Expression profiling of adult acute lymphoblastic leukemia identifies a *BCR-ABL1*-like subgroup characterized by high non-response and relapse rates

Outcome in adult precursor B-cell acute lymphoblastic leukemia (BCP-ALL) is unsatisfactory, with complete response rates of approximately 80% and event-free survival at 5 years of only 40%-50%.[1] This inferior survival in adult compared with pediatric patients might be explained by a higher frequency of poor prognostic cytogenetic subtypes, such as *BCR-ABL1* translocation and *MLL* rearrangements, and a lower frequency of better prognostic subtypes, such as *ETV6-RUNX1* and high-hyperdiploidy. Secondly, treatment intensity varies markedly between protocols in children and adults. Improved outcome was observed for adolescents and adults receiving intensified chemotherapy inspired by a pediatric regimen.[2,3] Based on gene expression similar to *BCR-ABL1*-positive cases, a new high-risk group in pediatric BCP-ALL was identified, '*BCR-ABL1*-like', which included approximately 15% of BCP-ALL cases and was associated with higher relapse incidence and lower event-free survival.[4,5] The frequency of *IKZF1* deletions, which is approximately 75% in *BCR-ABL1*-positive adults[6,7] and children,[8] is also higher in the pediatric *BCR-ABL1*-like group (~40%) than in other BCP-ALL (~16%).[4,5] Recently, the *BCR-ABL1*-like signature and *IKZF1* deletion were shown to be independent prognostic

Table 1. Characteristics of adult BCP-ALL patients included in the molecular analysis.

Characteristic	*BCR-ABL1*-positive n=42 (33%)	*BCR-ABL1*-like n=21 (17%)	*MLL*-rearranged n=14 (11%)	Remaining BCP-ALL[a] n=50 (39%)	All BCP-ALL patients n=127 (100%)	*P*[c]
Age median (range)	38 (17-71)	25 (16-59)	39 (20-66)	34 (16-68)	36 (16-71)	0.15
16-20 years	5 (12%)	6 (29%)	1 (7%)	12 (24%)	24 (19%)	
21-39 years	18 (43%)	9 (43%)	6 (43%)	15 (30%)	48 (38%)	
40-71 years	19 (45%)	6 (29%)	7 (50%)	23 (46%)	55 (43%)	
Immunophenotype						
Pro-B-ALL	1 (2%)	4 (19%)	10 (71%)	10 (20%)	25 (20%)	<0.001
Common B-ALL	32 (76%)	14 (67%)	–	31 (62%)	77 (61%)	
Pre-B-ALL	7 (17%)	–	2 (14%)	5 (10%)	14 (11%)	
B, not further specified	2 (5%)	3 (14%)	2 (14%)	4 (8%)	11 (9%)	
Risk group[b]						
Standard	–	12 (57%)	–	35 (70%)	47 (37%)	<0.001
High	42 (100%)	9 (43%)	14 (100%)	15 (30%)	80 (63%)	
White blood cell count						
< 30x10^9/L	19 (45%)	16 (76%)	5 (36%)	41 (82%)	81 (64%)	<0.001
≥30x10^9/L	23 (55%)	5 (24%)	9 (64%)	9 (18%)	46 (36%)	
IKZF1 status						
No deletion	10 (26%)	13 (65%)	12 (86%)	31 (63%)	66 (54%)	<0.001
Deletion (*)	29 (74%)	7 (35%)	2 (14%)	18 (37%)	56 (46%)	
Not detected	3	1	–	1	5	
(*) Type of *IKZF1* deletion						
Haploinsufficient (incl. exon 2)	17	3	2	15	37	
Dominant-negative (DEL 4-7)	9	2	–	3	14	
Other (DEL 3-8 or DEL 4-8)	3	2	–	–	5	
Treatment intensity						
Intensive (1993-2005)	32 (76%)	18 (86%)	9 (64%)	32 (64%)	91 (72%)	0.24
High-intensive (2005-2009)	10 (24%)	3 (14%)	5(36%)	18 (36%)	36 (28%)	
Imatinib						
No	25 (60%)	21 (100%)	14 (100%)	50 (100%)	110 (87%)	<0.001
Yes	17 (40%)	–	–	–	17 (13%)	
CR on first-line treatment						
No (**)	12 (29%)	6 (29%)	–	3 (6%)	21 (17%)	0.003
Yes	30 (71%)	15 (71%)	14 (100%)	47 (94%)	106 (83%)	
(**) Reason for no CR on first-line treatment						
Early death	4	3	–	3	10	
Refractory	8	3	–	–	11	
Allogeneic SCT in first CR						
No	10 (33%)	10 (67%)	6 (43%)	30 (64%)	56 (53%)	0.04
Yes	20 (67%)	5 (33%)	8 (57%)	17 (36%)	50 (47%)	

