Clinical features and prognostic implications of *TCF3-PBX1* and *ETV6-RUNX1* in adult acute lymphoblastic leukemia

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ABSTRACT

Background

The t(9;22) and t(4;11) chromosomal translocations, which generate the *BCR-ABL* and *MLL-AF4* fusion genes, define high-risk subtypes of acute lymphoblastic leukemia in adults. However, the prognostic impact of other rarer fusion genes is less well established in adult acute lymphoblastic leukemia than in the childhood form.

Design and Methods

In the context of the German Multicenter Therapy Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) we used reverse transcriptase polymerase chain reaction to investigate 441 cases of *BCR-ABL*- and *MLL-AF4*-negative B-precursor acute lymphoblastic leukemia for the *TCF3-PBX1* (*E2A-PBX1*) and *ETV6-RUNX1* (*TEL-AML1*) fusion transcripts generated by the t(1;19)(q23;p13.3) and t(12;21)(p13;q22) translocations. Both are well-known molecular alterations in pediatric acute lymphoblastic leukemia in which they have favorable prognostic implications.

Results

We identified 23 adult patients with *TCF3-PBX1* and ten with *ETV6-RUNX1*. In contrast to previous reports we found no significant difference in overall survival between *TCF3-PBX1*-positive and -negative patients. At 2 years after diagnosis all the *ETV6-RUNX1*-positive patients were alive and in continuous complete remission, but their long-term outcome was negatively affected by late relapses. *TCF3-PBX1*-positive patients exhibited a characteristic $CD34^-/CD33^-$ and mostly $cyIg^+$ immunophenotype. *ETV6-RUNX1* only occurred in patients under 35 years old and was associated with a significantly lower white blood count.

Conclusions

In contrast to previous suggestions, adult patients with *TCF3-PBX1*-positive acute lymphoblastic leukemia do not appear to have a worse outcome than their negative counterparts. *ETV6-RUNX1*-positive patients had a very favorable performance status during the first few years but their long-term survival was negatively affected by late relapses. Both groups of patients are characterized by distinct clinicobiological features which facilitate their diagnostic identification.

GMALL therapy studies 05/1993, 06/1999 and 07/2003 have been registered at www.clinicaltrials.gov: NCT00199069, NCT00199056, NCT00198991.

Key words: homeobox gene, core binding factor, helix-loop-helix transcription factor, pre-B ALL, common ALL.

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Introduction

The t(1;19)(q23;p13.3) translocation in acute lymphoblastic leukemia (ALL) was first cytogenetically detected in 1984.[1] It involves two genes: *TCF3* (synonym: *E2A*) on chromosome 19 and *PBX1* on chromosome 1.[2] *TCF3* encodes two proteins, E12 and E47, which are basic helix-loop-helix transcription factors that play critical roles in B-cell maturation. *PBX1* is a homeobox gene that appears to be expressed in all tissues but not in B or T lymphocytes. The *TCF3-PBX1* gene fusion causes unphysiological activation and truncation of *PBX1*, leads to trans-activation of several genes and, finally, generates a malignant cell phenotype.[3]

The t(12;21)(p13;q22) translocation was first described in 1994[4] and is not detectable by conventional cytogenetic methods. It leads to the fusion of two genes, *RUNX1* (synonyms: *AML1*, *CBFa2*) on chromosome 21 and *ETV6* (synonym: *TEL*) on chromosome 12.[5,6] *RUNX1* belongs to the core binding factor family of transcription factors.[7,8] *ETV6* is involved in chromosomal translocations in a wide variety of hematologic malignancies.[9] It appears to be an important transcription factor required for hematopoiesis in the bone marrow.

Data on the prognostic impact of the *TCF3-PBX1* transcript are discordant, and few data are available on the *ETV6-RUNX1* transcript in adult ALL. On the other hand pediatric ALL patients with either transcript have an above-average clinical outcome.[10] We performed retrospective and prospective investigations within the framework of the German Adult ALL (GMALL) Therapy Study Group to assess a large number of adult ALL patients for these two transcripts.

