Atypical *BCR-ABL* mRNA transcripts in adult Acute lymphoblastic leukemia

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ABSTRACT

RT-PCR detects chimeric *BCR-ABL* mRNA in approximately 25% of adult acute lymphoblastic leukemia (ALL) cases. Minor breakpoint transcripts (e1a2) are found in about 70% of positive cases and major breakpoint transcripts (e13a2, e14a2) in about 30% of cases. However, other *atypical* transcripts are sometimes observed. We report experience gained in the GMALL Study Group and identified 8 *BCR-ABL*-positive adult ALL cases with such atypical transcripts: 5 with e1a3, 2 with e13a3 (b2a3), and 1 with e6a2. This corresponds to a prevalence of 1-2% of all *BCR-ABL*-positive cases. The clinical courses are reported and diagnostic proposals are made.

Key words: acute lymphoblastic leukemia, *BCR-ABL*, PCR.

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The *BCR-ABL* fusion gene is found in around 25% of adult ALL cases. Nearly all these cases are immunologically common or pre-B ALL, the fusion gene is rare in immature pro-B ALL and absent in mature (Burkitt-type) B-ALL. *BCR-ABL* is of interest in ALL for two reasons. First, those affected are known to have a poor prognosis under conventional therapy[1,2] and are therefore, considered *high-risk* patients and primary candidates for intensified therapy regimens, including allogeneic transplantation. Second, *BCR-ABL*-positive patients can have a significantly better prognosis under treatment regimens with *ABL* tyrosine kinase inhibitors such as imatinib[3] or dasatinib[4] and should therefore receive them whenever possible. The three *typical BCR-ABL* mRNA transcripts are e1a2, e13a2 and e14a2, but several *atypical* ones have also been observed.[5] However, these observations were made almost exclusively in CML cases, and very little is known about atypical transcripts in ALL. We report here our experience at the central diagnostic laboratory of the German Multicenter ALL (GMALL) Study Group.

Design and Methods

Patients' samples

All samples were obtained within the framework of the GMALL studies at primary diagnosis. Diagnosis of ALL was made or confirmed at our institution by immunophenotyping. Our investigations conformed to the principles of the Helsinki Declaration.

PCR method

Two RT-PCR methods were used, a nested PCR and a single-round multiplex PCR (later referred to as *multiplex PCR*). The conditions of the nested PCR have been previously described.[5] The multiplex PCR was adopted from Cross *et al.* (1993),[7] but primer B2B was substituted by primer *5'CML-IL*[6] (both primers are located in *BCR* exon 13). The HotStarTaq kit (QIAGEN, Hilden, Germany) was used, and a 15-minute denaturation step was added at the beginning of the cycler program. PCR was performed on a PerkinElmer 9600 or 2400 cycler. Each PCR was analyzed on a 1% TBE agarose gel. PCR products were identified by their size after electrophoresis.

Immunophenotyping

Immunophenotyping was carried out using standard methods as previously described.[2,8] All samples exhibiting a B-precursor immunophenotype (pro-B, common, pre-B) were subjected to PCR analysis for *BCR-ABL*.

Sequencing

DNA sequencing of purified PCR products was carried out on an ABI sequencer using standard techniques.

Results and Discussion

Between 2000 and 2007, a total of 1,214 adult B-precursor ALL cases were diagnosed and investigated for *BCR-ABL* at our institution using two different RT-PCR methods (one nested and one single-round multiplex PCR) as described. Four hundred and sixteen cases (34%) were *BCR-ABL*-positive (310/795 common=39%, 101/274 pre-B=36.9%, 5/145 pro-B=3.4%). The duplicate investigation not only improved diagnostic safety but also disclosed atypical transcripts which could not have been detected using only one method.

