients	678	247		105	35	
Sex			0.65			0.23
Male	371 (54.7)	131 (53.0)		73 (69.5)	28 (80.0)	
Female	307 (45.3)	116 (47.0)		32 (30.5)	7 (20.0)	
Age at diagnosis (years)			<0.0001			0.92
1-9	549 (81.0)	171 (69.2)		49 (46.7)	16 (45.7)	
≥10	129 (19.0)	76 (30.8)		56 (53.3)	19 (54.3)	
WBC count (x10^{10}/L)			<0.0001			0.0003
< 1	387 (57.1)	81 (32.8)		16 (15.2)	3 (8.6)	
1-4.99	228 (33.6)	95 (38.5)		37 (35.2)	3 (8.6)	
5-9.99	39 (5.8)	36 (14.6)		20 (19.0)	4 (11.4)	
≥ 10	24 (3.5)	35 (14.2)		32 (30.5)	25 (71.4)	
NCI risk group			<0.0001			0.01
Standard risk	496 (73.2)	124 (50.2)		23 (21.9)	1 (2.9)	
High risk	182 (26.8)	123 (49.8)		82 (78.1)	34 (97.1)	
Prednisone response[3]			0.002			0.01
Good	646 (95.3)	221 (89.5)		67 (63.8)	14 (40.0)	
Poor	29 (4.3)	24 (9.7)		34 (32.4)	20 (57.1)	
No information	3 (0.4)	2 (0.8)		4 (3.8)	1 (2.9)	
MRD risk group[4]			<0.0001			0.02
Standard	250 (36.9)	86 (34.8)		11 (10.5)	1 (2.9)	
Intermediate	372 (54.9)	109 (44.1)		53 (50.5)	11 (31.4)	
High	56 (8.3)	52 (21.1)		41 (39.0)	23 (65.7)	
DNA index[5]			<0.0001			0.81
<1.16	368 (54.3)	194 (78.5)		89 (84.8)	27 (77.1)	
≥1.16	152 (22.4)	13 (5.3)		8 (7.6)	2 (5.7)	
No information	158 (23.3)	40 (16.2)		8 (7.6)	6 (17.2)	
ETV6/RUNX1			<0.0001			
Negative	429 (63.3)	198 (80.1)				
Positive	194 (28.6)	32 (13.0)				
No information	55 (8.1)	17 (6.9)				
BCR/ABL1			0.007			
Negative	668 (98.5)	236 (95.5)				
Positive	10 (1.5)	11 (4.5)				
MLL/AF9			0.002			
Negative	677 (99.9)	236 (95.5)				
Positive	1 (0.1)	11 (4.5)				
B-other[6]			<0.0001			
Negative	357 (52.7)	67 (27.1)				
Positive	209 (30.8)	156 (63.2)				
No information	112 (16.5)	24 (9.7)				

[1]As a cut-off to distinguish the CD45-low from the CD45-high expression group, the 75th percentile was used. In BCP-ALL the 75th percentile corresponds to a CD45 expression ratio (expression on leukemic blasts/normal lymphocytes) of 11%, in T-ALL to a CD45 expression ratio of 70%. [2]χ^2 test comparing CD45-high and CD45–low groups, patients with no information excluded from test; [3]good: less than 1000 leukemic blood blasts /μL on treatment day 8, poor: more than 1000 /μL. [4]Minimal residual disease (MRD) risk groups: standard risk: negative at time points 1 and 2 (TP1 and TP2), intermediate risk: TP1 and/or TP2 <10^{-3}, high risk: TP2 ≥10^{-3}. [5]DNA-Index≥1.16 indicates high hyperdiploidy. [6]B-other: defined as negative for high hyperdiploidy, ETV6/RUNX1, BCR/ABL1, *and* MLL/AF4 *rearrangements.*