Design and Methods

Patients' samples

All samples investigated were obtained for diagnostic purposes at the time of primary diagnosis within the framework of the GMALL therapy studies 05/1993, 06/1999 and 07/2003 between May 1993 and January 2005. Immunophenotyping and reverse transcriptase polymerase chain reaction (RT-PCR) analysis for *BCR-ABL* and *MLL-AF4* were done at the time of diagnosis. Other RT-PCR analyses were performed retrospectively for the period prior to 1993 and prospectively for the period from 2004-2005. Residual material was archived in liquid nitrogen. Patients had given their written consent to scientific investigations at the time of inclusion in the GMALL studies.

Immunophenotyping

Immunophenotyping of fresh samples was essentially performed as described elsewhere, using standard procedures.[11] Samples exhibiting B-cell lineage commitment, but were not mature B-ALL, were included for further analysis.

Reverse transcriptase polymerase chain reaction analysis

Total RNA isolation and reverse transcription were performed as recently described.[12] Two different RT-PCR analyses were used to assess all samples for *BCR-ABL* mRNA as previously described.[12] Samples with a pro-B immunophenotype were additionally analyzed for *MLL-AF4* by RT-PCR[13] and for other *MLL* fusions by multiplex RT-PCR.[14] Residual material was archived in liquid nitrogen. Only samples that tested negative in these RT-PCR were included for further analysis.

PCR for *TCF3-PBX1* and *ETV6-RUNX1* was performed basically using the standardized primers and single-round PCR procedure proposed by the BIOMED-I Initiative.[15] Using the HotStarTaq kit (QIAGEN, Hilden/Germany), the PCR included an initial 15-min denaturation step. *TCF3-PBX1*- or *ETV6-RUNX1*-positive samples were additionally analyzed by real-time quantitative PCR on an RG-3000 cycler (Corbett Research) using Europe Against Cancer conditions[16] to confirm the positive result and exclude cross-contamination. Reverse transcribed RNA isolated from 10^{-3} dilutions of KASUMI-2 and REH cell lines served as positive controls for the PCR.

Cell lines

KASUMI-2 (established in 1990 by M Asou, Hiroshima University, Japan, *unpublished*) and REH[17] cell lines were obtained from the DSMZ Braunschweig/Germany (ACC 240 and ACC 22). Cell lines were cultured using standard procedures, and serial dilutions were prepared with buffy coat leukocytes. RNA isolated from these dilutions was reverse transcribed as described above, and the 10^{-3} dilution was used as a positive control in the RT-PCR.

Statistical analysis

Statistical analysis was done using SPSS version 16.

Results

A total of 441 patients with a B-cell precursor immunophenotype were identified for the analysis. *BCR-ABL*-positive cases (36% of B-cell precursor ALL) and *MLL-AF4*-positive cases (52% of pro-B ALL) were excluded. The 441 samples comprised 88 with a pre-B immunophenotype, 314 with common ALL and 39 with pro-B ALL. All were assessed for *TCF3-PBX1* and *ETV6-RUNX1* by RT-PCR. The pro-B patients were excluded from further analysis, given that all 39 pro-B ALL patients were *TCF3-PBX1*- and *ETV6-RUNX1*-negative, and that pro-B ALL is considered a high-risk entity according to the GMALL protocols. The cohort for final analysis comprised 402 patients with pre-B or common ALL: 235 (58.5%) males and 167 (41.5%) females with a median age of 30 years (range, 15-64 years). These patients were treated according to the protocols of the GMALL 05/93 (123 patients), 06/99 or 07/2003 (280 patients) therapy trials. These protocols included an intense two-phase induction therapy (prednisolone, vincristine, daunorubicin, L-asparaginase, cyclophosphamide, cytarabine, 6-mercaptopurine), followed by consolidation (high-dose methotrexate, asparaginase, 6-mercaptopurine), re-induction, and six alternating cycles of consolidation therapy (high-dose cytarabine, high-dose methotrexate/asparaginase, cyclophosphamide, teniposide). All patients received repeated prophylaxis with intrathecal methotrexate and cranial irradiation. Allogeneic transplantation was recommended for high-risk patients in first remission. Criteria defining high-risk B-cell precursor ALL were a white blood count (WBC) greater than 30×10^9/L, detection of *BCR-ABL*/Philadelphia chromosome, a pro-B immunophenotype, detection of the *MLL-AF4* fusion/ t(4;11)(q21;q23)