Atypical transcripts with a fusion of *ABL* exon 3 to *BCR* only yielded a PCR product in the multiplex PCR, since the *ABL* primer of the nested (second-round) PCR was located in *ABL* exon 2. The e1a3 transcript is not distinguishable from e13a2 by product size in the multiplex PCR.[9] In contrast to the e13a2 transcript, however, it does not yield a product in the nested PCR. Before 2000, diagnostics were performed using only the nested PCR, and no atypical transcripts were observed. Therefore, multiplex PCR was used to retrospectively reinvestigate 130 archived B-precursor ALL samples from the years 1995-1999 that had been confirmed *BCR-ABL*-negative by nested PCR. Atypical transcripts were identified and characterized by sequencing in 8 cases (Table 1, Figures 1 and 2): e1a3 in 5 cases, e13a3 in 2, and e6a2 in 1 case. The chromosomal breakpoints of the 2 e13a3-*BCR-ABL*-positive patients were identified in *ABL* intron 2 and *BCR* intron 13 by direct PCR amplification from genomic DNA and subsequent sequencing (EMBL Acc No AM400881 and AM600680). One patient (e1a3) had a pre-B immunophenotype and all others had a common B-precursor immunophenotype. Five patients displayed aberrant antigen expression (Table 1). Cytogenetic analyses revealed a t(9;22) translocation in 7 cases while in 1 case no cytogenetic data were available. Patient median age was 47 years (range 29-64). Four of the 8 patients had a leukocyte count of >30/nL at diagnosis and were therefore at high risk according to the GMALL criteria. Four of them presented with marked splenomegaly. Otherwise, the overall clinical features did not differ significantly from those of other adult ALL patients. All were essentially treated according to the GMALL 5/93, 6/99 or 7/03 and Elderly Therapy protocols[2,3] (Table 1). The 5 most recently diagnosed patients also received concomitant imatinib. One patient underwent allogeneic transplantation, and imatinib treatment was then discontinued because of side effects and the possible risk of interference with a T-cell-mediated GVL effect. In a second patient the imatinib dose had to be reduced because of hepatotoxicity. One patient received an autologous transplant and is still in CCR on imatinib. Three patients who did not receive imatinib died following hematologic relapse within 18 months of diagnosis while those 5 who received imatinib are still alive, although with a short follow-up time (130-540 days, median 280 days).

Table 1. Characteristics of patients with atypical *BCR-ABL* transcripts. Immunologic, genetic, and notable clinical features of the 8 identified patients. Cytogenetic analysis revealed no metaphases in 2 cases. Five patients received imatinib up-front with concomitant chemotherapy.

	Age (yrs), m/f	*BCR-ABL transcript*	*Immuno-phenotype*	*Cytogenetics*	*WBC (/nl)*	*Clinical features at diagnosis*	*Imatinib (mg/day)*	*Therapy, clinical course*
1	62m	e13a3	common[1]	46,XY, t(9;22)(q34;q11) FISH: BCR-ABL-positive	10	Splenomegaly (16 cm)	-	According to *GMALL 5/93*, CR after induction, auto Tx, relapse and death month 18
2	64f	e1a3	common	46,XX	1.8		-	According to *GMALL 5/93*, CR after induction, relapse month 8, death month 16
3	31m	e1a3	common[2]	44,XY, dic(3;9)(q27;p11) t(9;22)(q34;q11), -7	31	Hepato-splenomegaly	600 (before allo Tx)	According to *GMALL 7/03*, CR after induction, allo Tx day 154, in CCR month 13
4	61f	e1a3	common	No metaphases	14	Splenomegaly	400	According to *GMALL Elderly*; CR after induction, under maintenance therapy in CCR month 18
5 *5/93*,	48m	e1a3	common	46,XY	62		–	Start according to *GMALL* therapy interrupted, relapse and death month 6
6	37f	e13a3	common[3]	45,XX,-7, t(9;22)(q34;q11)	70		600/300	According to *GMALL 7/03*, CR after induction, allo Tx day 120, in CCR day 140
7	45f	e1a3	pre B[4]	No metaphases, FISH: BCR-ABL-positive	5		600	According to *GMALL 7/03*, allo Tx day 100, in CCR day 130
8	29f	e6a2	common[3]	46,XX, t(9;22)(q34;q11) FISH: BCR-ABL-positive	40	Splenomegaly (20 cm)	600	According to *GMALL 7/03*, CR after induction, allo Tx day 180, in CCR day 200

WBC: white blood cell count at diagnosis. [1]with myeloid co-expression (CD33, CDw65); [2]with aberrant CD117 (c-kit) expression; [3]with myeloid co-expression (CD33, CD13); [4]with myeloid co-expression (CD13).