CR: complete response; SCT: stem cell transplantation. [a]Remaining BCP-ALL group contains eight cases with high-hyperdiploidy (50-62 chromosomes), three TCF3-PBX1*-rearranged cases, and 39 remaining BCP-ALL cases without known recurrent aberrations, and not identified by the BCR-ABL1-like signature. [b]High risk: white blood cell count ≥30 x 10^9/L, and/or* BCR-ABL1 *positivity,* MLL *abnormality, and/or no CR after remission induction course 1. [c]For categorical variables, the Fisher exact test was used. Age was tested as a continuous variable using the Kruskal-Wallis test.*

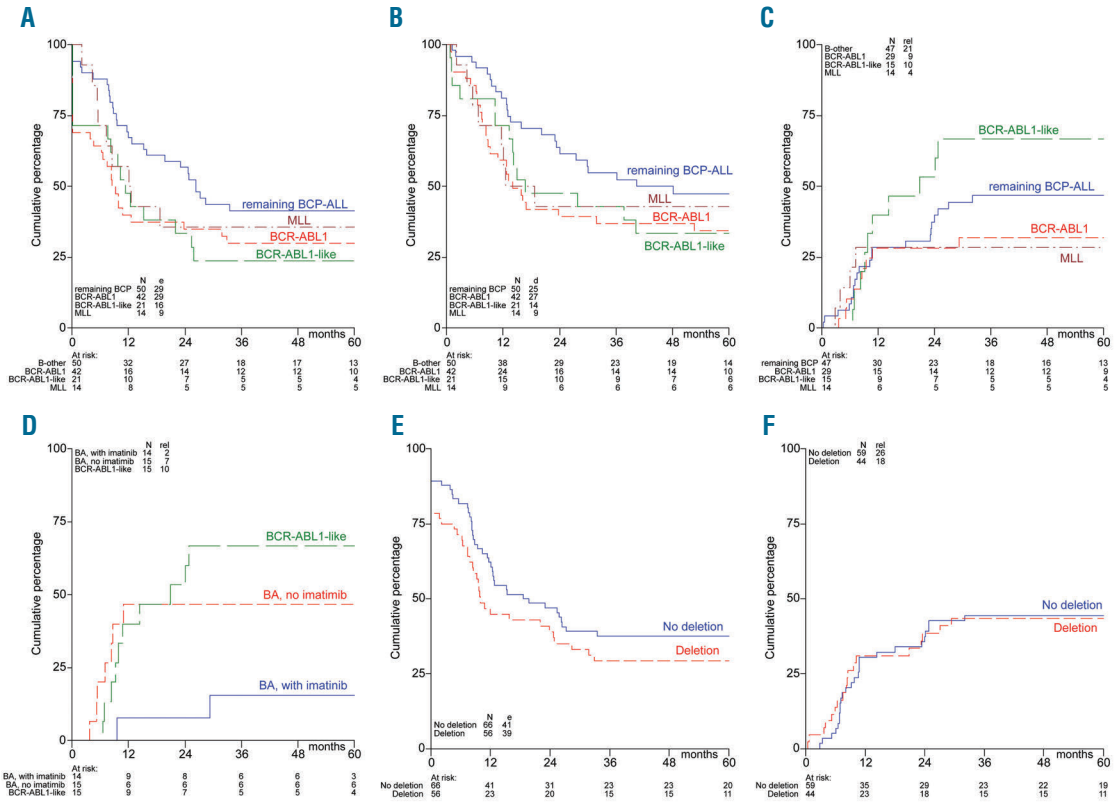

Figure 1. Association of *BCR-ABL1*-like subtype with clinical outcome. (A) Event-free survival curves comparing *BCR-ABL1*-positive, *BCR-ABL1*-like, *MLL*-rearranged and remaining BCP-ALL. The events were no complete response on protocol, relapse, and death. (B) Overall survival curves comparing the same four groups. (C) Cumulative incidence of relapse curves for the same four groups. (D) Cumulative incidence of relapse comparing patients positive for *BCR-ABL1* treated or not with imatinib, and *BCR-ABL1*-like BCP-ALL. (E) Event-free survival curves for *IKZF1*-deleted *versus* non-deleted patients. (F) Cumulative incidence of relapse curves for *IKZF1*-deleted *versus* non-deleted patients. For cumulative incidence of relapse analyses, relapse was taken as an event, and death as a competing event.

factors for event-free survival in pediatric BCP-ALL.[9] Here, we investigated the presence of a *BCR-ABL1*-like group in adult BCP-ALL and its association with *IKZF1* deletions and prognosis. Our findings suggest that *BCR-ABL1*-like BCP-ALL is a new subgroup of adult BCP-ALL characterized by more frequent refractory disease and a high relapse rate.

This study comprised 127 consecutive patients (age ≥16 years) with newly diagnosed BCP-ALL with available molecular data. Patients were included in one of four consecutive clinical trials of the Dutch-Belgium HOVON study group (n=102) or were treated accordingly (n=25) in intensive (HOVON-18 or HOVON-37, n=91, 1993-2005) or pediatric-inspired high-intensive (HOVON-70 or HOVON-71, n=36, 2005-2009) protocols.