and persistence of blasts after induction therapy. The main differences between the 6/99 or 7/03 protocol and the 5/93 protocol were a condensed induction phase, the use of dexamethasone instead of prednisolone and pegylated asparaginase instead of *E. coli* asparaginase. The details of these trials have been reported elsewhere.[18,19]

RT-PCR detected a *TCF3-PBX1* fusion in 23 patients and an *ETV6-RUNX1* fusion in ten patients. In terms of clinical characteristics, 12 (52%) of the *TCF3-PBX1*-positive patients were male and 11 (48%) female with a median age of 27 years (range, 15-60 years) (P=0.1423, Mann-Whitney U test). Six (60%) *ETV6-RUNX1*-positive patients were male and four (40%) female with a median age of 24 years (range, 17-34 years) (P=0.0322, Mann-Whitney U test). Nine out of ten *ETV6-RUNX1*-positive patients were under the age of 30. The median WBC count at diagnosis was 35.5×10^9/L (range, 4-238.5×10^9/L) in *TCF3-PBX1*-positive patients (n=22; P=0.10, Mann-Whitney U test) and 3.75×10^9/L (range, 1-12.9×10^9/L) in *ETV6-RUNX1*-positive patients (n=10; P=0.0015, Mann-Whitney U test), while *TCF3-PBX1*- and *ETV6-RUNX1*-negative patients with pre-B and common ALL (n=363) had a median WBC count of 10×10^9/L (range, 0.5-720×10^9/L). Thirteen (59.1%, n=22) of the *TCF3-PBX1*-positive and none of the *ETV6-RUNX1*-positive patients had a WBC count greater than 30×10^9/L, one of the high-risk criteria in the GMALL protocols, while the WBC count was above this level in 92 (25.3%, n=363) of the *TCF3-PBX1*- and *ETV6-RUNX1*-negative patients with pre-B and common ALL. Central nervous system involvement was found in only one of the ten *ETV6-RUNX1*-positive patients and none of 19 *TCF3-PBX1*-positive patients, as compared to 18/325 (5.5%) patients in the control group.

With regards to response to therapy, 21 *TCF3-PBX1*-positive patients (91.3%, n=23) achieved complete remission after induction I, one patient achieved complete remission after induction II, and the other one did not achieve remission. Two *TCF3-PBX1*-positive patients died during induction therapy. All ten *ETV6-RUNX1*-positive patients achieved complete remission after induction I.

Of the 23 *TCF3-PBX1*-positive patients, 20 had a pre-B immunophenotype (22.7% of all pre-B cases; P<0.0001, Fisher's test) and three had a common ALL immunophenotype (1.0% of all cases of common ALL), while all ten *ETV6-RUNX1*-positive patients had a common ALL immunophenotype (3.2% of common ALL cases; P=0.13, Fisher's test). All 23 *TCF3-PBX1*-positive patients were negative for CD34, CD13 and CD33. The corresponding values in *BCR-ABL*-negative patients, as determined in a historical cohort of 257 patients with common ALL/pre-B ALL in a GMALL study, were 66%, 14% and 18%, respectively.[18] The differences were statistically significant for CD34 (P<0.0001) and CD33 (P=0.032) but not for CD13 (P=0.054; Fisher's test). Immunophenotypic features did not differ markedly between *ETV6-RUNX1*-positive and *ETV6-RUNX1*-negative patients.

