Clinical Features and Outcome of Children With First Marrow Relapse of Acute Lymphoblastic Leukemia Expressing BCR-ABL Fusion Transcripts

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Although the Philadelphia chromosome (Ph1) has been identified as an adverse prognostic factor in acute lymphoblastic leukemia (ALL), little is known about the incidence and clinical course of relapsed Ph1-positive ALL in children. The incidence was determined by screening of 170 consecutive children with first bone marrow relapse of ALL using the reverse transcriptase-polymerase chain reaction (RT-PCR) and comparison with cytogenetic analysis. Among these 170 children, 20 (12%) were found to be BCR-ABL-positive, representing a rate that is about three times higher than that reported for newly diagnosed ALL. Ten of the cases were identified by RT-PCR only. In none of the 21 patients with T-cell immunophenotypes could an expression of the BCR-ABL transcript be detected. BCR-ABL positivity was associated with a significantly shorter duration of first remission (P = .0036) and higher white blood cell (P = .0157) and blast cell counts (P = .0304) at relapse diagnosis. All patients were treated according to the ALL-REZ BFM 87 and 90 relapse trials of the BFM Relapse Study Group. The intensive multiagent chemotherapy induced a second complete remission in only 60% of children with BCR-ABL-positive ALL compared with in 91% of those without BCR-ABL expression (P = .0023). The prognosis of BCR-ABL-positive ALL in children is poor, with a probability of event-free survival at 2 years of 8% versus 50% in those without BCR-ABL expression (P = .0003). Molecular screening for the BCR-ABL mRNA or cytogenetic analysis should become part of the routine diagnostic panel for all children with newly diagnosed ALL and is fundamental for children presenting with an early bone marrow relapse.

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Molecular and cytogenetic studies have documented the prognostic influence of specific chromosomal abnormalities in leukemia. One of the most striking and well-characterized abnormalities is the translocation t(9;22)(q34;q11), known as the Philadelphia chromosome (Ph1). As a consequence of this translocation, the coding sequence of the ABL gene is almost completely fused to one of three remaining BCR exons I through III on chromosome 22, resulting in the expression of leukemia-specific, chimeric BCR-ABL messenger RNAs and proteins. Ph1 is characterized in more than 90% of patients with chronic myelogenous leukemia (CML). However, in initially diagnosed acute lymphoblastic leukemia (ALL), the translocation occurs in 3% to 5% of children and 25% to 50% of adults. Typically, the fusion variant in ALL is different from that in CML and involves the minor breakpoint cluster region (mBCR), ie, BCR exon I is fused to ABL exon II. The corresponding protein (p190bcrabl) is considered to be involved in malignant transformation of hematopoietic cells. In adults as well as in children, Ph1 has been identified as an adverse prognostic factor.

In recent years, risk-adapted treatment protocols using classical risk factors such as white blood cell (WBC) count, age, and immunologic phenotype have improved the outcome of most patients with ALL, except for patients with Ph1. Despite intensified induction and consolidation therapy, patients with Ph1-positive ALL are at high risk for relapse. There are no reports on prospective studies, and few published data exist that demonstrate the course of Ph1-positive ALL in patients after relapse.

Therefore, a prospective study was initiated to determine the incidence of Ph1-positive ALL in a population of relapse patients and to compare clinical features and outcome between children with and without BCR-ABL fusion transcript expression. About 90% of all children with relapse of ALL in Germany and Switzerland are enrolled in the ALL-REZ BFM Relapse Study Group trials and have been treated accordingly. The ALL-REZ BFM trials were approved by the Institutional Review Board of the Freie Universität Berlin (Berlin, Germany), and written, informed consent was obtained from all patients or their parents.

MATERIALS AND METHODS

Patients and Treatment

Between 1987 and April 1995, bone marrow samples from 170 children and adolescents aged up to 18 years with first bone marrow relapse of ALL were available for molecular analysis of the BCR-ABL fusion transcript for a prospective study on minimal residual disease associated with German ALL-REZ BFM trials. The following report refers to 148 patients with first bone marrow relapse of non-T-ALL who were treated according to protocols ALL-REZ BFM 87 and 90. In study ALL-REZ BFM 87, treatment was based on alternating short-term multidrug chemotherapy courses: four R1 and R2 courses. In study ALL-REZ BFM 90, course R3 (main constituents: etoposide and high-dose cytarabine) was introduced. Three of each R1, R2, and R3 courses were administered up to a total of nine courses. In study ALL-REZ BFM 87, as well as in the previous studies ALL-REZ BFM 83 and 85, children with early or very early marrow relapse received an initial induction protocol.

The study design differed in the methotrexate (MTX) dose used within the R courses. However, no differences in outcome depending on MTX dosages could be shown.
Patients with ALL in second complete remission (CR) having a matched sibling donor were scheduled for allogeneic bone marrow transplantation (BMT) after completion of at least two courses of chemotherapy. If BMT was not available, chemotherapy was continued according to the protocol until BMT could be performed. Autologous BMT (ABMT) was optional, as was the decision for matched unrelated donor (MUD) BMT.