[1,2,10] Written informed consent was obtained from all participants. The HOVON studies were approved by the ethical committees of the participating centers and were conducted in accordance with the Declaration of Helsinki. Standard cytogenetics and EGIL immunophenotypes were determined (Table 1). Since 2003, imatinib was added to the standard regimen in patients with *BCR-ABL1*-positive BCP-ALL. Allogeneic stem cell transplantation (SCT) was performed in eligible patients with an HLA-identical sibling. In patients with high-risk disease (white blood cell count ≥30 x 10^9/L, and/or *BCR-ABL1* positivity, *MLL* abnormality, and/or no complete response after remission induction course 1) an alternative donor could be used. Patients included in the molecular study had a higher white blood cell count and a related higher proportion of high-risk disease compared with the total cohort. Importantly, all other clinical characteristics and outcomes (complete response rate, event-free survival, disease-free survival and overall survival) were similar between the two groups (*Online Supplementary Table S1*). Gene expression profiles were generated using Affymetrix HG-U133 plus 2.0. Adult *BCR-ABL1*-like cases were identified using the 110 probe sets and clustering procedure with the previous study cohort of 107 pediatric Dutch Childhood Oncology Group cases as reference.[9] *IKZF1* deletions were detected using the SALSA P202 version B1 IKZF1 multiplex ligation-dependent probe amplification assay.[9] Tyrosine-kinase activating fusion genes were screened by reverse transcriptase polymerase chain reaction analysis (*Online Supplementary Table S2*).[11]

We considered four groups: *BCR-ABL1*-positive, *BCR-ABL1*-like, *MLL*-rearranged and remaining BCP-ALL patients. The cumulative incidence of relapse was estimated using a competing risks model. Among patients who reached complete remission, relapse was considered as an event, with death as a competing event. To test for equality of cumulative incidence of relapse, the Gray test was applied. Event-free survival was calculated from the start of treatment until no complete response on protocol (consid-

ered as an event on day 1), relapse or death, whichever came first. Overall survival was measured from the start of treatment to death. Patients still alive were censored at the date of last contact. Event-free survival and overall survival were estimated using the actuarial Kaplan-Meier method. Survival data between the groups were compared using univariate Cox regression. The proportions of patients without a complete response on first-line treatment were compared using the Fisher exact test. All *P*-values are two-sided, and a significance level $\alpha = 0.05$ was used.