With regard to clinical outcome, *TCF3-PBX1*-positive patients had a slightly better overall survival (51% *versus* 40%) and remission duration (48% *versus* 40%) than the *TCF3-PBX1*-negative group (Figures 1 and 2). The overall survival (57% *versus* 40%) and remission duration (50 *versus* 40%) were also better in *ETV6-RUNX1*-positive patients than in *ETV6-RUNX1*-negative patients (Figures 3 and 4). However, neither of these differences was statistically significant. All ten *ETV6-RUNX1*-positive patients were in complete remission 2 years after diagnosis, but four of them subsequently relapsed, and three of these died.

One *ETV6-RUNX1*-positive patient was diagnosed with molecular relapse on day 623, received an allogeneic transplant in second complete remission and died on day 1605. Two *ETV6-RUNX1*-positive patients relapsed on day 1447 and day 1593 and died on day 1663 and day 1815 (*i.e.* roughly 4-5 years after diagnosis). One patient with a relapse diagnosed on day 1077 achieved a second complete remission under conventional therapy and was still alive on day 2348.

Regarding the *TCF3-PBX1*-positive patients, 13 of the 23 died during the course of their disease. There were four early deaths during therapy between day 69 and day 152. Six patients received a transplant in first complete remission and three of them died between day 273 and day 417. Another six *TCF3-PBX1*-positive patients had a relapse between day 109 and day 873 and four of them died between day 266 and day 1092.

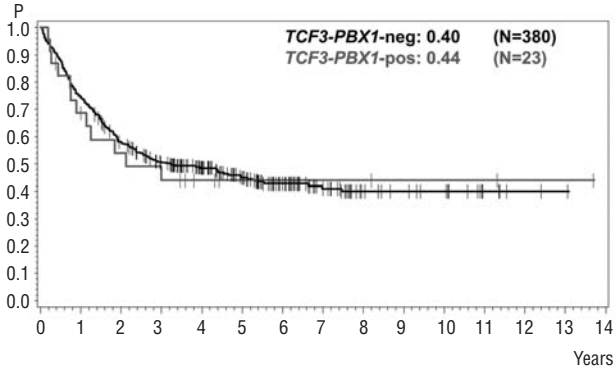

Figure 1. Overall survival of patients with *BCR-ABL*-negative common/pre-B ALL according to *TCF3-PBX1* status: GMALL studies 05/93, 06/99, and 07/03.

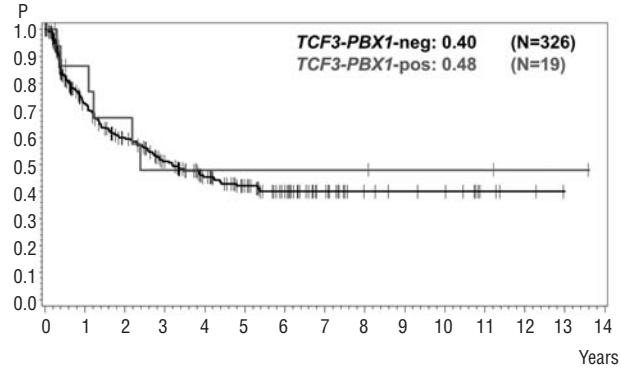

Figure 2. Remission duration in patients with *BCR-ABL*-negative common/pre-B ALL according to *TCF3-PBX1* status: GMALL studies 05/93, 06/99, and 07/03.