Molecular Detection of the BCR-ABL Fusion Transcript

RNA isolation. Cells from bone marrow aspirates, peripheral blood, or cell lines were lysed for 30 minutes in 5 vol of erythrocyte lysis buffer (155 mmol/L NH₄Cl, 10 mmol/L KHCO₃, 1 mmol/L EDTA) and pelleted by centrifugation. Total cellular RNA was isolated from 5 × 10⁶ to 1 × 10⁷ cells using acid guanidium thiocyanate-phenol-chloroform extraction.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. The cDNA reactions were performed for 45 minutes at 37°C in 20 µL containing 50 mmol/L Tris-HCl (pH 8.3 at room temperature), 75 mmol/L KCl; 3 mmol/L MgCl₂; 20 mmol/L dithiothreitol (DTT), 20 U RNA-Guard (Pharmacia, Freiburg, Germany); 1 mmol/L deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP); 20 U RNA-Guard (Pharmacia, Freiburg, Germany); and 1 vol of mineral oil. PCR was performed with a DNA thermal cycler (Autogene; CLF, Analytische Laborgerate GmbH, Emersacker, Germany). The statistical analysis was performed using SAS Software (SAS Inc., Cary, NC) version 6.10 for Windows. The analysis entailed univariate statistics, Wilcoxon rank sum tests (WRST), analysis of variance (ANOVA), and Spearman rank correlation. Crossclassification was tested using two-tailed Fisher's exact test (FET). Survival analysis included product limit estimates (Kaplan-Meier), and the log-rank test (LRT), as well as the multivariate Cox regression model with proportional hazards and Wald $\chi^2$ tests (WCT). The level of significance was set to .05.

Survival time was calculated from the date of relapse to the last follow-up event. In patients undergoing BMT, survival time and EFS were censored with the date of the transplantation. None of the patients was lost to follow up.

RESULTS

Of 148 analyzed samples, 20 were found to be BCR-ABL-positive. Only one child showed findings consistent with the p210 subtype; the remaining were of the p190 subtype. Results from cytogenetic analyses were available in 13 patients.
of the BCR-ABL-positive and in 57 of the BCR-ABL-negative patients. Chromosome analysis could be successfully performed in 10 children with BCR-ABL-positive ALL. Ph<sup>+</sup> was detected in nine, and one child had a partial deletion of the short arm of chromosome 9. In 15 of 57 children with BCR-ABL-negative ALL, the karyotype was found to be normal; nine were hyperdiploid (N > 50), and 33 showed irregular karyotypes.

The two groups of patients with BCR-ABL-positive and -negative ALL did not differ regarding age (Fig 1A; ANOVA, P = .8694), sex (FET, P = 1.000), site of relapse (FET, P = .160), French-American-British (FAB) classification (FET, P = .058), immunologic phenotype (FET, P = .064), or use of front-line therapy at first diagnosis of leukemia. Fourteen of 20 patients with BCR-ABL-positive and 91 of 128 patients with BCR-ABL-negative patients were treated according to BFM front-line protocols (Table 1).

The duration of first CR differed significantly (ANOVA, P = .0086; Fig 1B). Additionally, 11 of 20 positive patients relapsed on therapy, but only 39 of the negative group did (FET, P = .042). Both the WBC and the absolute peripheral blast counts were significantly higher in BCR-ABL-positive than in BCR-ABL-negative children at diagnosis of first relapse (WRST, P = .0157 and P = .0304, respectively). In contrast, the percentage of blasts was not different across both groups (ANOVA, P = .2789; Fig 1C and D).

All patients were treated according to the ALL-REZ BFM therapy protocols. The observation times in both groups did not differ significantly (WRST, P = .2764; Fig 1E).

Most importantly, the groups were different regarding response to therapy and outcome. Whereas in 104 of 114 patients with BCR-ABL-negative ALL, a second remission could be achieved, only 11 of 18 positive patients responded to therapy (FET, P = .00231). Furthermore, 50 children in the BCR-ABL-negative group are in second continuous CR, but 18 of the 20 positive patients had an adverse event (FET, P = .011). The detailed outcome is compiled in Table 2.

EFS and survival curves are shown in Figs 2 and 3. Note that three of the BCR-ABL-positive and 32 of the negative children are accounted for as censored observations because they underwent BMT. One positive patient is still at risk, ie, censored, whereas another one already had an adverse event and, thus, is censored for the survival analysis but not for the analysis of EFS (Tables 3 and 4). The differences regarding EFS and survival between both groups are obvious.

The univariate analysis showed a significant dependence of EFS on expression of BCR-ABL fusion transcripts (Fig
2). Furthermore, significant effects of the variables timepoint of relapse (LRT, \(P = .0001\)), duration of first remission (LRT, \(P = .0001\)), blast count (LRT, \(P = .0107\)), and WBC count (LRT, \(P = .0222\)) could be detected in the univariate survival analysis over all patients. In contrast, the variables age at initial diagnosis, age at relapse, blast count, percentage of blasts, and location of relapse were not significant (LRT, \(P > .05\)). The significant variables (LRT, \(P < .05\)) of the univariate analysis comprised the covariates for the multivariate analysis. The two variables that remained significant when adjusted for the other factors were timepoint of relapse (on/off therapy; WCT, \(P = .0009\)) and the duration of initial remission (WCT, \(P = .0076\)); all other variables were removed backwards from the model (WCT, \(P > .05\)).