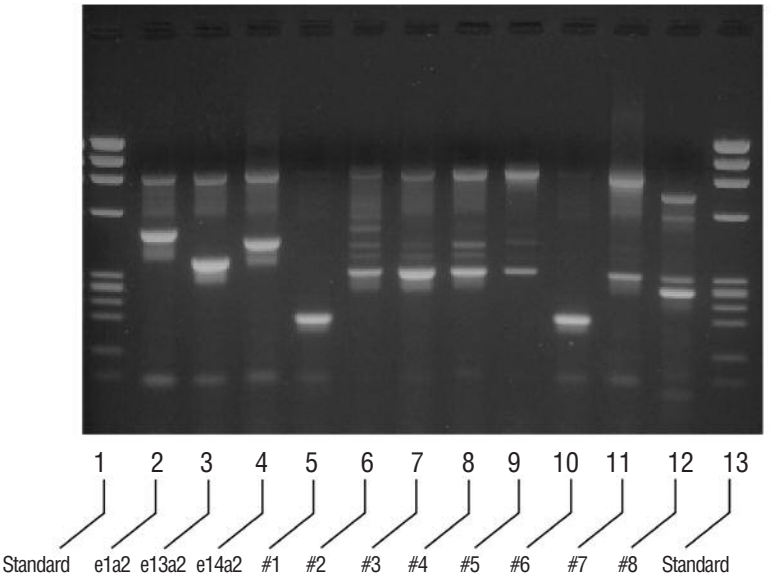

Figure 1. Atypical transcripts. The agarose gel shows the 3 typical *BCR-ABL* transcripts, e1a2, e13a2 and e14a2 (lane 2-4), and the 8 patients (#1-#8) with atypical transcripts (lane 5-12). Lanes 2-11 were produced using the multiplex PCR described in the text, while case #8 (lane 12) was recently identified using a newly developed optimized *BCR-ABL* PCR [*Burmeister, manuscript submitted*]. Patients are numbered as in Table 1. Lane 1 and 13: °174/Hae III digest size standard.

Since e1a2, e13a2 and e14a2 are the three variants comprising the vast majority of *BCR-ABL* transcripts in adult ALL, many diagnostic PCRs are designed to focus on their detection. However, several reports of CML patients have drawn attention to a number of *variant BCR-ABL* transcripts such as e1a3, e6a2, e8a2, e13a3, e14a3, e19a2 and others.[5,9-12] These atypical transcripts in CML have a reported prevalence of around 1-2%,[13] although this may be underestimated.[12,14]

To our knowledge, only 2 adult ALL patients with atypical *BCR-ABL* transcripts have been described so far.[15] The patients were a 39-year-old female with common ALL and e1a3, and a 61-year-old male with pre-B ALL and an e13a3 transcript. A lymphoid CML blast crisis could not be ruled out in the latter case. Both patients died 9 and 14 months after diagnosis in disease progression. Wilson *et al.*[14] also reported an e1a3 transcript in an ALL patient but provided no clinical data. There have been no systematic investigations in larger ALL patient cohorts.

The present investigation detected atypical transcripts in roughly 1-2% of all *BCR-ABL*-positive patients. The atypical transcripts detected here were: e1a3 in 5 cases, e13a3 in 2, and e6a2 in 1 case. Of particular interest is the e6a2 *BCR-ABL* transcript found in our study. This transcript has only been reported in 7 patients with myeloid leukemias,[10] 4 with chronic-phase CML, 1 with myeloid blast crisis, 1 with CMML, and 1 with acute basophilic leukemia. Our patient is the first ALL case with this rare *BCR-ABL* transcript variant. It has been hypothesized that CML patients with e6a2 *BCR-ABL* may have a worse prognosis[16] possibly because they lack the (GET)/*dbl*-like domain of *BCR*. However, the massive splenomegaly in our ALL patient is not in line with the conspicuous absence of splenomegaly in previously described e6a2-positive CML cases.[10] This transcript variant may therefore be associated with a broad spectrum of different leukemia types. The other atypical transcripts found – e1a3 and e13a3 - may be regarded as variants of the typical minor and major breakpoint transcripts e1a2 and e13a2 but with a chromosomal breakpoint at 562 bp *ABL* intron 2 instead of 140 kb *ABL* intron 1. The µ-bcr e19a2 transcript was not detected in our series of patients. It is believed to be associated with a relatively