Discussion

Conflicting data have been published regarding the prognostic impact of the t(1;19)/*TCF3-PBX1* translocation and/or fusion mRNA transcript in adult ALL. Most authors reported that adult patients with t(1;19) had a poor prognosis[20-24] and were suitable candidates for allogeneic transplantation.[25] This is in striking contrast to the situation in children, in whom the t(1;19) initially also predicted a poor outcome[26] but with newer therapy regimens is now associated with a good or even excellent prognosis.[10,27,28] The reason for this improvement in prognosis is not completely clear and it is mostly ascribed to the early intensive remission induction in newer therapy protocols.[28,29] Recently published data from St Jude Children's Research Hospital suggest that, despite their favorable overall outcome, pediatric patients with *TCF3-PBX1* may have an increased risk of central nervous system relapse.[30,31] Most studies in adults were based on cytogenetics which may underestimate the frequency or misdiagnose this aberration at the molecular level.[32,33] Some investigators found an adverse prognosis in patients with a cytogenetically balanced t(1;19) translocation,[34] but others could not confirm these findings.[35,36]

The t(12;21) with *ETV6-RUNX1* fusion cannot be detected by conventional cytogenetics and thus RT-PCR or fluorescence *in situ* hybridization must be used. Few data exist on *ETV6-RUNX1* in adult ALL. All published studies investigated only small populations of adult *BCR-ABL*-negative patients and reported incidences of 0 to 3%. The clinical implications of this fusion gene in adult ALL were not clear and no detailed data were given.[37-39] In pediatric ALL, however, the *ETV6-RUNX1* fusion gene is a well-established favorable prognostic factor.[10,40-42]

Survival and remission analysis in our cohort of adult *ETV6-RUNX1*-positive patients showed four late relapses between 2 and 5 years after diagnosis and three late deaths due to relapse. One relapsed patient achieved a long-term second remission under conventional therapy, as has also been frequently observed in relapsed pediatric *ETV6-RUNX1*-positive patients.[43] Thus the long-term prognosis of *ETV6-RUNX1*-positive adult patients might not be as good as expected from the findings during the first 2 years after diagnosis when all ten patients were in complete remission. The frequency of *ETV6-RUNX1* in pediatric patients with B-cell precursor ALL has been reported to decrease with age from 24% (age, 1-4 years) to 18.6% (age, 6-9 years) and 4.5% (age, 10-14 years).[44] The corresponding frequencies for our series of adult patients with B-cell precursor ALL (including *BCR-ABL-/MLL-AF4*-positive and pro-B patients) were 2.9% (age, 15-34 years) and 0% (age, ≥35 years). If only *BCR-ABL-/MLL-AF4*-negative common ALL/pre-B ALL patients were considered, the frequency of *ETV6-RUNX1* was 4.3% in the group aged 15 to 34 years.

As mentioned earlier, some previous studies suggested that adult patients with *TCF3-PBX1* might have an adverse prognosis. Foà *et al.* reported on ten *TCF3-PBX1*-positive adult patients who were treated in GIMEMA multicenter studies.[24] The ten patients had no particular high-risk factors: nine were under 30 years old and nine achieved a complete remission after induction therapy. One died during induction. Only two had a WBC count over 30×10^9/L, and none had central nervous system disease. However, five of the remaining nine patients suffered a hematologic relapse (one with concomitant central nervous system relapse) after a median time of 7 months. The *Groupe Français Cytogénétique Hématologique* reported that nine of 11 t(1;19)-positive adult patients with a median WBC count of 11×10^9/L and a median age of 22 years had a treatment failure within the first 12 months.[20] In our cohort of *TCF3-PBX1*-positive patients, 13 of 22 patients had a WBC count greater than 30×10^9/L and were, therefore, by definition, at high risk. Nevertheless the response to therapy was good (>90% in complete remission after induction I), and the overall survival and remission duration in our *TCF3-PBX1*-positive patient cohort was slightly, albeit not significantly, better than in the *TCF3-PBX1*-negative group of patients.