We applied the gene expression signature used to discover *BCR-ABL1*-like cases in pediatric BCP-ALL[4,9] in a cohort of 127 adult BCP-ALL, and identified 21 *BCR-ABL1*-like cases (17%). This frequency is similar to the 15-20% reported in pediatric cohorts.[9] The median age of the adult *BCR-ABL1*-like patients was 25 years, which was lower than the median age of *BCR-ABL1*-positive patients (n=42, median 38 years), *MLL*-rearranged cases (n=14, median 39 years), and the remaining BCP-ALL patients (n=50, median 34 years). The *BCR-ABL1*-like patients had similar white blood cell counts and were treated similarly to the remaining *BCR-ABL1*-negative, *MLL* wild-type BCP-ALL cases (Table 1). According to the current risk group classification (see Table 1), 43% of the *BCR-ABL1*-like patients were classified as high risk.

The non-response rate, defined as the proportion of patients who had not achieved complete response after first-line induction or consolidation, was higher in the *BCR-ABL1*-like (6/21, 29%) and *BCR-ABL1*-positive (12/42, 29%) groups than in the *MLL*-rearranged cases (0/14, 0%) and remaining BCP-ALL cases (3/50, 6%) (*P*=0.003; Table 1) and the difference remained statistically significant after correction for white blood cell count (*P*=0.01). The remaining BCP-ALL cases included eight hyperdiploid and three *TCF3-PBX1* cases; exclusion of this possibly better prognostic group still resulted in a significant difference in non-response rate (*P*=0.007). The difference in complete response rate did not result in statistically significant differences in event-free or overall survival, although both event-free and overall survival rates were low in the *BCR-ABL1*-like patients (Figure 1 A,B).

The cumulative incidence of relapse curves were not statistically different between the four subtypes of BCP-ALL, although the relapse rate was highest in the *BCR-ABL1*-like group (*P*=0.23; Figure 1C). Among standard-risk cases, a similar trend in higher relapse rate for the *BCR-ABL1*-like group (n=12; 5-year cumulative incidence of relapse 67%) *versus* the remaining BCP-ALL group (n=35; 5-year cumulative incidence of relapse 44%, *P*-value=0.23) was observed. Overall, the outcome for pediatric-inspired, high-intensive treatment seemed better than for intensive treatment (*Online Supplementary Figure S1*), which is in agreement with previous studies.[2,3] Since only three *BCR-ABL1*-like cases were treated in pediatric-inspired protocols, we cannot evaluate a possible improvement of outcome with more intensive treatment. The 5-year cumulative incidences of relapse in the *BCR-ABL1*-like group (67%) and the *BCR-ABL1*-positive group without imatinib treatment (47%) were higher than that in the imatinib-treated *BCR-ABL1*-positive group (21%) (overall *P*=0.09; Figure 1D). Together, these data suggest that the *BCR-ABL1*-like subtype is associated with an aggressive form of adult BCP-ALL characterized by a high rate of non-response to first-line treatment and a high relapse rate.