The same procedure was performed for the analysis of survival time. Identically, the univariate analysis showed a significant relation of timepoint of relapse (LRT, \(P = .0001\)), duration of first remission (LRT, \(P = .0001\)), blast count (LRT, \(P = .0052\)), and WBC count (LRT, \(P = .0165\)) with survival time. Again, the two variables, timepoint of relapse (WCT, \(P = .0001\)) and duration of first remission, remained significant in the multivariate analysis of survival time (WCT, \(P = .0144\)).

**DISCUSSION**

The incidence of Ph' in initially diagnosed childhood ALL has been reported to be as high as 2.3% to 6% and to be a negative prognostic factor associated with other risk factors such as high WBC and peripheral blast cell counts, FAB L2 morphology, and age at diagnosis. A BCR-ABL transcript is rarely expressed. In general, the prognosis of relapsed T-cell leukemia is poor. These patients were excluded to prevent a bias.

There were no differences regarding age, sex, immunophenotype, site of relapse, or FAB classification between both groups. However, BCR-ABL-positive children presented with significantly higher WBC and blast counts and a significantly shorter duration of first remission. Eleven of 20 BCR-ABL-positive children relapsed on front-line therapy, whereas 89 of 128 BCR-ABL-negative patients had finished the front-line therapy.

Most importantly, the outcome of both groups could be clearly distinguished. First, in only 11 BCR-ABL-positive children, a second remission could be achieved compared with in 104 of 114 BCR-ABL-negative children; that is, 39% versus 9% did not respond to therapy at all. The 1-year EFS probability of the negative group is still about 60% in contrast with 24% of the other. Although the observation time in both groups is almost equal, as of yet, none of the positive patients reached an EFS of 5 years (Figs 1F and 2).

<table>
<thead>
<tr>
<th>BCR-ABL Fusion Transcript</th>
<th>EFS (yr)</th>
<th>Observations (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>0.618</td>
<td>0.497</td>
</tr>
<tr>
<td>Positive</td>
<td>0.241</td>
<td>0.090</td>
</tr>
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</table>

Log-rank test: \(\chi^2; 14.1981\); degrees of freedom, 1; \(P > \chi^2\) under H0, 0.0002; H0, EFS of both groups is equal.
The following were participating clinical centers of the ALL-REZ BFM study: Aachen (R. Mertens, MD), Aalen (B. Höhmann, MD), Augsburg (P. Heidemann, MD), Berlin (G. Henze, MD), Berlin-Buch (W. Dörfel, MD), Berlin-Charite (G. Gaedicke, MD), Bielefeld (V. Schöck, MD), Bonn (U. Bode, MD), Braunschweig (G. Mau, MD), Bremen (H.I. Spaar, MD), Chemnitz (I. Krause, MD), Coburg (J.D. Thaben, MD), Datteln (W. Andler, MD), Dortmund (H. Breu, MD), Düsseldorf (U. Göbel, MD), Erfurt (G. Weimann, MD), Erlangen (J.D. Beck, MD), Essen (W. Havers, MD), Frankfurt (B. Kornhuber, MD), Freiburg (M. Brandis, MD), Giessen (F. Lampert, MD), Göttingen (M. Lakomek, MD), Graz (C. Urban, MD), Hamburg (G. Janka-Schaub, MD), Kiel (C. Klinggräff, MD), Köln (F. Berthold, MD), Koblenz (M. Rister, MD), Krefeld (U. Gobel, MD), Leipzig (K.M. Debatin, MD), Herdecke (C. Tautz, MD), Homburg (N. Graf, MD), Innsbruck (K. Dengg, MD), Jena (F. Zintl, MD), Karlsruhe (J.T. Fischer, MD), Kassel (H. Wehinger, MD), Kiel (C.v. Klinggräff, MD), Köln (F. Berthold, MD), Koblenz (M. Rister, MD), Krefeld (P. Thomas, MD), Leipzig (M. Domula, MD), Löbeck (I. Mutz, MD), Ludwigshafen (H.C. Dominick, MD), Magdeburg (U. Mittler, MD), Mönchengladbach (W. Müller, MD), Münster (J. Hürjens; J. Ritter, MD), Nürnberg (A. Jobke, MD), Nürnberg (H. Gröbe, MD), Rostock (M. Eggers, MD), Saarbrücken (R. Grieb-König, MD), Schwerin (P. Hagemeister, MD), Stuttgart (J. Treuner, MD), Trier (W. Rauh, MD), Tübingen (D. Niethammer, MD), Wiesbaden (J. Weber, MD), Würzburg (J. Kuhl, MD), Germany.

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