In conclusion, our data do not support the assumption that *TCF3-PBX1* is a specific high-risk entity in adult ALL. Differences in the prognostic implications may be due to the different therapy regimens applied. One could speculate that, in analogy to the situation in pediatric ALL, early more intensive remission induction may also lead to a better outcome in adult *TCF3-PBX1*-positive patients. *ETV6-*

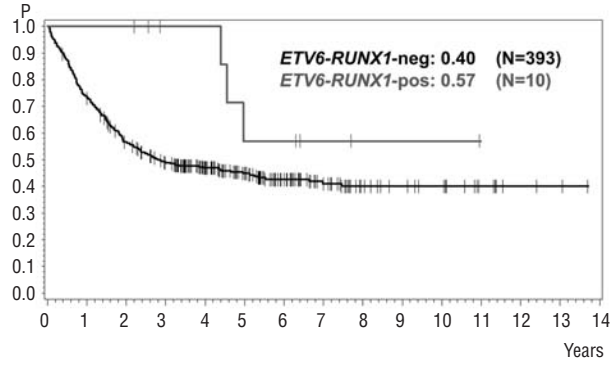

Figure 3. Overall survival of patients with *BCR-ABL*-negative common/pre-B ALL according to *ETV6-RUNX1* status: GMALL studies 05/93, 06/99, and 07/03.

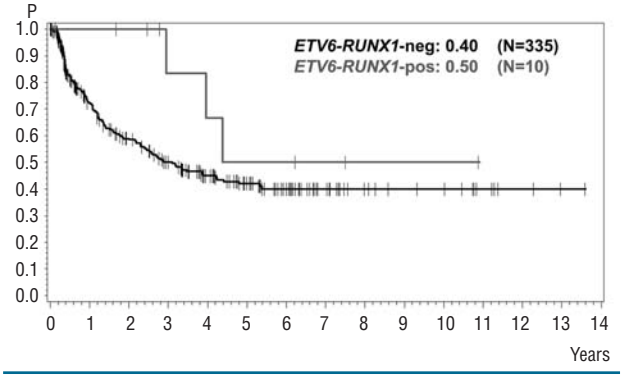

Figure 4. Remission duration in patients with *BCR-ABL*-negative common/pre-B ALL according to *ETV6-RUNX1* status: GMALL studies 05/93, 06/99, and 07/03.

RUNX1-positive adult patients had a better overall survival than the control group but the difference was not statistically significant. Remarkably, all *ETV6-RUNX1*-positive adult patients were in complete remission 2 years after diagnosis, but their long-time overall survival was negatively influenced by late relapses. However, these conclusions are still preliminary, and larger long-term studies are needed to obtain more information on the prognostic impact of these two aberrations. An association with certain immunophenotypes has been described for many specific genetic alterations found in B-cell precursor ALL.[45] *TCF3-PBX1* is known to be associated with a pre-B immunophenotype in adult ALL, while the data for *ETV6-RUNX1* in adult ALL are scant. We found that *TCF3-PBX1* was significantly associated with a $cyIg^{+}/CD34^{-}/CD33^{-}$ immunophenotype. All *ETV6-RUNX1*-positive patients had a common B-cell precursor immunophenotype, but this was not statistically significant.

Although minimal residual disease assessment based on clonally rearranged *TCR/IG* genes is the current gold standard in ALL,[46] *TCF3-PBX1* and *ETV6-RUNX1* might be other useful complementary targets for such assessment. Consensus standards have been issued for detecting them by real-time PCR.[16] For this reason and for the purpose of learning more about the biology of these aberrations, we propose that they should be included in the molecular diagnostic panel in adult ALL. To reduce the number of investigations we suggest considering only specific subsets of patients. Only *BCR-ABL*-negative patients should be investigated. All pre-B patients and all patients with common ALL and a CD34/CD33-negative immunophenotype should be assessed for *TCF3-PBX1*. Only pre-B/common ALL patients under 35 years old should be assessed for *ETV-RUNX1*. This should enable identification of nearly all affected patients with a justifiable effort.

Authorship and Disclosures

TB was the principal investigator, designed the research, analyzed data and wrote the paper. NG analyzed data and is head of the GMALL study center. DHo is head of the GMALL study group. ET is a member of the GMALL study board and supervised the immunophenotyping. SS and LF performed the immunophenotyping. AS and DHu performed RT-PCR analyses.

The authors have no potential conflicts of interest.

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