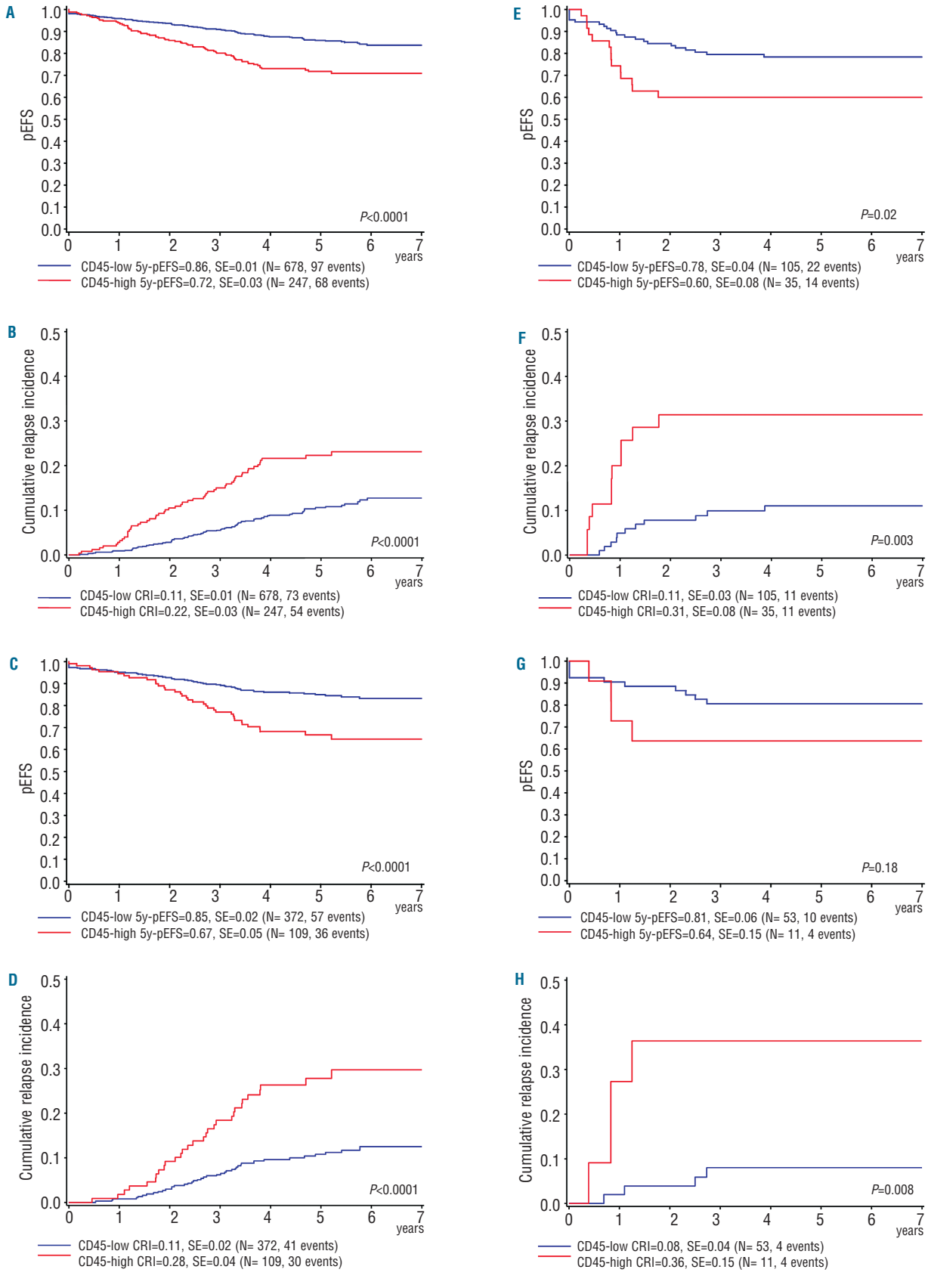

Figure 1. Treatment outcome comparing pediatric ALL patients with CD45-high and CD45-low expression. Kaplan-Meier estimates are shown for BCP-ALL on the left side (A-D) and T-ALL on the right side (E-H). For the whole cohort: (A and E) event-free survival (EFS) at 5 years, and (B and F) cumulative relapse incidence (CRI) at 5 years. For the intermediate risk group: (C and G) EFS, (D and H) and CRI. The same cut-off to distinguish CD45-high from CD45-low expression was used for all analyses in BCP-ALL (A-D) and T-ALL (E-H).