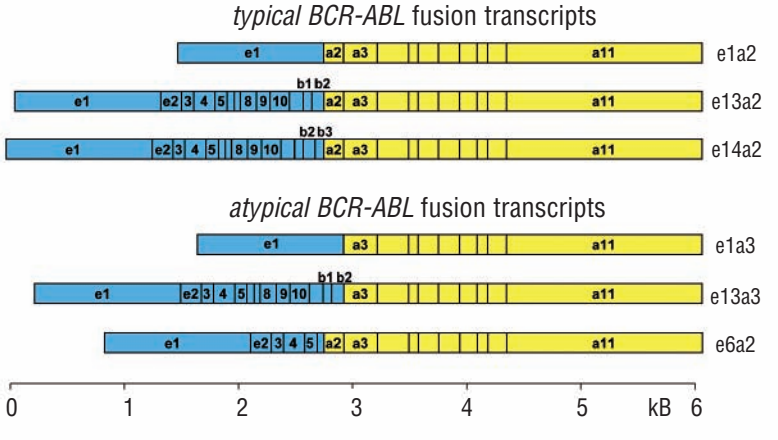

Figure 2. Typical and atypical *BCR-ABL* transcripts in adult ALL. Typical transcripts in ALL are e1a2, e13a2 and e14a2. Atypical transcripts lacking *ABL* exon 2 are e1a3 and e13a3. The atypical e6a2 transcript arises from a breakpoint outside the major or minor *BCR* breakpoint region.

mild myeloproliferative disorder (*neutrophilic CML*) and has never yet been observed in an ALL case.[17] The transcript levels in affected patients are very low, and the transforming capacity of the resulting p230 chimeric bcr-abl protein appears to be relatively weak. Most CML cases with atypical *BCR-ABL* transcripts reported in the literature had a more benign course and were responsive to imatinib and/or interferon α,[9,11,18,19] but myeloid blast crises have also occasionally been noted.[18,20] Theoretically, all atypical *BCR-ABL* transcripts should in principle be responsive to imatinib treatment, regardless of whether *BCR* sequences are fused to *ABL* exon 2 or 3, since they all encode the ATP-binding pocket (i.e. the imatinib binding site) of the *ABL* kinase. Transcripts lacking *ABL* exon 2 are thought to be associated with a more benign clinical course because they have a truncated *src homology 3* (SH3) domain and may therefore be less leukemogenic, possibly due to decreased STAT5 activation.

In conclusion, our results suggest that most of the atypical transcripts known from CML can also be found in *BCR-ABL*-positive ALL, and that their relative frequency is similar. The most frequent atypical transcript in adult ALL appears to be e1a3. Although atypical *BCR-ABL* transcripts are rare in adult ALL, care should be taken not to overlook them. PCR-based *BCR-ABL* diagnostics should be designed to also detect atypical transcripts, at least those with fusion of *ABL* exon 3 to *BCR*. A complementary cytogenetic analysis remains mandatory. Since the first submission of this manuscript, 4 additional e6a2-positive cases have been described, all myeloid leukemias.

Authors' Contributions

All authors have critically read and approved the manuscript. TB: designed and performed research (PCR), wrote the paper; SS: performed immunophenotyping; AT, HT, HD, EJ, TL, FS, UJMM, JE: treated patients; HR: performed cytogenetic analysis; DH: head of the GMALL Study Group, performed GMALL study design; NG: head of the GMALL Study Center, analyzed data; ET: member of the GMALL study board, analyzed data.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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