We identified 56 (46%) patients with *IKZF1* deletions; 37 deletions included the start codon in exon 2 or encompassed all exons, resulting in haploinsuffiency, and 14 deletions involved exons 4-7, resulting in the dominant-negative isoform (Table 1). The *IKZF1* deletion frequency in adult BCP-ALL with *BCR-ABL1* was 74%, in line with the frequencies in previous studies,[6,7] and significantly higher than frequencies in *BCR-ABL1*-like (35%) and remaining BCP-ALL cases (37%; *P*<0.001). The frequency of *IKZF1* deletions in *BCR-ABL1*-negative, *MLL* wild-type cases was higher in adults (36%) than in children (20%).[9] Non-response rates were not significantly different between *IKZF1*-deleted patients (11/56, 20%) and non-deleted patients (7/66, 11%; *P*=0.2). Deletion of *IKZF1* was not associated with event-free survival (*P*=0.16) or cumulative incidence of relapse (*P*=0.84; Figure 1E,F). *IKZF1* has been associated with an unfavorable prognosis in childhood BCP-ALL,[5,12,13] and in adult BCP-ALL with *BCR-ABL1*.[14] It is possible that mutations of *IKZF1* were present in our non-deleted group. However, such mutations have been found in pediatric cohorts at a very low frequency.[15]

The reported HOVON trial analysis showed that patients with an HLA-identical sibling donor had a lower cumulative incidence of relapse at 5 years compared with patients without a donor (24 *versus* 55%, *P*<0.001).[1] Strikingly, only one of five *BCR-ABL1*-like patients who received allogeneic SCT in complete response relapsed, compared with nine of ten *BCR-ABL1*-like patients who did not undergo allogeneic SCT in complete response. We compared disease-free survival for chemotherapy only, censoring patients who underwent allogeneic SCT at the date of the transplant. In the *BCR-ABL1*-like group, nine of the 15 patient treated only with chemotherapy relapsed compared with 15/47 of the remaining BCP-ALL patients treated only with chemotherapy. The *BCR-ABL1*-like group had a worse 5-year disease-free survival rate (10% *versus* 37% in the remaining patients), although this difference was not statistically significant (*P*=0.14), probably due to the small number of patients. A possible approach to improve outcome in *BCR-ABL1*-like patients could, therefore, be allogeneic SCT with an alternative donor if there is no suitable sibling, as already occurs in patients with high-risk disease.

In addition, immediate targeted treatment of *BCR-ABL1*-like patients seems an attractive option to reach higher response and lower relapse rates, e.g. with immunotherapeutics or tyrosine kinase inhibitors. In children, 30-40% of *BCR-ABL1*-like BCP-ALL show activated kinase pathways, in part caused by kinase-activating fusions such as *EBF1-PDGFRB* and *PAX5-JAK2*.[11,15] Patients' leukemic cells carrying kinase fusions are sensitive to tyrosine kinase inhibitors in cell culture and in xenograft models.[11,15] Promising results have been obtained by recent studies in pediatric and adult patients with refractory BCP-ALL positive for tyrosine kinase fusions who reached complete remission upon treatment with imatinib or dasatinib.[11,16,17] Another possible way to improve outcome for adult *BCR-ABL1*-like patients is, therefore, to screen for activating kinase mutations or translocations that could be targeted by specific inhibitors. We tested the 21 *BCR-ABL1*-like cases for fusions involving *JAK2, ABL1, ABL2, PDGFRB, CSF1R, IL2RB, NTRK3, TSLP* and *TYK2* reported by Roberts *et al.*[11] We did not detect these fusions, suggesting the presence of alternative kinase activating lesions. Further research is needed to study outcome in larger cohorts of patients and to identify possible genomic lesions underlying the *BCR-ABL1*-like BCP-ALL subtype in adults which may provide a rationale for targeted or intensified treatment.

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Acknowledgments: the authors would like to thank A. van der Veer and M.E. Willemse for interpreting the MLPA assay.

Funding: this work was supported by the Center for Translational Molecular Medicine BioChip program, the Foundation Pediatric Oncology Center Rotterdam (KOCR), and the European Union's Seventh Framework Programme (FP7/2007-2013) under the project European Network for Cancer research in Children and Adolescents (ENCCA, grant agreement HEALTH-F2-2011-261474).

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doi:10.3324/haematol.2014.117424

Key words: B-cell precursor acute lymphoblastic leukemia, BCR-ABL*-like acute lymphoblastic leukemia, expression profiling, outcome.*

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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