IL7R mutations had higher CD45 expression (*P*=0.002). Here, the number of patients with *IL7R* mutations was more than 2-fold higher in the CD45-high group than in the CD45-low group (12.9% *versus* 5.3%, *P*=0.225). Together, these data suggest a connection between activating *IL7R* mutations, JAK/STAT pathway activation, and high expression of CD45.

Discussion

In a previous study, we compared gene expression profiles of minimal residual disease-resistant (high risk) and -sensitive (standard-risk) ALL in order to identify potential new prognostic markers.[6] Subsequently, we tried to confirm the potential prognostic relevance of genes identified and their respective proteins in representative study populations. One of these genes was *PTPRC* which encodes for the protein tyrosine phosphatase CD45. CD45 is part of the immunophenotyping marker panels for the diagnosis of acute leukemias recommended by the EGIL and the European LeukemiaNet.[14,24] It is used to define the blast population, on which the expression of differentiation antigens is to be focused. In the routine diagnostics of the ALL-BFM 2000 trial, CD45 expression was reported in terms of percentage of positive cells. This approach does not, however, allow for quantification of the brightness of expression. In fact, using this approach we were previously unable to demonstrate an association between CD45 expression and prognosis in the setting of past ALL-BFM-based protocols.[25] By contrast, our recent gene expression study based on a continuous scaling of *PTPRC* gene expression indicated its potential prognostic significance. In order to provide a full-scale estimation of CD45 expression at a protein level we, therefore, re-analyzed the flow cytometric data and quantified CD45 expression in terms of fluorescence intensity ratio between blasts and normal lymphoid cells.

Interestingly we observed significantly higher CD45 expression in immature pro-B ALL as compared to the more mature common and pre-B ALL, contrasting the data on CD45 expression according to the maturation stage in normal lymphopoiesis.[12] This difference might in part be explained by the presence of a *MLL/AF4* rearrangement.[11] However, CD45 expression was also high in those pro-B ALL-samples that tested negative for the *MLL/AF4* rearrangement, and some of these cases may be characterized by other *MLL*-gene rearrangements which were not routinely analyzed.

With respect to the potential prognostic value of measuring CD45 expression, our analysis of an unselected set of 1065 patients treated according to the ALL-BFM-2000 protocol demonstrated that high CD45 expression on leukemic blasts was associated with a poor prognosis. The high CD45 expression was associated with treatment response and outcome, mainly due to a higher cumulative incidence of relapse, and had additive value as a prognostic factor - especially in the intermediate-risk group of patients in whom the majority of relapses still occurs. Of interest, Borowitz and colleagues showed that bright CD45 expression (quantified in terms of mean equivalents of soluble fluorochrome using calibrated fluorescence beads) was associated with a poor prognosis in BCP-ALL patients treated according to the protocols of the Pediatric Oncology Group study group.[11] Our data indicate that high levels of CD45 expression on leukemic blasts are not only associated with a poor prognosis in BCP-ALL, but also in T-ALL. Moreover, whereas in BCP-ALL the prognostic weight of CD45 expression was relatively low as compared to established risk factors in our study population, the prognostic relevance of CD45 expression was much higher in T-ALL. This is of special interest, as

Table 2. Multivariate Cox regression analysis for the risk of relapse in BCP-ALL.

	Whole cohort			Intermediate-risk group		
Feature	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
CD45-high	1.93	1.28-2.91	0.002	1.82	1.24-2.68	0.002
Female sex	0.58	0.39-0.87	0.008	0.54	0.36-0.79	0.002
Age ≥ 10 years	1.16	0.75-1.80	0.507	1.32	0.87-1.98	0.188
WBC ≥ $5x10^{10}$/L	2.49	1.63-3.81	<0.0001	2.88	1.92-4.31	<0.0001
ETV6/RUNX1	0.52	0.29-0.94	0.029	0.41	0.24-0.72	0.002
BCR/ABL1 or *MLL/AF4*	2.17	1.00-4.71	0.051	-	-	-
MRD-standard risk	2.56	1.58-4.16	<0.0001	-	-	-
MRD-high risk	1.73	1.02-2.94	0.044	-	-	-

Table 3. Multivariate cox regression analysis for the risk of relapse in T-ALL.

	Whole cohort			Intermediate-risk group		
Feature	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
CD45-high	2.86	1.14 – 7.21	0.025	3.93	1.60 – 9.68	0.003
Female sex	0.7	0.23 – 2.12	0.534	0.63	0.21 – 1.88	0.406
Age ≥ 10 years	2.06	0.82 – 5.20	0.125	2.17	0.87 – 5.39	0.094
WBC ≥ $5x10^{10}$/L	0.78	0.30 – 1.99	0.598	0.74	0.30 – 1.86	0.526
MRD-high risk	1.34	0.51 – 3.55	0.553	-	-	-

patients with T-ALL have a particularly dismal outcome once they have relapsed.[4,5]

CD45 is a key regulator of antigen receptor signaling in T and B cells and inhibits cytokine receptor signaling by negatively regulating JAK kinases in anti-apoptotic JAK-STAT signaling.[7-10] Common aberrations leading to activation of JAK-STAT signaling are the *P2RY8-CRLF2* fusion in BCP-ALL, and activating mutations of *IL7R* in T-ALL.[18-23,26-29] We analyzed the association of high CD45 expression and these aberrations and found that the presence of a *P2RY8-CRLF2* fusion in BCP-ALL as well as of *IL7R* mutations in T-ALL is associated with high CD45 expression. It has also been shown that an activated kinase gene expression signature (the so called Ph-like or BCR-ABL-like signature) could be associated with a poor prognosis irrespectively of the underlying genomic aberration.[30-32] It would, therefore, be worth analyzing CD45 expression in patients with this signature in order to assess whether CD45 might serve as a screening tool for this important group of patients.

CD45 negatively regulates Src and JAK kinases which are involved in the maintenance and proliferation of cells and increased CD45 expression should inhibit cell proliferation, since proliferation is increased in cells that do not express CD45.[8,13] We did find that there is an inverse correlation between the percentage of CD45-positive leukemic cells and the percentage of blasts in S-phase.[25] Recently, we and others have shown that a low proliferation gene expression signature in primary leukemic samples is associated with *in vivo* treatment resistance.[6,33] Given that the function of the majority of cytotoxic agents currently used in the treatment of acute leukemias is cell cycle-dependent, reduced cell proliferation could explain decreased sensitivity of these blasts to treatment and subsequently a higher rate of treatment failure.

In summary, high CD45 surface expression is associated with a poor prognosis in BCP-ALL as well as in T-ALL. Different prognostic parameters have already been identified for these diseases and probably more will be discovered in the near future. We are convinced that a combination of classical and new risk parameters will create an integrated prognostic classifier which will contribute to a further improvement of risk-adapted treatment in childhood ALL. Measurement of CD45 expression may become one component of this classifier. However, there is a clear need for further analysis of CD45 expression, combined with other potential markers, in larger study populations. As CD45 expression is routinely measured in diagnostics of acute leukemias, its use as a prognostic parameter is attractive not only in developed countries, but could be of particular interest in countries with limited financial resources.

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